FACSS PRESENTS

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Nugget Casino Resort

Sparks, Nevada

ABSTRACT BOOK

Sunday, October 8, 2023

Oral Presentations

Keynote: "The Remarkable Correspondence with Advances in Vibrational Spectroscopy," Peter Griffiths, Sierra 5

(SUN-01.1)**Fifty years of FACSS and SciX Conferences: The remarkable correspondence with advances in vibrational spectroscopy**

Peter Griffiths, *Griffiths Consulting*

Prior to 1973, there were many small analytical chemistry conferences in the Fall, with Pittcon maintaining its predominant position in the Spring. In an attempt to reduce the number of Fall meetings, several of the organizers of these conferences decided to merge several of them to form the Federation of Analytical Chemistry and Spectroscopy Societies (FACSS). The location of FACSS meetings would be selected so that hotel rooms would be sufficiently affordable that a week's stay would not break the budgets of university faculty. As a result, graduate students and post-docs would be more readily able to attend. The first FACSS conference was held in Atlantic City, with subsequent conferences held in such locations as Indianapolis, Detroit, St. Louis and Kansas City. At one stage, it looked as if the organizers had settled on Philadelphia as a permanent site, with FACSS being held in the Adams Mark Hotel from 1979 through 1985. However, from 1986 to the present day, it reverted to its original goal of varying the location, albeit often in a relatively expensive hotel. However, only once in the first 25 years of its existence was the conference held west of St. Louis in 1991 when a joint meeting with the Pacific Spectroscopy conference was held at the Disneyland Hotel. Even though it would have been beneficial to the attendees, a joint meeting with EAS never took place. Attendance at all 50 meetings was usually between 1000 and 2000. To increase the registration, the organizing committee rebranded the conference and changed the name to The Great Scientific Exchange in 2011, but this had little effect on the attendance. Analytical chemistry underwent major changes in the 50 years of FACSS's existence, as instruments became smaller and more powerful. This was certainly the case for vibrational spectroscopy with FT-IR instruments dominating the market after about 1975. This allowed a wide range of techniques to be introduced that were not achievable with grating spectrometers. In several respects, the development of analytical instruments was mirrored by the development of FACSS/SciX and examples of this correspondence will be shown in this talk.

Monday, October 9, 2023

Oral Presentation

23ART01: Student Research in Archaeological Chemistry, Southern Pacific F
Chair: Juan Santiago
Co-Chair: Md Nazibul Islam

(ART-01.1)**The Mobility of Fecal Stanols in Soils**

AJ White, Varenka Lorenzi, Lora Stevens; *University of California, Berkeley, California State University Long Beach*

Fecal stanol molecules identified in water bodies and sub-aqueous sediments are used as human biomarkers in diverse applications from sewage monitoring to archaeological studies. However, the rate and mode of transportation of these molecules to their point of deposition are poorly understood. Specifically, a potential lag between the time of defecation and time of eventual deposition would create chronometric error in archaeological studies. This talk presents preliminary data from an ongoing field experiment in San Dimas Experimental Forest, California that attempts to identify and track the movement of fecal stanol molecules through soils. We established two field plots in different slope settings, applied pig dung, and sampled plot soil at fixed depths and lateral distances over six-

month intervals. Our initial results show high vertical mobility in the flat plot, some vertical and lateral movement in the sloped plot, and no lateral migration in the flat plot. These results indicate that fecal stanol molecules are mobile in terrestrial sediments, but their mobility is uneven due to multiple topographic, hydrological, and pedological factors.

The Effects of Archaeological Fires on Volcanic Glass Shards: An Experimental Approach

(ART-01.2)**Jayde N. Hirniak**, Panagiotis Karkanas, Eamonn Needham, Eugene Smith, Christopher Campisano, Curtis Marean; *Arizona State University; American School of Classical Studies at Athens; University of Nevada, Las Vegas.*

Fire usage is a common behavior preserved at archaeological sites during the Middle and Later Stone Age throughout South Africa. In fact, many sites preserve multiple combustion features throughout the stratigraphy, demonstrating continuous and habitual use of fire. Because of the abundance of combustion features throughout the record, it is critical to understand the taphonomic effects of direct heat and burning on materials present within the sediment. Here, we present an experimental study that tests the effects of heat and burning on volcanic ash. Volcanic ash is used at various sites as a dating and correlation tool, and the geochemical signature of the glass within the deposits is used to correlate to an eruption in time. However, there is a lack of understanding of how fire conditions affect the geochemistry of the volcanic glass, presenting an issue when using this tool at archaeological sites with abundant fire use. Therefore, we explore the effects of indirect heating using a kiln and direct heating through actualistic fire experiments. We placed 0.5 grams of volcanic ash in a kiln and heated it in 100-degree increments ranging from 200-900 degrees Celsius. We also mixed volcanic ash in experimental fires to examine the changes in an environment more realistic to an archaeological fire. Following experiments, we measured the major and trace element chemistry to understand the changes that occurred during these processes. The results of this study are important for understanding the taphonomic processes that affect the geochemistry of volcanic glass, which is essential for the success of this dating and correlation tool.

(ART-01.3)**The Application of Trace Metal Stable Isotopes to Hominid Physiology and Behavior**

Renee Boucher, Linda Godfrey, Paul Koch; *University of California, Santa Cruz, Rutgers University, New Brunswick*

Previous work has shown that the stable isotope ratios of Fe and Cu (δ56Fe and δ65Cu) are potential proxies for sex-related differences among mammals. Two underlying mechanisms have been proposed: the relative abundance and speciation of these elements in blood and mass-balancing caused by blood loss in menstruating females. However, additional evidence is needed to determine which mechanism applies to non-human primates. The analysis of δ56Fe and δ65Cu has been applied to modern and historical human bone, as well as to the bone of non-human primates. Rhesus macaques (Macaca mulatta) are unique in that they have a similar ovarian cycle to humans, which often involves the same degree of menstruation. Here, we present a modified Fe and Cu column chromatography protocol for use on bioapatite. We discuss the applications of trace metal stable isotope analysis to a free-ranging population of rhesus macaques ($n = 20$) and show sex-related differences in δ65Cu values ($T = 2.94$, $p = .01$), which is in line with data reported from mice. Like rhesus macaques, chimpanzees are often used as comparative models for the socio-ecological context of early humans, or hominins. However, unlike rhesus macaques, they do not menstruate and are often pregnant or lactating for much of their lifespan. To validate which mechanism contributes to sex-related differences in non-human primates, we also apply these novel methods to bioapatite ($n = 30$) from a historical museum collection of chimpanzees (P. t. troglodytes) and bonobos (Pan paniscus). Thereby, we can explore the potential mechanisms in a model that is more akin to early hominins, shedding light on what the Fe and Cu isotopic signature might look like evolutionarily and how sex-related differences in the metabolism of transition metals affects this. We establish a reference dataset with the potential to expand the use of these systems to address broader evolutionary questions on female reproductive biology in modern humans.

(ART-01.4)**Paint Production in the Chaco World: Revealing Networks of Power from the Chemical to the Cosmological**

Kelsey Hanson; *University Of Arizona*

Paint is one of the oldest human technologies, but one of the most misinterpreted and undertheorized in archaeological contexts. In Pueblo communities of the northern U.S. Southwest, color is crucial for expressing and initiating core cosmological tenants, like cardinal directions, seasons, and sociopolitical affiliations. Paint is an important material expression of these cosmologies and is used widely in pottery, murals, pictographs, performance regalia, and altars. The recipes for paint often follow strict adherence to sodality-controlled rules, making it an ideal material to explore relationships between knowledge and power. Many scholars argue that the roots of these chromatic sociopolitical systems originate in the Chaco World, centered in northern New Mexico (A.D. 850–1150). Drawing from the analysis of 1,566 paint-related objects from museum collections from Chaco-era archaeological sites, I explore the diversity of paint recipes in use, and ask to what extent these recipes were shared, centralized, or controlled through time. I accomplish this by characterizing paint recipes through a combination of polarized light microscopy, X-ray fluorescence, Fourier transform infrared spectroscopy, Raman spectroscopy, and scanning electron microscopy with energy dispersive spectroscopy. Social network analysis of shared compositional signatures illustrates the circulation of shared recipes and accompanying knowledge within and between Chaco communities. In this paper, I will share the preliminary results of these analyses to discuss networks of sociopolitical power through time, offering new histories of shifting power dynamics in the Chaco World.

(ART-01.5)**Embodiment, Identity, and Infant feeding Practices in the Ancient Andes: Analysis of δ13C and δ15N in Serial Samples of Permanent Molars from Tiwanaku, Bolivia**

Marcos de la Rosa-Martinez; *Arizona State University, School of Human Evolution and Social Change*

Understanding the complex roles and meanings of breastfeeding practices and childhood provisioning may help anthropologists contextualize paleodietary studies and the role of foodways in the construction and maintenance of social identities in human antiquity. Here, I employ stable isotope measures (δ13C and δ15N) of weaning age and early childhood diet derived from serial micro-samples of first molar dentin from 30 individuals dating to the Middle Horizon (ca. CE 500-1000) interred in the urban core of the ancient Tiwanaku state, in the Titicaca Basin, to examine interpersonal variation in breastfeeding and weaning practices, along with childhood provisioning, across site sectors, osteologically determined sex, and human body modification. The expansion of the Tiwanaku polity (ca. CE 700-1050) encompassed the foundation of state-level, multiethnic communities utilizing variable subsistence strategies throughout differing environments in the South-Central Andes. Relatively little work has focused on childhood diet and development within the pre-Hispanic Andes, where numerous cultural and ecological factors feed into the decision-making regarding childhood diet and development. Isotopic analysis of incrementally growing dental tissues will provide biogeochemical evidence of weaning and dietary histories, and how these processes changed over the months and years at the inter- and intra- individual level. This novel biogeochemical data will be combined with more traditional archaeological and paleopathological datasets, as well as Bayesian modeling, to address the variation of early life experiences within the pre-Hispanic Tiwanaku polity and to identify distinct dietary histories, and their relationship with early life stressors and child-rearing decisions made by communities of high-altitude populations in the Andes.

23ATOM03: From Humble Beginnings – The Great Diversity of Glow Discharge Spectrometries, Central Pacific A/B/C
Chair: R. Kenneth Marcus

(ATOM-03.1)**A Long Way to Go - 50 Years of Permanent Progress from Glow Discharge Emission Spectroscopy to Glow Discharge Mass Spectrometry**

Norbert Jakubowski; *Spetec GmbH*

This lecture will focus on the historic development of glow discharge techniques for direct analysis of solid materials and the strength and weaknesses of these analytical methods will be discussed. Our story will start in the early 1970tees with the development of continuous DC discharges for direct analysis of metals by optical emission spectroscopy, will cover the capability for depth profiling and will discuss the need for rf-discharges for analysis of ceramic materials. Finally we will discuss why for decades most of the discoveries in emission spectroscopy were transferred to mass spectrometric techniques. The advantages of the latter analytical method are very obvious. For analysis not thousands of lines have to be investigated but just a few isotopes are sufficient to get all analytical information, thus analysis is simple, spectroscopic interferences well known in optical emission spectroscopy are less pronounced and so limits of detection are lowered from the ppm range (in emission spectroscopy) down to the ppb range.
The second part of this lecture is dedicated to instrumental developments in glow discharge mass spectrometry starting with a dc glow discharge instrument, the VG 9000, a double focusing sector field device, extending the field to quadrupole and time-of-flight mass analyzers for direct analysis of solid and semiconducting materials and discussing the need for pulsed and rf-discharges for the analysis of ceramics and polymer materials.
Finally new trends for fast flow discharges and new commercial devices will be discussed and future trends will be highlighted to demonstrate that 50 years of permanent progress in instrumentation had opened the door to novel applications of glow discharge mass spectrometry with tremendous impact on the development and analytical characterization of novel and innovative materials which are the basis for future technical improvements.

(ATOM-03.2)**Glow Discharge Optical Emission Spectroscopy from 1852 to the present - still going strong**

Arne Bengtson; *Swerim AB*

A glow discharge (GD) is a plasma formed by the passage of electric current through a gas. The (probably) first publication describing a GD device is from 1852 by W.R. Grove who studied the electrical conductivity of gases. He observed that the cathode was eroded by the discharge and thereby also discovered sputtering. The first GD lamp was designed by Geissler 1857, producing different colours with different gases. Plücker and Hittorf did the first studies of optical emission spectroscopy (OES) using Geissler tubes in 1858, thereby pioneering GD-OES. A more recent well-known type of GD is the Hollow Cathode lamp, extensively used as a line emission source for Atomic Absorption spectroscopy. The birth of GD-OES for chemical analysis in the metallurgical industry was the development of the Grimm type GD for flat metal samples, commercialised in 1968 as part of a complete spectrometer system. This was a direct current (DC) source, very stable, with spectra of excellent quality. For routine analysis of metallurgical samples the Grimm GD has had considerable success for several types of complex alloys, but never posed a real challenge to the spark source for routine analysis of e.g. low alloy steel. Soon after the introduction of the Grimm lamp, several researchers started the development of what has become the major type of application – compositional depth profiling (CDP) of technical surface layers. The “flat” sputtering process combined with fast time-resolved detection enable the analysis of chemical depth profiles, very fast combined with advanced high vacuum techniques, at considerably lower cost. At present, instruments for CDP are common in industrial laboratories around the world. The development of CDP, with particular emphasis on methods for quantification, will be presented and discussed. A major improvement of the technique was the introduction of Radio Frequency (RF) powered GD lamps in the late 1980s, allowing analysis of non-conductive surface layers. More recently, the introduction of spectrometers with solid state array detectors has further increased the flexibility of the technique considerably. The state-of-art of GD-OES instrumentation and analysis methods will be discussed.

(ATOM-03.3)**Radiofrequency (Em)Powered Glow Discharge Spectrometry: Enabling novel analysis methods from materials to biological sciences**

Gerardo Gamez, Harsshit Agrawaal, Rajendra Joshi, Hanuk Kwon; *Texas Tech University Dept. of Chemistry and Biochemistry*

After their initial demonstration for mass spectrometry in the early 70s [1], it took almost two decades for rf powering schemes in glow discharge to re-emerge and be implemented for optical emission spectroscopy [2, 3]. Their incorporation into commercial instruments in the early 90s gave way to a swell of research output. The advantage was clear, as opposed to its d.c. counterpart, rf glow discharge did not required samples to be conductive, thus opening the door to a plethora of applications in many different fields [4]. Here, we will discuss the range of applications enabled by rf glow discharge spectrometries, from materials to environmental to biomedical sciences. In addition, we will present how these advances have opened the way to the latest developments in glow discharge optical emission spectroscopy elemental mapping, including the most recent methods for ultrahigh throughput nanoparticle characterization in terms of composition, distribution, and size, with potential enhancements via novel sample deposition and hyperspectral imaging techniques.

1. J.W. Coburn, E. Kay, A new technique for the elemental analysis of thin surface layers of solids, Appl. Phys. Lett. 19 (1971) 350–352.
2. M. Chevrier, R. Passetemps, Proce´de´ et dispositif d'analyse de surfaces non-conductrices, European Patent No. 0 296 920 A1 (1988).
3. D.C. Duckworth, R.K. Marcus, Radio frequency powered glow discharge atomization/ionization source for solids mass spectrometry, Anal. Chem. 61 (1989) 1879–1886.
4. M.R. Winchester, R. Payling, Radio-frequency glow discharge spectrometry: A critical review, Spectrochimica Acta Part B 59 (2004) 607–66.

(ATOM-03.4)**New Developments and Strategies for Solution-Cathode Glow Discharge Atomic Emission Spectrometry**

Steven Ray, Nicholas Hazel, Mitchell Stry; *SUNY Buffalo Dept of Chemistry*

A new generation of liquid-based glow discharge plasmas are being developed for atomic spectrometry which hold a number of significant advantages over current technologies. One example, the solution-cathode glow discharge (SCGD) is a simple, low power (80W), portable plasma sustained in ambient atmosphere directly upon a sample solution that is being developed for atomic emission spectrometry (AES). The sample solution is directly sampled by the SCGD plasma, with analyte entering the plasma directly to be excited. The SCGD and similar liquid-based glow discharges can often offer limits of detection competitive with established techniques like Inductively-Coupled Plasma Atomic Emission Spectrometry (ICP-AES). However, it has been widely reported that particular elements do show compromised limits of detection, limiting the applicability of the strategy in particular applications.

Here, we will critically examine several new instrumental strategies designed to advance the capabilities of SCGD-AES and similar liquid-based glow discharge experiments. These capabilities coupled with the minimal operating requirements of the plasma have made the SCGD-AES experiment an attractive candidate for on-line, continuous, or in-field applications. Finally, the utility of these liquid-based glow discharge systems for other applications in analytical chemistry analyses will be discussed.

(ATOM-03.5)**Mass Spectrometry of Solution Glow Discharges: Combined Atomic and Molecular (CAM) Ionization**

R. Kenneth Marcus; *Clemson University*

Often overlooked, the very first mass spectrometry ionization sources were, in fact, glow discharges. Taken a step farther, J. J. Thomson would be the father of glow discharge mass spectrometry (GDMS). Beyond his work of the early 20th century, there was actually not a commercially available GDMS until the early 1980s. To date, low-pressure, sputtering-based GD sources are still the only ones that are commercially available, sold predominately for the analysis of high-purity materials or samples requiring depth-resolved analysis.

The early 2000's saw the advent of atmospheric pressure plasmas, operating in a modality where an electrolytic solution acted as one of the electrodes, was shown to possess i-V characteristics which fit the definition of a glow discharge. To date, there have probably been over 20 different

designs fitting the classification of a solution electrode glow discharge, all operating at atmospheric pressure and employed for optical emission analysis; each with its own name/acronym. Likewise, in 2001 this laboratory developed its own, unique design called the liquid sampling-atmospheric pressure glow discharge (LS-APGD). That project ultimately died in 2007 or so.

A collaborative project between this laboratory and the Pacific Northwest National Laboratory (PNNL) was initiated in 2010, investigating the potential of coupling a microplasma (the LS-APGD) to an ultrahigh resolution Orbitrap mass spectrometer. That effort was successful from the very beginning, demonstrating excellent capabilities towards elemental and isotope ratio analysis, while also reaping the benefits of mass resolution exceeding m/□m ~70,000. Since that time, over 25 refereed publications have described the MS sampling of the LS-APGD microplasma, predominately in the areas of isotope ratio mass spectrometry performed on the Orbitrap. Completely unexpected, but now demonstrated in many chemical systems, is the fact that the LS-APGD can also produce relevant mass spectral from molecular species, ranging across pharmaceuticals, inorganic complexes, polyaromatic hydrocarbons, proteins, and now polyfluorinated alkyl substances (PFAS). The overall capacity is termed 'combined atomic and molecular', or CAM, ionization. Indeed, other solution glow discharges have demonstrated this capability. We will describe here the diverse applications and capabilities that these microplasma have in CAM-MS; clearly an untapped market opportunity.

23BIM06: Spectroscopy and The Role it Plays in Commercialization of NextGen Therapeutics, Sierra 2
Chair: Linda Kidder

(BIM-06.1)**Estimating the optimal number of components in PARAFAC models of complex fluorescence data sets**
Helene Fog Froriep Halberg, Marta Bevilacqua, **Åsmund Rinnan;** *University of Copenhagen, Department of Food Science*

The measurement of autofluorescence by excitation-emission matrices combined with Parallel Factor Analysis (PARAFAC) has successfully been applied for the analysis of food and beverages. For the optimal decomposition of such data, it is vital to establish the proper number of components of the PARAFAC model, which is not trivial when the sample complexity increases. The two main diagnostic tools for the choice of the number of PARAFAC components are the core consistency and the split-half analysis. In this talk I will show that the core consistency is a too conservative diagnostic, and that the split-half becomes too unstable. I will show when and why they do not work, and come with some suggestions as to what could be used instead.

(BIM-06.2)**Assessment of Fluorescence Impact on Low-Level Detection in Drug Product**
Vladimir Villanueva-Lopez, David Wilsdon; *Worldwide Research and Development, Pfizer Inc.*

The ability to quantify a component accurately and precisely in a moving blend depends on the measurement technique, the molecules' intensity of response (and surrounding matrix), and the sample presentation. Near Infrared (NIR) spectroscopy has been widely used across the industry and within Pfizer to measure the quantity of API present in blends by integrating a spectrometer to a tablet press feed frame for the PCMM (Pfizer Continuous Manufacturing Module) platform. The powder is measured seconds before the material can be compacted into tablets. NIR spectroscopy is limited when the material is present at levels around 3.00 % w/w and below. To support the portfolio and expand the sensing envelope, we require additional tools to support PCMM.
This presentation will cover an assessment of the inherent fluorescence challenge in developing low-dose sensing based on Raman Spectroscopy using a Science of Scale (SoS) tool known as the Benchtop Feed frame. The SoS is a small replicate of the tablet press filling chamber and enables running experiments with a small amount of material. A design of experiments was established to include excipients fluorescence, lot-to-lot materials variability, and day-to-day variability. Several chemometrics approaches and data preprocessing were tested to enhance the predictive capabilities of the analytical method.

(BIM-06.3)**Making Chemical Imaging an Essential Part of Product Development**

Patrick Wray, Dimuthu Jayawickrama, **Zoë Whalley**, Gregory Lane, Lucy Hawarden, Venkata Bobba, David Trinkle, Elyse DiMaso, Gary McGeorge; *Bristol-Myers Squibb, The University Of Birmingham, BMS.*

Spectroscopic imaging techniques have the power to provide incomparable insights into any chemical and physical phenomena surrounding pharmaceutical manufacture, stability and dissolution due to their ability to provide highly resolved spatial and chemical information.
Until now these advanced techniques have mostly been viewed as problem solving tools due to their complexity of operation, analysis and perceived time-consuming operation.
There have been significant recent advances to overcome these limitations to enable chemical imaging to become an integral part of product development. Importantly, modern imaging systems are orders of magnitude faster which allows for statistically significant amounts of data to be gathered in a matter of seconds.
Through the development of software and workflows, the complex process of analysis has been transformed into an automated flow. This process can handle large amounts of generated data and rapidly carry out statistical analyses of the samples and transfer the results into a database.
The work in question focuses on statistics to record the size, frequency and shape of domains of API or polymer found in samples. These statistical analyses can also carry out more global sample analyses to assess sample homogeneity and texture metrics. The process can be applied to blends, granules and tablets and has shown significant utility in monitoring the effects of changes to manufacturing processes or to trending the stability of a process over time.

(BIM-06.4)**Multidimensional Fluorescence (A-TEEM) For The Characterization Of Challenging Samples - From AAVs To Exosomes**

Jeffrey Julien, Linda Kidder, Adam Gilmore; *Horiba Instruments Incorporated*

A-TEEM is a multimodal spectroscopic approach that incorporates; 1) UV/Vis spectroscopy ('A-T' for Absorbance-Transmission); and 2) 3D fluorescence ('EEM' for Excitation Emission Matrix) and for a 2-in-1 measurement approach that has shown significant capability in rapid and cost-effective analysis of biotherapeutics and natural products. A key attribute of A-TEEM that differentiates it from vibrational spectroscopy is the very low limits of detection, in the PPB to PPT range, which is several orders of magnitude better than NIR, FT-IR, and Raman spectroscopy. Additionally, the sensitivity and specificity of the A-TEEM technique is very high and is conducive to measurements taken in aqueous solutions. All of these characteristics distinguish it as a robust tool for biopharmaceuticals characterization, where compounds of interest are present in low concentration and sample matrices tend to be very complex. Separations techniques tend to dominate given these sample characteristics, where the components of interest must be separated from the matrix for detection. A-TEEM has demonstrated capability to characterize these complex samples prior to separations, which if feasible is preferable, saving both time and money. We will show practical examples for biopharmaceuticals, highlighting the potential for robust classification and quantification in areas as diverse as vaccines, viral vectors, and exosomes.

(BIM-06.5)**Using Polarized Excitation Emission Matrix (pEEM) for monitoring protein conjugation reactions.**

Alan Ryder; *Nanoscale Biophotonics Laboratory, University Of Galway.*

Conjugation of small molecules to proteins is important for many therapeutic applications including Antibody Drug Conjugates (ADCs) and PEGylation. Ideally one needs a non-destructive, in-situ based method for monitoring the conjugation reactions as well as the final products. By observing intrinsic protein emission using polarized Excitation-Emission Matrix (pEEM) spectroscopy, one can get a more detailed and informative measurement of protein changes in solution. pEEM is a 4D (λex, λem, IF, r) extension of conventional 3D EEM measurements (λex, λem, IF) where polarization (or anisotropy, r) can provide unique information about protein size and mobility.

pEEM measurements can be separated into parallel (EEM∥) and perpendicular (EEM⊥) polarized EEMs which provide size information about particle content. This can be coupled with the Rayleigh scatter signals to provide additional information about changes in particle size. The big advantages of pEEM protein analysis in an industrial context is that it is label free, needs minimal sample handling, and can be implemented using conventional bench-top fluorometers.

In proteins, pEEM measures the complex, intrinsic emission of tyrosine and tryptophan fluorophores that integral to the structure. pEEM data analysis and interpretation using multivariate data analysis, can extract a wide range of useful information about structure and size providing the basis of a robust conjugation reaction monitoring method for therapeutic proteins. Here we will discuss some of the significant advantages of pEEM as an analytical method for monitoring conjugation reactions including: it can be implemented on small reaction volumes (in a cuvette), the use of the parallel (EEM∥) polarised spectral measurements provide information about particle content and size, and the non-destructive aspect allows for sample retention for orthogonal analytical testing. Here we show in detail several case studies involving the in-cuvette monitoring of PEGylation [1] and ADC linker conjugation [2]. We show how pEEM reveals the presence of transient aggregates and can quantify certain critical aspects such as degree of PEGylation and Drug to Antibody ratio.

References:
(1) de Faria e Silva, AL.; Elcoroaristizabal, S.; Ryder, AG. Biotechnol. Bioeng. 2020, 117, 2969-2984.
(2) de Faria e Silva, AL.; Ryder, AG. Biotechnol. Bioeng. 2022, 119, 3432– 3446.

23CHEM01: Recent Advances in Chemometrics, Southern Pacific E

Chair: Peter Harrington

(CHEM-01.1)Uncovering Chemical Information using Virtual Reality

John Kalivas, Jordan Peper; *Idaho State University*

Chemical data exploration by visualization is an open-ended research area when considering how to move forward from traditional flat screen graphics. Immersive virtual reality (VR) offers incredible opportunities allowing significant expansion of human decision involvement. Adding human data analysis decisions helps algorithms succeed where automated algorithms fail. It is documented that as the degree of data complexity increases, the likelihood of automated algorithms failing also increases compared to incorporating more human decisions. One example is the computer game Foldit where human players compete with each other to discover high-scoring protein folds representing a protein's physical structure. Presented are our approaches for dynamic data analysis using interactive VR for 3D data visualization that also includes feeling data shapes with texture using haptic gloves as well as hearing the data by sonification. With VR, the human interacts with a computer to explore data uncovering hidden patterns for chemical attributes and sample relationships e.g., correlations, outliers, chemical properties such as for quantitative activity structure relationship (QSAR) studies. Current multivariate data visualization methods are limited to 2D and 3D graphics observed on flat computer screens, for instance, heatmaps, 3D bar plots, networks, or projection methods such as principal component analysis (PCA). The human brain is more efficient at making decisions when processing complex data than computers, especially when shape, texture, and sound are part of the decision. The user is immersed in the data with interactive zooming, rotation, and angle of view capabilities such as viewing and touching data from inside a data cloud outward in addition to inspecting data from the standard flat screen perspective of an outside observer peering inwards. Presented are applications of VR enhancing discriminant analysis for complex classification problems and model selection for calibration methods such as PLS. Opening up data analysis with VR allows humans to visually, haptically, and sonically immerse themselves inside data thereby reducing reliance on automatic and black-box procedures. Using VR in the touch and sound modes also provides new tools for data analysis by the vision impaired.

(CHEM-01.2)**Graphing a New Path Forward: Network Analysis in Chemometrics**

Caelin Celani, William Gilbraith, Karl S. Booksh; *University of Delaware, Savannah River National Laboratory*

Graph-based analysis has found a niche across many academic disciplines including synthetic chemistry, social psychology & sociology, computer science, mathematics, biology, and more. Despite broad utilization, analytical chemists and chemometricians have been slow to adopt the use of graphs in the analytical data analysis toolbox. This presentation will discuss a combination of well-established and novel graph-based methods and how they can be used for a variety of tasks in the analytical chemistry laboratory and data analysis workflow. Some examples that may be discussed include the use of graphs for chemical plant monitoring, environmental chemistry, temporally evolving systems, synthetic and computational chemistry, as well as graphs' potential for use in variable selection, calibration transfer, visualization, and data compression.

(CHEM-01.3)**Analysis of the Electrochemical Depolymerization of Lignin using Chemometrics**
Gobind Sah, John Staser, Peter Harrington; *Ohio University, Harrington Center for Intelligent Instrumentation.*

The economics of electrochemical depolymerization of lignin are most likely unfavorable without some control over the oxidation mechanism because depolymerization generates many unwanted compounds. Therefore, it is crucial to understand whether hydroxyl radicals mediate the oxidation process instead of a direct electrochemical route to depolymerization. Control over the depolymerization process can lead to high-yield chemical products like aromatic phenols and carboxylic acids. In this study, lignin compounds were electrochemically oxidized using a Nickel-Cobalt (Ni-Co) electrocatalyst at several electrode potentials. The oxidation products were analyzed using headspace solid-phase micro-extraction (SPME) gas chromatography-mass spectrometry (GC-MS), and chemometric tools, including singular value decomposition (SVD), principal component analysis (PCA), and multivariate curve resolution (MCR). The results revealed that both direct electrochemical oxidation and hydroxyl radical attack govern the electrochemical oxidation of the lignin. Direct electrochemical oxidation furnished the more desirable products, while the radical-mediated reaction produced a wide variety of products.

(CHEM-01.4)**Simple Procedure to Synthesize Data for Machine Learning**
Peter Harrington; *Harrington Center for Intelligent Instrumentation*

Machine learning (ML) and artificial intelligence (AI) are popular buzzwords today. Yet, they are extensions of chemometric methods that have existed for decades. By adding more layers of processing units, models can solve more complex problems. As the number of adjustable parameters increases, so does the need for increased numbers of model-building objects, such as spectra or chromatograms. However, insufficient model-building objects may exist, especially for rare samples or expensive measurements, resulting in poorly generalized models.

Another problem arises when the experimental design is unbalanced, which may result in skewed or biased models. A simple method has been developed for synthesizing normally distributed objects with the same eigenstructure as the actual templates. This approach is beneficial because it fills in the data space of sparsely distributed objects, the synthesized objects follow a normal distribution which is an assumption for many algorithms, and unbiased models can be constructed from the balanced distribution.

23FORENS02: Food Forensics, Southern Pacific A/G
Chair: Luis Rodriguez-Saona; *The Ohio State University*

(FORENS-02.1)**Potential of LIBS in Food Analysis**

Ismail Boyaci; *OHIO STATE UNIVERSITY*

Food is the main source of different proteins, carbohydrates, fats and minerals that are essential, for human nutrition and health. The amount of these components in the food shows the quality of the food in terms of consumption. Therefore, the determination of the amounts of food components is of great importance in terms of food safety and quality. To meet the growing demand for multi-component information for product monitoring, rapid and sensitive analytical techniques are required that can detect major and trace components with good sensitivity and accuracy. Due to that, producers have an attention on rapid and reliable, eco-friendly technologies to follow the food processes and final products. Also, consumers demand to be informed about the composition, origin and quality of food and they should choose their diet according to this information. With the globalization of food markets, food authentication has become a significant concern worldwide to ensure food safety and to avoid origin and quality fraud. At this respect, different analytical methods have been developed for monitoring food safety and quality which have main technological challenges. Majority of them depends on DNA, proteomics and isotopic analysis. Which are time-consuming, lab-scale methods and require certain hazardous chemicals, rigorous pre-processes and specialized person. Despite that, rapid, basic and practical on-line analysis in food industry gain popularity by the development in the spectroscopic analysis. From these methods; LIBS have ability to determine elemental composition with high potential for rapid food analysis. In our research group, it was shown that potential usage of LIBS for analysis of quality parameters and determination of food adulteration. In this context, it was shown that applicability of LIBS in identification of meat species, determination of whey adulterated milk powder, determination of ash and protein contents of cereals and NaCl analysis in bakery products. In addition to the promising results of LIBS on food applications, future respects of these methods and ongoing studies for food safety and quality discussed.

(FORENS-02.2)**Optical Trade Space Analysis for Handheld Near-Infrared Spectroscopic Sensors**
Christopher Ball, Siyu Yao, Luis Rodriguez-Saona; *The Ohio State University*

Recent advances in miniaturized near-infrared spectrometers and advanced spectroscopic analysis algorithms have enabled the development of handheld sensor systems that can be used in the field for a wide variety of applications. Our team has previously reported on the development of such a sensor system to analyze food and agricultural products, including measurement of protein, amino acids, and fatty acids in soybeans; lycopene, sugars, and acidity in tomatoes; beta-glucan and fiber in oats; major cannabinoids in hemp; and many others. One of the key design constraints in these sensor systems is the optical interface between the sample material and the spectrometer, which has major implications on the accuracy of results, ease of use, and sensor form factor. This talk presents an analysis of how small spectroscopic sensors can optimize the design for illumination source, sample handling, and detection of reflected radiation. We summarize our experiences developing our current sensor, results from recent test data, and design considerations for future prototypes.

(FORENS-02.3)**Bright Path for Spectroscopy Devices to Safeguard Foods**
Luis Rodriguez-Saona; *The Ohio State University*

Current methods for testing foods are time-consuming, expensive, and labor-intensive, requiring complex procedures of sample treatment and well-trained technicians to operate expensive instrumentation. Vibrational spectroscopy (NIR, IR and Raman) technology provides rapid and cost-effective tools for effective food surveillance. This presentation covers the current state of research on applications of vibrational spectroscopy for monitoring raw materials, screening for adulteration and detection of food contaminants. Optical technology is rapidly developing into small size and compact devices allowing for robust, high-throughput and ease of operation for in-field routine analysis. This technology addresses stated needs by the industry for tools that can verify nutritional levels at point of sale, allowing detection and quantification of food components through spectral signature profiles enabling for real-time and field-based measurements for controlling the product stream, addressing risk management, and brand equity.

(FORENS-02.4)**Near-infrared spectrometers: Overview of state-of-the-art instrumentation**
John Gilmore; *Hamamatsu Photonics*

This 30-minute technical talk focuses on near-infrared vibrational spectroscopy, photon creation, and photon detection. Near-infrared spectroscopy is a powerful tool for analyzing molecular vibrations in a wide range of materials, including biological samples, pharmaceuticals, and food safety. The talk will cover the basic principles of near-infrared vibrational spectroscopy, including how it works and the types of information it can provide. Additionally, the talk will explore the process of photon creation and detection, which are critical components of any near-infrared spectroscopy experiment. Comprehensive analysis of various types of NIR spectrometers with an emphasis on portability. Attendees will gain a deeper understanding of the fundamental physics underlying near-infrared spectroscopy and the tools required to perform accurate and meaningful measurements. The talk will be suitable for researchers and scientists with a background in spectroscopy or related fields, as well as those who are new to the technique and looking to expand their knowledge.

(FORENS-02.5)**Microplastics in our drinking water - does the container really matter?**

Julie Chen-Nguyen, Sanga Kim, **Eunah Lee;** *HORIBA Scientific*

As the awareness of microplastics pollution rises, so does the confusion about how to react as a consumer. Some people insist boxed water is the better approach. Some opt to distill and re-mineralize water at home. However, the majority cannot afford nor do they have the time or propensity to treat water at home. And what about after exposure to Sun? Would UV and heat cause degradation of the container materials?
To determine, at least as a guide for selected samples, the impact of container martials, we compare water in various containers (e.g. cartons, glass bottles, plastic bottles, aluminium cans). We then compare them again after a week long exposure to sun light. We have followed the Southern California Coastal Water Research (SCCWRP) recommended methods for sample preparation and analysis methods for drinking water.

23IR07: Applications of Photothermal IR Spectroscopy and Imaging in the Life Sciences, Sierra 3

Chair: Rohith Reddy; *University Of Houston*

(IR-07.1)**Advances in Cancer Tissue Analysis using Photothermal Mid-infrared Spectroscopic Imaging**

Chalapathi-Charan Gajjela, Ragib Ishrak, Xinyu Wu, Reza Reihani, Sharmin Afrose, David Mayerich, **Rohith Reddy;** *University of Houston*

The application of vibrational spectroscopy has dramatically enhanced biochemical profiling within tissue sections. It is especially pivotal in the biomedical field when dealing with chemically diverse samples, such as cancerous tissues, where traditional spectroscopy often falls short. In this setting, we examine the evolution in Mid-Infrared Spectroscopic Imaging (MIRSI), a breakthrough integration of vibrational spectroscopy's molecular precision and the fine spatial resolution of microscopy, transforming the landscape of biomedical imaging. Traditional use of Fourier transform infrared (FT-IR) imaging tools within MIRSI has evolved through the intersection of machine learning, enabling label-free, quantitative discernment of tissue sub-types and cancer grades. The advent of Quantum Cascade Lasers (QCLs) has further advanced MIRSI, fostering the development of state-of-the-art technologies like discrete frequency infrared (DFIR) and photothermal IR imaging. These techniques offer superior resolution and flexibility, holding significant advantages over FT-IR. Our presentation aims to deliver an in-depth comparative assessment of these MIRSI technologies within the framework of biomedical imaging, elucidating their respective benefits.

Ovarian cancer stands as one of the most lethal cancers affecting women in the U.S., with annual diagnoses exceeding 22,000. The key to improving survival rates lies in early detection of the disease. To automate disease diagnosis, we employ MIRSI imaging paired with machine learning. However, this necessitates data of superior quality and resolution. To meet this need, we leverage the super-resolution potential of optical photothermal infrared imaging (O-PTIR) for ovarian tissue analysis and

subsequent tissue subtype segmentation. Furthermore, bone disorders such as osteosclerosis can be recognized by their spectroscopic signatures via MIRSI. We are set to present high-resolution MIRSI imaging data and corresponding results from bone samples. Notably, we are also unveiling the inaugural study utilizing polarization MIRSI to identify thin collagen fibers (≈1μm in diameter) and their orientations — an essential factor in accurately grading human bone marrow fibrosis.

(IR-07.2)**Probing metabolic heterogeneities within microbial populations at single-cell level with O-PTIR imaging**

Cassio Lima, Howbeer Muhamadali, Royston Goodacre; *University of Liverpool*

Phenotypic heterogeneity is commonly found among bacterial cells within microbial populations due to intrinsic factors as well as a response to external perturbations. The development of phenotypic heterogeneity in bacterial populations acting as microbial cell factories is a major issue from an industrial bioprocessing perspective as it may negatively affects the overall productivity by the growth of subpopulations with inefficient producer cells. Monitoring the spread of phenotypes across bacterial cells within the same population at single-cell level is key to the development of robust and high-yield bioprocesses. Here we discuss the use of optical photothermal infrared spectroscopy (O-PTIR) to probe phenotypic heterogeneity within Bacillus strains by monitoring the production of the bioplastic poly-3-hydroxybutyrate (PHB) at single-cell level. Measurements obtained on single-point and imaging mode show a significant variability in the PHB content within bacterial cells, ranging from whether or not a cell produces PHB to variations in the intra-granular biochemistry of PHB within bacterial cells. Our results show the ability of O-PTIR spectroscopy to study PHB metabolism at single-cell level in a rapid, label-free and semi-quantitative manner. These findings highlights the potential of O-PTIR spectroscopy in single-cell microbial metabolomics as a whole-organism fingerprinting tool that can be used to monitor the dynamic of bacterial populations as well as understanding their mechanisms for dealing with environmental stress, which is crucial for metabolic engineering research.

(IR-07.3)**Spatially Resolved Photothermal Infrared Spectroscopy of Antiparallel Amyloid Aggregates in Cerebral Amyloid Angiopathy**

Ayanjeet Ghosh; ***The University Of Alabama***

Cerebral Amyloid Angiopathy (CAA), which involves amyloid deposition in blood vessels leading to cerebral hemorrhage and recurring strokes, is present in the majority Alzheimer's disease (AD) cases. Familial mutations in the amyloid β (Aβ) peptide are correlated to higher risks of CAA, and some of these mutants have been shown to form transient and/or intermediate fibrils with antiparallel beta sheet structure. This is contrast to wild type Aβ, which is well known to form parallel cross beta fibrils. While the structure of the wild type Aβ peptide has been investigated in great detail, less is known about the structure of mutants involved in CAA. It is also not well understood if these transient intermediates can exist in AD tissues and how translatable in-vitro structural models are to amyloid aggregates in AD tissues. We address these critical gaps in knowledge using two different modalities of spatially resolved photothermal infrared spectroscopy, namely AFM-IR and O-PTIR. Coupling infrared spectroscopy with microscopic techniques allows for investigating structures of individual fibrils in vitro or specific amyloid deposits ex-vivo, and thus enables identification of aggregate structures that can otherwise be averaged out in spatially resolved measurements. Photothermal AFM-IR, wherein the localized thermal response from resonant excitation of the sample is translated to infrared absorbance, enables infrared spectroscopy with nanoscale resolution. O-PTIR uses the same photothermal effect in optical microscopy, thus allowing for transitioning from isolated fibrils to amyloid deposits in tissues. We show using AFM-IR that familial mutants of Aβ can form stable fibrils with antiparallel structure, which undergo structural evolution into parallel beta sheets. Using O-PTIR, we extend these observations to AD tissues, and demonstrate that not only do the antiparallel structures can persist in vascular amyloid aggregates, the structural evolution of fibrils in-vitro is also mirrored in disease progression, thus providing a direct validation of the translatability of in-vitro structural models of amyloid aggregates to AD and CAA.

(IR-07.4)**High-Resolution Infrared And Raman Microscopy Applied To Brain Hippocampal Sections Of A Transgenic Mouse Model of Tauopathy**

Francesca Palombo, Hao Meng, Jessica Mansfield, Michelle Bailey, Francesco Tamagnini, Nick Stone; *University Of Exeter, University Of Reading*

Tauopathy is one of the two major hallmarks of Alzheimer disease, the most common form of dementia and the 7th leading cause of death worldwide [1]. It is characterized by abnormal deposition of tau protein in the brain, mainly in neurons as neurofibrillary tangles (NFTs). Since there is no single test alone that can diagnose dementia, there is an evermore pressing need to develop new healthcare technologies which can address the problem. Vibrational spectroscopy is a family of techniques that, based on vibrations within molecules, provide label-free high specificity for the detection of chemical and structural properties of matter.
This study aimed to address key challenges in the detection of tauopathy signatures in ex vivo hippocampal sections of the rTg4510 mouse model of tauopathy which recapitulates key features of the human diseases, including progressive age-related NFTs, memory impairment and a dramatic loss of neurons in young mice [2]. We applied both Infrared and Raman microspectroscopy techniques across various spatial scales to investigate the discriminatory features within the Cornu Ammonis (CA1) region of the hippocampus between transgenic (rTg4510) and littermate control (wild type) mice. The obtained results provide new insight into the distribution of activated microglia within the tau mouse hippocampus [3] which can aid in detection of tauopathy in a clinical setting.

References
[1] Dementia - World Health Organization (WHO), 15 March 2023, https://www.who.int/news-room/fact-sheets/detail/dementia
[2] J. Gamache et al. Nature Communications, 10: 2479 (2019)
[3] H. Meng et al. Manuscript in preparation.

(IR-07.5)**Multiplexed Mapping of Endogenous Synthesis and Exogenous Uptake of Fatty Acids by Optical Photothermal Infrared Microscopy**

Sydney Shuster, Hannah Castillo, **Caitlin Davis;** *Yale University*

To survive, cells must sense and rapidly adapt their metabolism to environmental stress. Fatty acid metabolism, including endogenous de novo biosynthesis and exogenous uptake, plays a central role in meeting fluctuating cellular energy needs. Understanding the dynamics of competing fatty acid pathways across the cell, their variations across cell types, and their responses to cellular stresses is crucial, as dysregulation of fatty acid metabolism is implicated in diseases including cancer and metabolic syndrome. However, measuring fatty acid metabolism inside the cell is difficult because labeling lipids and their precursors is not trivial. Existing techniques only measure parts of fatty acid metabolism, like glucose uptake, or do not provide subcellular spatial resolution. Recently, we showed that optical photothermal infrared microscopy (O-PTIR) is a powerful tool for tracking de novo lipogenesis with spatial resolution exceeding 500 nm. Here, we employ orthogonal isotopic labeling of glucose and oleic acid to concomitantly monitor endogenous synthesis and exogenous uptake of fatty acids in live and fixed hepatocytes and adipocytes. Leveraging O-PTIR's high spatial resolution, we observe differences in rates of de novo lipogenesis and fatty acid scavenging at distinct locations across the cell. Furthermore, we assign lipid composition, including lipid saturation levels using hyperspectral imaging. This comprehensive approach provides a foundation for understanding lipid dysregulation and engineering effective metabolic treatment strategies.

23LIBS02: LIBS Throughout the FACSS History, Southern Pacific B/C
Chair: Hunter Andrews; *Oak Ridge National Laboratory*

(LIBS-02.1)**Excitation temperature with Boltzmann plot: Significance, accuracy and correct use**

Bruno Bousquet, Vincent Gardette, Vincent Motto-Ros, Rosalba Gaudiuso, Marcella Dell'Aglio, **Alessandro De Giacomo;** *University Of Bordeaux, Université de Lyon, ILM, University of Bari, CNR-IFN*

In laser-induced breakdown spectroscopy (LIBS) as well as in the most of the plasma based techniques, the plasma temperature is a parameter of major importance related to physical characteristics of the laser induced plasma as well as to the experimental conditions involved in laser ablation, laser-plasma interaction and plasma dynamics. When the local thermodynamic equilibrium (LTE) condition can be assumed, the plasma temperature derived from plasma emission (excitation temperature) it is assumed to be the electron temperature as a result of the electron collision regime. In any case the determination of excitation temperature is not restricted only to the LTE and further considerations on the thermodynamics state should be discussed.
In this presentation, several considerations will be taken into account in order to discuss how accurate can be the plasma temperature derived from the Boltzmann plot method, i.e. how to assess trueness and precision and it will be also discussed the limit of the plasma thermodynamic state in the application of the Boltzmann plot technique.

(LIBS-02.2)**The Evolution of LIBS Calibration Curves**

Matthieu Baudelet; *University Of Central Florida*

Since its establishment and use for analytical chemistry in 1962/1963, Laser-induced breakdown spectroscopy (LIBS) has evolved to become one of the standards for elemental analysis of low-Z elements, high resolution chemical imaging and a strong field deployable technology. A large part of its use is the quantitation of analytes of interest in sometimes complex matrices. This means creating a calibration framework where matrix effects are large (due to laser ablation and background gas interactions). While the traditional univariate calibration curve still represents a large part of LIBS quantitative applications, this presentation will review the evolution of the quantitative performance of LIBS over the years, from modeling to empirical approaches, from univariate linear regression to machine learning, from ultra-low concentrations to large stoichiometric ratio, and from single point analysis to large chemical maps.

(LIBS-02.3)**Isotopic LIBS**

Richard Russo, Jhanis Gonzalez, George Chan; *Applied Spectra, Inc., Lawrence Berkeley National Laboratory*

As a laboratory instrument, LIBS (Laser Induced Breakdown Spectroscopy) analytical capabilities are comparable to ICP-OES for many elements, without requiring high argon gas flow. This value added feature makes LIBS attractive as a field instrument. To go one step further is to measure isotopes in the laser plasma in a field instrument. Disruptive applications include nuclear forensics, safeguards, mining, geochemistry, point of care, and others. LAMIS (Laser Ablation Molecular Isotopic Spectroscopy) was developed to measure isotopes in laser plasmas from molecular emission band spectra, to circumvent the generally small splitting in in atmospheric pressure atomic and ionic spectra, and has been demonstrated by measuring the isotopic composition of B, C, H, Sr and other elements. However, for some elements like uranium, the atomic and ionic line spectra themselves exhibit relatively large isotopic splitting, on the order of tens of picometers. With laboratory spectrometers, these lines can be resolved and fitted to extract the isotopic ratio. Extending laboratory uranium LIBS isotopic measurements to field based instrument platforms will be the basis of this talk. The use of LIBS for measuring uranium isotope ratios at atmospheric pressure in both bulk and gas samples will be presented.

(LIBS-02.4)**Improved Data Processing for Accurate Plasma Diagnostics and Calibration-Free LIBS**

Igor Gornushkin; *BAM*

Many applications of LIBS require the measurement of plasma temperature and electron density, which in turn requires knowledge of the integrated line intensity and the shape of the spectral lines. While the integral intensity is preserved as light passes through the spectrometer, the shape emitted by an individual atom or ion is greatly distorted. This is due, firstly, to the transfer of light through the plasma (self-absorption), secondly, to the influence of the instrumental function of the spectrometer, and, thirdly, to the aberrations of the optical system. In addition, processing of spectral information, such as background removal, noise reduction, deconvolution, and line fitting, introduces additional errors in the reconstructed linewidth and line integral, which leads to erroneous temperature and electron density values.
This communication will be devoted to the general shortcomings of spectral data processing and the resulting inaccuracies in determining the plasma parameters. The analysis is based on the use of synthetic spectra generated by plasma with known temperature and particle density. The estimation of errors caused by inadequate processing of the spectral data is made by comparing the initial and determined plasma parameters. As a result, an improved data processing method will be proposed that takes into account the spectrum distortion by the instrumental function and integration on the pixel detector. The former is accounted for by convolution (instead of deconvolution) of the estimated line profile using a predetermined slit function, and the latter is achieved by piecewise integration of the line profile by the pixel detector, taking into account the pixel size and uniform or non-uniform pixel separation. Recommendations will be made for which analytic function best approximates the observed spectral lines and examples will be given for the application of this routine to calibration-free LIBS using both synthetic and experimental data.

(LIBS-02.5)**Coupling Microwave Excitation with Laser Induced Breakdown Spectroscopy: Wave of the Future or Doomed Approach?**

Steven Ray, Kelsey Williams, Buddhika Kumara; *SUNY Buffalo Dept of Chemistry, The State University Of New York at Buffalo*

A variety of approaches have been developed that seek to improve the performance of the Laser-Induced Breakdown Spectroscopy (LIBS) experiment by adding supplemental microwave excitation. The potential benefits of this marriage are significant. Whereas the laser-induced plasma (LIP) formed by the laser pulse is necessarily short-lived, microwave energy could be used to excite and sustain the plasma over a longer time period and more efficiently use sputtered material. The LIP is well recognized as a highly energetic plasma, and while an atmospheric pressure microwave plasma would be less energetic than the LIP, microwave plasma optical emission spectrometry would also benefit from the LIBS sampling solid nebulization of the solid sample, and it is a well-known atomic spectrometry technique in its own right. Moreover, the microwave power sources are inexpensive, simply modulated, efficient, and available in relatively high average powers. With the recent introduction of laser ablation molecular isotopic spectrometry (LAMIS), the potential of microwaves excitation to increase molecular emission in the long-lived LIP structure could also improve LAMIS measurements. However, realization of these benefits has been difficult to achieve experimentally. Despite many examples in the literature of microwave-enhanced LIBS experiments increasing the atomic emission signal observed from 10-1000 times, reproducible analysis has been elusive. In part, this difficulty can be traced to the complexity of coupling microwave radiation into the evolving LIP, and particularly the influence of the plasma frequency changes over the lifetime of the LIP and the difficulty inherent in energy absorption for radiation in the microwave GHz regime (e.g. 2.45GHz). Here, we will review prior results in this research area, and introduce a new strategy to couple microwaves and LIBS that employs purpose-designed microstrip and stripline architectures. These microstrip antenna are created as a copper circuits laid atop a thin dielectric resonant structures using standard PCB-like construction techniques. As important, new microwave power supplies original developed for communications are now available, which allow the microwave waveform applied to be tailored in frequency, duration, and power.

23RAM02: SERS 1, Cascade 3
Chair: Roy Goodacre; *University of Liverpool*
Co-Chair: Zac Schultz: *The Ohio State University*

Co-Chair: Sian Sloan Dennison; *University of Strathclyde*

(RAM-02.1)**Surface-Enhanced Raman Spectroscopy (SERS)-Assisted Gradient Detection in a Gut-on-a-Chip Fluidic Device**

Alexis Lebrun, Antoine Girard, Flavie Lavoie-Cardinal, Denis Boudreau; *Université Laval*

There is strong evidence that potential disruptors such as toxins or viral infections may compromise intestinal permeability and cause pathogen intrusion into the body, leading to mechanisms associated with cardiometabolic disease. Surface-enhanced Raman spectroscopy (SERS) is an exalted molecular identification technique that produces highly specific spectra allowing the analysis of biological mixtures of related chemical species in a relatively short measurement time. Combining SERS spectroscopy with microfluidic devices may improves the sensitivity and efficiency of the measurements while providing better control over various parameters of the sample medium being analyzed.

The present project aims to develop and implement a hyperspectral imaging approach based on SERS spectroscopy within a custom gut-on-a-chip (GOC) model to study intestinal permeability. Once integrated into the GOC, this approach will allow to visualize and identify locally the molecular gradients of key cellular transport pathways in the GOC gut barrier with unparalleled spatial and spectral resolution.

The developed GOC device is a superposed two channels sandwich-like device consisting mainly of double-sided laser-cut adhesive tape for the fluidic channels and a polydimethylsiloxane top part to connect the tubing and enclose the device. Pre-grown Caco-2 cells were introduced into the GOC apical channel, and subsequently kept alive during the measurements following an established protocol . Gold nanostars (AuNS) were used as the SERS substrate due to their good biocompatibility and high SERS enhancement factors, and to maximize the coupling efficiency with a HeNe excitation source at 632.8 nm. AuNS were electrostatically immobilized within the GOC at the gut barrier/basal channel interface to capture and enhance the Raman signal of various gut-derived molecules that cross the gut barrier. SERS hyperspectral images were acquired within the GOC while flowing molecular mixtures composed of potential markers of gut barrier permeability, such as short- fatty acids. Machine learning-based data analysis strategies were then applied to the hyperspectral images to quantify the molecular signature measured in the GOC model.

The results obtained with this project will lead to a greater understanding of intestinal permeability, as well as the various interactions that occur in the gastrointestinal tract.

(RAM-02.2)**Deep Learning-enabled Classification of Extracellular Vesicles Using SERS**

Colin Hisey; *The Ohio State University*

Extracellular vesicles (EVs) are micro- and nanoscale particles that are produced by all cells and circulate in all bodily fluids. Their protective lipid membranes and complex molecular cargo have encouraged the development of using EVs as therapeutics and liquid biopsy biomarkers. However, the inherent heterogeneity and often low abundance of EVs indicate that ultrasensitive and information-rich characterization techniques are needed to fully realize their clinical translation. Recent studies have shown that surface-enhanced Raman spectroscopy (SERS) may meet this need by rapidly producing label-free molecular fingerprints of EVs that can then be processed and analyzed using machine learning algorithms. In this presentation, we will discuss several technologies developed by our group including multiple SERS substrate designs and analytical approaches such as deep learning-based preprocessing and bottleneck classifiers. These technologies have shown promise when applied to EVs relevant in cancer, infectious and reproductive diseases, and EV mixtures, while also impacting several other Raman spectroscopy applications.

(RAM-02.3)**SERS Investigation of the Impact of Common Nanoparticle Surfactants on the Transfer of Hot-Carriers**

Chelsea Goetzman, Zachary Schultz; *Savannah River National Lab, The Ohio State University*

The field of plasmonics has grown since the discovery of the incorporation of roughened metal surfaces was shown to increase in Raman signal leading to a technique known as surface enhanced Raman spectroscopy (SERS). The excitation of a localized surface plasmon resonance (LSPR) in metal nanoparticles with a laser suitable for Raman generates a localized enhanced electric field and increases the Raman signal. The LSPR is generated due to the in-phase oscillation of a metal's electrons in resonant energy with the laser wavelength. In addition to SERS, the plasmonic properties of the nanoparticles can act as photocatalysts, where SERS can be utilized as an in-situ monitoring technique. Plasmonic catalysis facilitates chemical reactions through the transfer of energetic hot carriers generated from the excited LSPR. While the field of plasmonic catalysis continues to grow, it is crucial to understand the mechanisms and conditions for the transfer of these hot carriers as well as how the SERS signal is impacted. Different shapes of nanostructures suitable for SERS have been developed using specific surfactants to direct particle growth and stabilize the resulting colloidal suspension. These surfactants are reported to impact the hot carriers generated. This work studies the impact of various surfactants on simple, spherical gold nanoparticles utilizing the well-characterized plasmonic driven reactions of aminothiophenol and nitrothiophenol. Further we extend the impact of the surfactants to elaborate on the SERS signal changes we have previously observed from the amino acid Tryptophan.

(RAM-02.4)**SERS of cells: from status to physiological process**

Janina Kneipp, Cecilia Spedalieri, Yiqing Feng; *Humboldt Universität Zu Berlin*

Label-free SERS probing in cells has transitioned from proof-of-principle to the characterization of molecule-nanostructure and molecule-molecule interactions and cellular processes that involve a wide variety of biomolecules and cellular compartments. To gain a better understanding of cellular physiology and to harness the selectivity of SERS, different aspects must be addressed that involve (i) the use of good models, (ii) the development of appropriate targeting approaches and (iii) the application of appropriate data analysis tools. We will discuss these aspects for the example of the endolysosomal environment of cultured animal cells, and show data that evidence the possibility to probe lipids, drug action, and the processing of proteins in the living cells.

23RAM15: Raman in Regenerative Medicine, Cascade 1
Chair: Ioan Notingher; *University of Nottingham*
Co-Chair: Max Dooley; *University of Nottingham*

(RAM-15.1)**Raman Spectroscopy For In Vivo Longitudinal Monitoring Of Rodent Models**

Martin Aage Barsøe Hedegaard, Anders Runge Walther, Elzbieta Stepula, Nicholas Ditzel, Moustapha Kassem, Morten Østergaard Andersen, Mads Sylvest Bergholt; *University of Southern Denmark, King's College London, Odense University Hospital and University of Southern Denmark*

The ability to assess the progress of live rodent models longitudinally is one of the most important aspects of improving the use of animal models as longitudinal monitoring enable a significant reduction in the number of animals needed and reduce animal to animal variance. Here we will show two applications of longitudinal monitoring using Raman spectroscopy of implant adsorption and disease monitoring in rodent models.

The first application is the use of fiber-optic Raman spectroscopy for non-invasive, label-free, and non-destructive quantitative monitoring of tissue development in subcutaneous bone scaffolds in mice over 16 weeks. Raman spectroscopy was able to quantify the time dependency of different tissue components related to the presence, absence, and quantity of mesenchymal stem cells. Scaffolds seeded with stem cells produced 3-5 times higher amount of collagen-rich extracellular matrix after 16

weeks implantation compared to scaffolds without. These, however, showed a 2.5 times higher amount of lipid-rich tissue compared to implants with stem cells. Ex vivo micro-computed tomography and histology showed stem cell mediated collagen and bone development. Histological measures of collagen correlated well with Raman derived quantifications. The technique developed here could potentially be adapted for a range of small animal experiments for assessing tissue engineering strategies at the biochemical level.

The second application is monitoring Rheumatoid arthritis (RA) is a chronic inflammatory autoimmune disorder characterized by synovial inflammation and pannus formation leading to destruction of local articular structure, bone erosion and functional disabilities. We demonstrate in vivo assessment of biochemical changes in CAIA mice using a transflection Raman setup. Mice with induced arthritis and controls were clinically and spectroscopically assessed for 14 days. Raman derived measures of tibiotarsal joint bone density correlated well with volumetric bone mineral density (vBMD) from ex vivo CT scans with low vBMD in mice exhibiting clinical symptoms of arthritis. The technique could potentially lead to a reduction in the number of animals needed, while improving research and development of novel therapeutic agents within the fields of bone and joint disorders.

Both examples show a great potential for using Raman in vivo for improving the use of animal models.

(RAM-15.2)**Imaging Topical Drug Delivery With Stimulated Raman Scattering Microscopy**

Natalie Belsey, Dimitrios Tsikritsis, Vasundhara Tyagi, Panagiota Zarmpi, Anukrati Goel,Jean-Luc Vorng, Alex Dexter, Tao Chen, Richard Guy; *National Physical Laboratory, University of Bath, University of Surrey*

Confocal Raman spectroscopy is a well-established tool to map chemical distribution in formulated products, for example pharmaceutical tablets. However, for high resolution imaging, or investigating dynamic processes, the relatively long acquisition times to generate 3D maps can be limiting. Stimulated Raman scattering (SRS) microscopy is a 3D imaging technique based on Raman-contrast capable of real-time examination of formulated products and their permeation into biological tissues such as skin, allowing the detailed, time resolved investigation of chemical absorption. The high sensitivity modulation transfer detection mechanism permits imaging which is free from emitted fluorescence, with the added benefit that the signal intensity is linear with concentration, simplifying quantitative analysis.
SRS has shown excellent promise as a tool for the study of a wide range of materials, including the study of topical drug formulations, including the real-time disposition of actives and excipients, the "metamorphosis" of applied formulations, and nanoparticle distribution. This technique offers unique insight into formulation properties and behavior post-application and can reveal mechanistic information regarding the penetration pathway.
In addition, SRS can be performed simultaneously to other optical imaging modalities such as second harmonic generation (SHG) and fluorescence microscopies to image connective tissues and other endogenous species. Workflows have also been developed to perform correlative SRS-SHG-secondary ion mass spectrometry imaging. This combination of spectroscopic imaging approaches facilitates powerful visualization, combining high chemical specificity and sensitivity with sub-micron spatial resolution.

(RAM-15.3)**Optimisation Of Spatially Offset Raman Spectroscopy For Quantification Of The Fibrotic Tissue Response**

Max Dooley, Ioan Notingher; *University of Nottingham*

The foreign body response is a set of processes that affect how well an implanted object with be received by a body. If the FBR goes wrong it can lead to chronic inflammation, tissue damage, and fibrosis, resulting in device rejection and failure. A key indicator of the FBR is collagen deposition around the foreign body and correctly predicting and monitoring the growth of this layer, therefore, could be important in a range of medical fields. Spatially offset Raman Spectroscopy(SORS) has been

shown previously as a method suitable for the detection of collagen concentration at depths of 1-2mm ex-vivo. This was due to the combination of the chemical sensitivity of Raman spectroscopy with the depth information that can be gathered by diffuse optics. Here we show how a FEM optical simulation package (NIRFaster) can be used to model the configuration of laser excitation and Raman signal detection geometries in order to optimise the signal-to-noise ratio for a given sample. This optimised configuration was then tested on an ex-vivo blinded sample to show that the improvements in the limit of detection that these optimisations achieved were enough to measure the low levels of collagen change that are indicative of the early stages of the FBR. The ongoing drive behind this work is to bring SORS as a non-invasive tool for measuring time course studies in order to minimise the wastage of resources caused by having to dissect samples at each stage for histology.

(RAM-15.4)**Detection of the early response of viral infection to cell by Raman spectroscopy**

Keita Iwasaki, Momoko Imai, Rheta Elkhaira, Hidetoshi Sato; *Kwansei Gakuin University,*

Early containment is effective in preventing outbreaks of viral infections. If the presence of human infectious viruses in the environment can be detected in real time monitoring, alarm can be issued and restrict movements of people before patients appear and become as carriers of virus, or spreaders. In our previous research, adenovirus was detected indirectly, via HEK293 cells by Raman micro-spectroscopy (Moor et al., 2018). Here, we can propose new technique which is by measuring humans and human virus-susceptible cultured cells, as a sensor that specifically detects human pathogenic viruses. Since Raman spectroscopy can measure living cells in a minimally invasive manner, it is expected to be applied to real-time monitoring.
Molecular base of the detection is differences in composition of nucleic acid and phenylalanine which is found in early period, which is after infection of 3 hours. Although it is still under investigation, molecular composition changes in early period are postulated to response of cell against to virus invasion or suppression of host cell's gene expression. In this study, to clarify whether the changes in early period of infection are commonly detectable or not, in molecular compositional base.
In an experimental infection model in which HeLa cells were infected with a lentiviral vector (GenTarget), GFP, which has been integrated into the host cell genome as a marker gene, is expressed, but virus-derived proteins such as capsid are not expressed. Therefore, the detectable changes in molecular composition can be expected to be only due to the response to invasion. In the results of principal component analysis for data obtained from 2 to 3 hours after infection, the score plots made a different group from the control cells. In addition, multivariate curve resolution analysis revealed that some of infected cells showed a decrease in nucleic acids compared to not infected cells. The reduction of nucleic acid is consistent with a previous study, supporting the possibility that Raman spectroscopy can detect the response comprehensively to viral invasion in early period.

(RAM-15.5)**A non-destructive Raman quantification of fatty acid in non-alcoholic fatty liver organoids**

Sanghoon Cho, Hoeil Chung; *Hanyang University*

In the past, animal testing was conducted on living animals with the aim of evaluating the possible negative effects on humans. However, the effectiveness of animal testing is rather controversial since symptoms appeared only in humans and ethical issue on animal testing is of a great concern nowadays. As an alternative, the use of organoids, cultured in human stem cells and have cell composition similar to actual organs, has been suggested. In this study, normal liver organoids (control groups) were cultivated and other organoids were also prepared as non-alcoholic fatty liver (NASH) with fatty acid, and Raman spectroscopy was employed for non-destructive measurement of the living organoids. To prevent damage of the organoids by laser excitation and acquire Raman spectra representative of entire organoids, a wide area coverage scheme providing a laser illumination diameter of 1 mm was employed to decrease the laser power per a given area. Also, to increase the sensitivity, the organoids in a growth medium were transferred into a cone-shaped groove made of stainless steel to maximize the collection of generated Raman photons from the organoids. The intensities of organoids peaks

were analyzed according to the fed fatty acid concentrations to the organoids, and the results of quantitative analysis were discussed.

23SPECIAL03: Microplastic Analysis - Standard Operating Procedures to Understand the Environmental and Health Threats, Southern Pacific B/C
Chair: Andrew Whitley
Co-Chair: Andrew Patterson

(SPEC-03.1)**Current Status Of Microplastics Analysis Solutions: A California-Based Perspective**

Leah Thornton Hampton; *Southern California Coastal Water Research Project (SCCWRP)*

Microplastics, plastic particles less than five mm in size, are a complex class of environmental contaminants, comprising a variety of material types, morphologies, colors, chemical additives, and sizes. Researchers continuously detect microplastics in even the most remote habitats and within organisms, including humans, and such exposures may lead to negative health impacts such as inflammation and oxidative stress. Research has been ongoing for decades, but, in recent years, legislative mandates to develop environmental management strategies for microplastics have spurred a flurry of activity to standardize methodologies and initiate monitoring programs both for drinking water and the environment, particularly coastal habitats. The State of California has emerged as a leader in addressing microplastic pollution, having adopted a standardized method and laboratory accreditation program for analyzing microplastics in drinking water. Standardized methods for monitoring microplastics in complex matrices such as sediments are anticipated to follow in the coming months. While these methods represent an initial step towards better understanding the extent and magnitude of microplastic contamination, they are imperfect. Prior to adoption, method accuracy, precision, time, and costs were evaluated during an Interlaboratory Method Evaluation Study. The results of this study demonstrated two key areas for improvement. First, sample processing and analysis times remain a major challenge for microplastic monitoring on a large scale as current methods are labor-intensive, particularly when microplastic particles must first be extracted from complex matrices. Secondly, smaller microplastic particles, particularly those less than 50 microns are challenging to extract and analyze spectroscopically. Particles within smaller size ranges are of concern due to their increased likelihood to cause negative health impacts, and particle-based concentrations may increase with decreasing particle size. Thus, innovative methodologies and solutions are needed to address these shortcomings to increase the effectiveness and efficiency of current and future monitoring efforts.

(SPEC-03.2)**Analysis of microplastics in drinking water and other clean waters by vibrational spectroscopies – methodology challenges and opportunities for rationalizing the debate through harmonization & ISO normalization**

Nizar Benismail; *Nestle Waters*

The presence of microplastics in the surroundings has become a growing concern for people. From daily use objects to environment, microplastics may now be found in the environment we live, the air we breathe, the food we eat and the water we drink. Unfortunately, large number of scientific studies is still dubious because of the lack of quality aspects about the reliable measurements of data. Some scientists think this analytical investigation to be simple, but using this state of art technology at her limit, combined to the complexity to manage interference impose some fundamental optimization, precautions and fine tuning.
This presentation addresses the technical challenges and potential pitfalls that could interfere the reliability of data generated during the analysis of microplastics using spectroscopic techniques coupled with microscopy to enlighten scientist to reach correct sample characteristics as number, size, and identification of microplastics amongst microparticles with automatic and quite high throughput of samples.
We review all the analytical steps involved in the analytical processes; from adequate analysis equipment, laboratory preparation to reduce ubiquitous cross contamination, filter selection, sample

preparation, blank management, signals interference and data interpretation, algorithm matching, limit of reporting, recovery rates, false positives and negatives, verification and validation of analytical method up to the expression of results.
To address these numerous and diverse challenges, a technical working group of expert laboratories (from universities, technical institutes, authority labs, commercial labs, industry labs) have worked for over two years to share their experience, to improve, harmonize and establish a common set of minimum requirements and best practices for performing accurate and reliable analysis of microplastics in water. The defined guidelines for spectroscopy have been used to settle the backbone of the normalization document to deliver final international ISO standard on the analyses of microplastics in clean waters by 2024.
These different harmonized elements should be applied by each laboratory claiming its capabilities on microplastic analysis in water by spectroscopies, ensuring different laboratories applying the ISO norm to produce similar results on identical samples. This will allow the comparability of results and contribute to the accurate analyses of microplastics in various matrices.

(SPEC-03.3)**Automated identification of micron-scale microplastic particles identification using optical photothermal infrared spectroscopy (O-PTIR) and Raman**

Craig Prater, Eoghan Dillon, Andrew Stuart, Austin Tisor, Jay Anderson, Mustafa Kansiz, Frank Weston, Kevin Kjoller; *Photothermal Spectroscopy Corp, PerkinElmer*
Microplastic (MP) contamination have been recognized as a global environmental problem. MP particles are found globally in water, air, soil and regularly ingested by marine life. MPs can enter the human body by via contaminated water, beverages, and food, and by breathing airborne particles. The MP research community has grown quickly to address questions related to environmental/health risks. Spectroscopic analysis is frequently used to characterize populations of MPs, but most analyses have been limited to >20 μm particles due to issues associated with spatial resolution, sensitivity and/or autofluorescence. This has left a critical unmet need in the analysis of micron scale MP particles, which are of special concern for human and animal health, because these particles are able to pass through the gut wall to accumulate in tissue with potential impact to organ function.
We have developed an automated capability for the measurement and analysis of micron scale MP particles based on Optical Photothermal Infrared Spectroscopy (O-PTIR) and complementary Raman spectroscopy. Preparations of microplastic particles can be automatically screened via optical microscopy to identify particles of interest and then automatically measured by O-PTIR and/or Raman. While conventional infrared spectroscopy (and even Raman) can struggle to spectroscopically identify micron scale MP particles, the O-PTIR approach overcomes the spatial resolution limits of conventional infrared spectroscopy by using a photothermal detection mechanism that employs a separate visible probe beam to detect infrared absorption. Because of the smaller wavelength or the probe beam, O-PTIR can achieve spatial resolution 10-30X smaller than infrared diffraction limits, while also avoiding size and shape dependent scattering artifacts. Using O-PTIR, MP identification has been achieved on polymeric particles small as 0.5 μm with no upper size limit. Because O-PTIR can operate with very low probe beam power, it can analyze dark/colored MP particles without photodamage. O-PTIR is also insensitive to autofluorescence can be problematic with Raman. That said, O-PTIR and Raman measurements can be performed simultaneously on the same MP particles to provide enhanced discrimination/confirmatory analysis. This presentation will review O-PTIR technology and operating principles and then discuss the automated measurement and analysis of arrays of MP particles.

(SPEC-03.4)**Stimulated Raman Scattering Analysis of Nanoplastics in Flow: High Sensitivity Enables Multi-Parameter Analysis**

Maximilian Huber, Liron Zada, Freek Ariese, Natalia Ivleva; *Technical University of Munich - Institute of Water Chemistry, Vrije Universiteit Amsterdam, LaserLaB Amsterdam, Department of Physics and Astronomy*

The characterization of nanoplastics (plastic fragments <1 µm) is still quite challenging since most analytical techniques can deliver only limited information on these complex analytes. Many different properties(size, concentration, chemical composition) must be considered for a proper characterization. A technique that can deliver multiple parameters, mainly size and chemical information, from one measurement is online-coupled field flow fractionation (FFF) - Raman microspectroscopy. However, this hyphenated technique still has some limitations, e.g., low sensitivity and dependency on optical trapping (OT), and cannot deliver particle concentrations. Therefore, a coherent Raman technique, stimulated Raman scattering (SRS), was tested for its potential hyphenation with FFF. SRS employs two different laser wavelengths. Their difference in frequency must match a vibrational transition of the target compound to result in an enhanced signal. Compared to spontaneous Raman, measurement times can be significantly reduced from 10 s to 60.5 µs. Nano- and microplastics (PS, PE, PMMA) in a size range from 100 nm to 5 µm could be detected in flow with this setup using a flow cell with either a reflective or a transparent base. Due to the increased time resolution, individual signals per particle could be observed with SRS rather than an average sum signal for spontaneous Raman. Therefore, this method can be used to count particles while also giving chemical information on the material. Calibrations can be performed to quantify nanoplastics within a certain size range. The peak shape of the individual signals reveals that not all detected particles were optically trapped in the focus of the laser. Untrapped particles result in a symmetrical peak shape, while OT is indicated by an intensity spike followed by a longer, continuous signal caused by the equilibrium position of particles slightly below the focal spot. In case of untrapped particles the mean peak intensity and width can be used for size estimation. Both parameters further depend on the overlap between the particle and the focal volume which necessitates statistical evaluation of the data. Overall, with this method a broad characterization of nanoplastics is possible since information on size, concentration and chemical composition can be obtained within one measurement.

(SPEC-03.5)**Characterization of select table salts for microplastics using Raman spectroscopy**
Andrew Patterson, Nikita Kovalyov, Bijan Jafari; *Eurofins.*

As the awareness of microplastics pollution rises, so does the confusion about how to react as a consumer. Increasing reports of seafood containing microplastics add to the worry that the ocean-derived food supply is tainted at many trophic levels. One ocean-derived product that might be overlooked is table salt. Since all salt on Earth was once saltwater, the composition of constituents in the salt can vary based on when and where the salt was sequestered. Some table salt is mined deep within the Earth, while other production relies on the evaporation of seawater. While trace-levels of minerals in some salts might be a welcome inclusion, microplastics from the evaporation of seawater in the manufacturing process can compromise salt. In addition, the rise in popularity of bottle-top grinders for salts and spices should be investigated as a potential point-source of microplastics. This market-basket survey will evaluate consumer salts for potential microplastics, either from the salt itself, or from attached grinding mechanisms.
Several salt samples of different declared origin and, if possible, the same salt samples with an attached grinder will be evaluated for microplastic content via Raman spectroscopy. For samples with grinders, the mass of salt to be analyzed will be ground through the bottle-top grinder. Samples will be diluted with ultra-pure water prior to filtration and subsequent Horiba Raman analysis. Southern California Coastal Water Research (SCCWRP) recommended procedures for sample preparation of drinking water will be employed where possible with a goal of reporting the morphology and composition of microplastics down to 5µm. An attempt to identify non-plastic debris found in the salt samples will be a secondary goal of the study.

23SPSJ02: 50 Years of UV Raman Spectroscopy, Cascade 4
Chair: Igor Lednev
Co-Chair: Barbara Rossi

(SPSJ-02.1)**Deep-UV Raman Study of Exosomes for Cancer Diagnosis and Monitoring**

Sila Jin, Yeonju Park, Jongmin Park, **Young Mee Jung;** *Kangwon National University, University At Albany.*
In recent years, there has been a growing interest in using exosomes for early cancer diagnosis and anticancer therapy monitoring. Exosomes are small membrane-bound vesicles found in various body fluids that contain a variety of cellular components, including proteins, nucleic acids, and lipids, and reflect the physiological status of the cells. The use of exosomes for cancer diagnosis has the potential to be a sensitive and non-invasive approach that can reduce the pain and risks associated with traditional biopsy methods.
Raman spectroscopy is a powerful analytical tool used extensively in cancer diagnosis because it provides material-specific information about the samples in the fingerprint region. However, spectral analysis of biomaterials is complicated by the strong autofluorescence emitted by biological samples, particularly in the visible region, which interferes with Raman signal detection. Therefore, alternative excitation source is need to overcome the autofluorescence background in biological samples.
In this study, we used a deep-UV laser excitation of 244 nm to obtain Raman spectra of exosomes isolated from various cell lines. The deep-UV Raman spectroscopy can increase the Raman intensity while minimizing the autofluorescence background. The Raman spectra of exosomes were subjected to principal component analysis (PCA) to identify the spectral differences between the various cell lines and to explore the possibility of using exosomes for cancer diagnosis and monitoring. Our findings demonstrated the potential of deep UV Raman spectroscopy as a powerful tool for exosome analysis and cancer diagnosis.

(SPSJ-02.2)**Advanced Instrumentation for DUV Raman Microscopy: Finer, Faster, and Brighter**

Atsushi Taguchi; *Hokkaido University*

Deep UV (DUV) light has high energy and short wavelength, offering a unique opportunity to study the electronic and vibrational properties of electronically resonant materials with high spatial resolution. However, the spatial resolution of the DUV Raman microscope has remained moderate compared to what we expect from the diffraction limit of DUV light, due to the lack of optical systems in the DUV wavelength region. Breaking the technical barrier of spatial resolution in DUV imaging systems will bring DUV microscopy to the next level which can be used as a highly sensitive analytical tool of nanomaterials, biomolecules, and semiconductors with extremely high spatial resolution.

I discuss the recent progress of DUV Raman microscopy in view of spatial resolution, imaging speed, and sensitivity. A classical and straightforward approach for gaining high resolution is to increase the numerical aperture (NA) of the DUV objective lens. We have recently created a reflection-type objective that can be used as an immersion objective [1]. Using glycerine (n=1.5) as an immersion medium, an NA of 0.9 has been achieved. DUV Raman imaging of the hexagonal boron nitride (hBN) sample shows a spatial resolution of about 180 nm.

Further enhancing the spatial resolution beyond the diffraction limit becomes possible by using a plasmonic nano-tip that acts as a nano-antenna to confine DUV light. We have demonstrated the plasmonic-tip-enhancement effect at a wavelength of 266 nm by observing resonance Raman scattering of adenine nanocrystals near the aluminum tip [2,3].

[1] Y. Kumamoto, et al, Adv. Opt. Mater. 7, 1801099 (2018).
[2] A. Taguchi, et al, J. Raman Spectrosc. 40, 1324–1330 (2009).
[3] A. Taguchi, et al, Appl. Phys. Lett. 101, 081110 (2012).

(SPSJ-02.3)**Electron-phonon coupling in linear sp-carbon chains unveiled by UV resonance Raman spectroscopy**

Carlo S. Casari; *Politecnico di Milano*

Carbon atomic wires are the ultimate 1D carbon systems including the elusive allotrope Carbyne, the ideal infinite chain of sp-hybridized carbon atoms with two possible structures: semiconducting

polyyne with alternated single-triple bond and metallic cumulene with all double bonds. In finite systems, the length and the terminations affect the wire structure in a significant way opening the way to tune the electronic and optical properties [1].
Raman is the technique of election for the investigation of sp-carbon systems and surface-enhanced Raman scattering (SERS) even in situ can be adopted to enhance the signal when the amount of material is scarce. However, SERS introduces peak shifting and new peaks due making it difficult the interpretation [2].
We here discuss the use of UV resonant Raman to investigate short carbon atomic wires in the polyyne structure terminated by single heteroatoms as the prototypical system to study length-dependent conjugation effects. Polyynes produced by Pulsed Laser Ablation in Liquids show peculiar absorption in the UV range (200-350nm) strongly dependent on the wire structure and characterized by nice vibronic features [3]. Exploiting the tuneable UV source available at ELETTRA Synchrotron Light Source we were able to resonantly enhance the Raman process at each vibronic peak of size-selected polyynes. The main Raman mode related to collective vibration in the sp-carbon skeleton showed overtones up to the fifth order with surprising non-monotonic enhancements of the intensity depending on the specific vibronic absorption. These observations allowed us to retrieve the vibronic levels in the ground and excited state and to investigate size-dependent electron-phonon coupling in these systems [4].

[1] C.S. Casari et al. Nanoscale 8, 4414 (2016)
[2] P. Marabotti et al. Carbon 189, 219-229 (2022)
[3] S. Peggiani et al. Phys.Chem.Chem.Phys. 22, 26312 (2020)
[4] P. Marabotti et al. Nature Comm 13:5052 (2022)

(SPSJ-02.4)**Modeling Resonance Raman in Complex Environments**

Chiara Cappelli, **Sara Gomez,** *Scuola Normale Superiore*

Computational chemistry may substantially aid the understanding of spectral signals of molecular systems embedded in complex environments. In most cases, such as in Resonance Raman spectroscopy, the presence of the environment plays a substantial role, which needs to be appropriately considered by reliable and accurate computational approaches.
I will give an overview of newly developed multiscale protocols based on polarizable Quantum Mechanics/Molecular Mechanics (QM/MM) approaches, for the calculation of electronic absorption and Resonance Raman spectroscopies of systems embedded in aqueous solution or DNA.

Acknowledgment
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(SPSJ-02.5)**Deep UV Resonance Raman spectroscopy for selective characterization of biological specimens**

Barbara Rossi, Fatima Matroodi, Denis Rajnovic, Alessandro Marcello, Lamyaa Almehmadi, Igor Lednev; *Elettra Sincrotrone Trieste, Elettra, Elettra-Sincrotrone And Icgeb, ICGEB, Massachusetts Institute of Technology (MIT), University at Albany, SUNY.*

The advantages of Deep UV Resonance Raman spectroscopy (DUVRR) make it a suitable method for exploring the biological systems, starting from the detection and identification of their critical components such as proteins, DNA, RNA and lipids until to the characterization of more complex systems as virus and bacteria. Thanks to the resonance effect, DUVRR spectroscopy enables to overcome some of the limitations of conventional visible Raman spectroscopy. By tuning the excitation wavelength in the deep UV range, the Raman signals of amide, aromatic amino acids or nucleobases chromophores are enhanced in the spectra, providing the opportunity of a selective label-free detection of specific biomarkers in biological samples. The detailed analysis of the Raman spectra can give unique insights on the structural arrangement of specific molecular moieties, their interactions

with the solvent environment and the establishment of specific interactions. This has been demonstrated to be a very informative approach for the study, for instance, of the structural stability of nucleic acids in unconventional solvents or in the aggregation processes of proteins. In addition to the unique selectivity of signals coming from crucial biomolecules, DUVRR spectroscopy allows to obtain a significant increment of the detection limit and to collect high signal to noise Raman spectra even in the presence of very low concentrations of the biological sample in water or aqueous environment. Moreover, the absence of interfering fluorescence backgrounds in the Raman spectra collected using excitation wavelengths below of 250 nm makes easier and more effective the analysis of the spectra. In this contribution, we would like to give a wide overview on the opportunities and advantages offered by the multi-wavelengths DUVRR spectroscopy for the molecular characterization, in liquid suspension and operando conditions, of simple and more complex biological species. In particular, we will show how the DUVRR spectra of active Vesicular Stomatitis Virus collected at various excitation wavelengths are very informative on the viability, RNA and protein integrity of the viruses under different conditions of type, intensity and time of UV exposition.

Plenary Sessions: The Coblentz Society Coblentz Award; Wei Xiong, Sierra 5

(PLEN-L1.1)**Ultrafast Dynamics of Molecular Polaritons: How Can A Photonic Cavity Modify Molecular Dynamics?**
Wei Xiong; *University of California, San Diego*

When molecular vibrational modes strongly couple with virtual states of photonic modes, giving rise to the formation of new molecular vibrational polariton states accompanied by a significant population of dark reservoir modes. The polaritons bear resemblance to the concept of bonding and antibonding molecular orbitals, akin to how atomic orbitals combine to create molecular bonds, while the dark modes can be likened to nonbonding orbitals. Being hybrid entities, polaritons exhibit a dual character, part-matter and part-light, which results in a notable shift in energy from their parent states. This intriguing frontier of research, known as polariton chemistry, carries the exciting prospect of modifying chemical reactions under thermally activated conditions, potentially revolutionizing the landscape of chemistry itself.

Despite numerous published findings supporting the idea of polariton chemistry, unraveling its underlying chemical physics and mechanisms has remained elusive. A key challenge lies in distinguishing between polaritons and dark modes, as their contributions to chemical processes must be discerned to fully comprehend the scope and implications of polariton chemistry. Successfully overcoming this obstacle is vital for harnessing polariton chemistry's power to predict and design outcomes in reactions influenced by strong coupling.

To tackle this challenge head-on, our group harnessed the capabilities of ultrafast two-dimensional infrared (2D IR) spectroscopy. This cutting-edge technique allowed us to differentiate between the dynamic behaviors of polaritons and dark modes. Our groundbreaking results shed light on the pivotal role played by polaritons in facilitating intra- and intermolecular vibrational energy transfer, offering a promising avenue to manipulate and control vibrational energy flow in molecular systems operating in a liquid phase. Furthermore, our investigation of a single-step isomerization event provided clear evidence that polaritons do, indeed, modify chemical dynamics under strong coupling conditions, while the dark modes exhibit behavior similar to that of uncoupled molecules, without influencing the reaction dynamics. This discovery corroborated the fundamental concept of polariton chemistry, providing validation that polaritons are influential players in shaping the potential energy landscape of chemical reactions. These works laid a critical foundation for the design of future cavities that could advance the field of polariton chemistry.

Plenary Sessions: The Coblentz Society Clara Craver Award; Ishan Barman, Sierra 5

(PLEN-L1.2)**From Spectroscopy to Solutions: Transformative Biophotonics in Disease Detection and Monitoring**

Ishan Barman; *Johns Hopkins University*

In the quest to bridge the gap between spectroscopy and practical solutions, this plenary talk delves into the cutting-edge advancements in biophotonics for disease detection and monitoring. Leveraging plasmon-enhanced Raman spectroscopy, our journey begins with the exploration of ultrasensitive molecular sensing using metallic nanostructures. Specifically, plasmonic nano-assemblies featuring nanogaps between nanoparticles (NPs) manifest strong electromagnetic field localization, enabling spectroscopic enhancement approaching the single-molecule regime. We introduce the DNA-silicified template for Raman optical beacon (DNA-STROBE), a synthetic innovation that harnesses DNA-templated sol-gel chemistry for the creation of robust nanocavities. Next, the talk provides we take a peek into the quantum realm, introducing DNA self-assembled RING nanoprobes for quantum biosensing. By integrating nitrogen-vacancy centers with plasmonic nanocavities, these probes offer enhanced spatiotemporal resolution for detecting ion flux-induced weak magnetic fields and spin states, opening avenues for understanding spin dynamics in biological systems. Finally, enzyme-mediated precision sensing is explored through self-amplifying Raman nanoprobes, capable of in situ assembly for molecular characterization and disease visualization.These transformative biophotonics solutions exemplify the progression from spectroscopic principles to practical applications, offering exciting avenues for disease detection, cellular analysis, and quantum biosensing.

23AES02: Electrokinetic Fundamentals, Southern Pacific F
Chair:Rafael Davalos
Co-Chair: Alaleh Vaghef Koodehi

(AES-02.1)**Microscale Bioseparations Combining Linear And Nonlinear Electrokinetic Effects**

Blanca H. Lapizco-Encinas; *Rochester Institute of Technology*

Electrokinetics (EK) is a major pillar in the field of microfluidics, in particular for applications such as the sorting and separation of target particles, ranging from macromolecules and parasites. Many successful EK-based systems have been developed for the purification, enrichment, and isolation of a wide array of bioparticles. EK keeps gaining popularity in the field of microfluidics since it is a label-free method that relies solely upon physical mechanisms, i.e., no chemical reactions are needed. Furthermore, it is possible to combine linear and nonlinear EK effects to achieve challenging separations.

Insulator-based EK (iEK) microdevices are systems that feature insulating structures that distort the electric field distribution within the device creating zones of higher field intensity. These zones of higher field intensity are where nonlinear EK effects arise and can be then used to finetune a desired particle separation. This present work is focused on the combination of linear and nonlinear EK effects for carrying out bioseparations. We employ microchannels made from PDMS, that contain an array of insulating structures that alter the distribution of an electrical field when an electric potential is applied. We studied these systems with extensive mathematical modeling with COMSOL Multiphysics and careful experimentation. This presentation includes a summary of the latest developments from our laboratory, including a discussion of the importance of considering nonlinear EK phenomena. Finally, we will highlight the distinct strategies employed to achieve efficient separation and sorting of samples containing highly similar particles and cells.

Acknowledgments:
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(AES-02.2)**Zeta-potential as a biomarker in red blood cell (RBC) physiology**

Erin Henslee; *Wake Forest University*

Surface (zeta, ζ) potential is an electro-physical property of cells, characterized by the difference in charge between the cell surface and the surrounding media. The negative zeta potential in red blood

cells (RBCs) results from sialic acid on the cell surface and is associated with the formation of rouleaux, sedimentation rate (ESR), and storage lesions. We have begun to investigate RBC zeta potential alongside other electrophysiological parameters obtained by Dielectrophoresis (DEP) to better understand how changes in DEP-based parameters, such as membrane conductance, play a role in various RBC physiology.
Many RBC ionic pathways are usually dormant with activity observed in pathological situations. Oxidative stress (OS) in RBCs is important as it irreversibly damages the cells, impairs oxygen delivery and induces red blood cell aging and haemolysis. Further, OS is found to be a dominant factor in RBC-related and other diseases. Eryptosis is a form of programmed cell death akin to apoptosis and is a result of RBC disease and OS. There are critical gaps in the study of OS and Eryptosis in RBCs including mechanistic pathways, agent-specific biophysical effects, as well as the assessment of therapeutic agents. Even when no disease is present, such as in blood transportation, storage as well as circadian rhythms, zeta potential has shown to be a potential biomarker in overall RBC health.
Here we will present results on RBC zeta potential changes as a result of storage parameters, oxidative stress (OS), and induced eryptosis on RBCs. Our preliminary findings include zeta potential dosage response curves for storage time (up to 20 days) and temperature (4, 22 and 37 °C) , induced eryptosis using ionomycin (1 to 10 μM), and induced OS via H2O2 (0.5 to 10mM).
In all cases we were also able to detect shifts in zeta potential alongside changes to DEP-measured parameters, membrane capacitance, membrane conductance, and cytoplasmic conductivity. While not surprising, these results not only support the ongoing work by others to establish an interconnected RBC “electrome”, they also provide further insights into ionic currents during crucial RBC processes.

(AES-02.3)**Synthesis of Bacterial Cellulose under AC electric fields**

Rodrigo Martinez-Duarte, Sindora Baddam; *Clemson University*

We present initial results on using electric field gradients, in a technique called dielectrophoresis (DEP), for the spatiotemporal manipulation of the bacterium K. xylinus; and the impact of the electric field on the synthesis of bacterial cellulose (BC). These are crucial steps for the use of K. xylinus as a robotic printer of architected nanocellulose scaffolds that can be used as is or serve as a precursor to multiple carbonaceous materials.
K. xylinus can synthesize BC, an advantageous source of cellulose over plant cellulose in terms of strength and purity. Particularly, the extraction of biomass from plants and purification of cellulose to remove hemicellulose and lignin is not required in BC production. The use of a bioreactor to produce BC also eliminates the need for forestry operations to produce cellulose. While the fabrication throughput of BC may appear low, it must be compared to the growth rate of plants, with the advantage that pure BC can be directly obtained. By combining DEP techniques with K. xylinus, we attempt to architect BC from the bottom-up and create engineered films that can be subsequently shaped using film shaping techniques like folding and origami.

We present two major developments towards the use of K. xylinus as a tiny printer: 1) the use of light-induced DEP, or the use of light fields to enable an electric field gradient, to enable basic manipulation of K. xylinus cells; and 2) the use of a microfluidic reactor featuring microelectrodes to study how electrostimulation can potentially manipulate and influence the synthesis of BC. This second study features experiments running over fourteen days with an AC induced electric field at polarization voltages of 1 Vpp, 2 Vpp, and 5 Vpp at a frequency of 750 kHz. Results suggest that long-term BC synthesis is possible under electric fields necessary for K. xylinus manipulation.

(AES-02.4)**Monte Carlo Simulation of Polymer Electrophoretic Transport through Polydisperse Nanoscale Pores via Entropic Trapping**

Sourav Bandyopadhyay, Victor Ugaz; *TAMU, Texas A&M University*

Microscale transport of biomolecules through nanoporous surroundings is critical in applications such as electrokinetic DNA and protein separations. The selectivity of these separation processes is governed by the physical transport mechanism by which the macromolecular analytes navigate through the pore network and the rate at which their mobility and dispersion scale with their molecular

size. Previous studies have shown how a time-varying electric field driving force enables entropic trapping-dominated transport of DNA through heterogeneous hydrogel networks via an activated process of hops between larger-sized pores joined by narrow connecting spaces. But although entropic trapping can deliver improved size-selective resolving power compared with conventional constant-field reptation-based approaches, predictive transport models can only capture experimentally measured mobility over a limited analyte size range. Here we address this need by introducing a transport model that overcomes the limitations of prior approaches where DNA mobility was described using a simple function of the macromolecule's radius of gyration and the applied electric field. Our improved model incorporates a more detailed evaluation of the single-file DNA migration time through narrow space (constriction region) connecting neighboring larger pores. Our formulation adopts a master equation for the single-file motion that incorporates an entropic bias, the applied external electric field, and frictional drag due to hydrodynamic forces and polymer-pore wall interaction. When this framework is applied to calculate electrophoretic DNA mobility, we obtain results in close agreement with experimental measurements over a much broader range of DNA size and nanoporous network polydispersity than previously demonstrated. Our ability to capture the dual effects of entropic trapping (in large pores) and entropic bias (during single-file motion through tiny pores) sheds new light on the impact of each process on separation selectivity, revealing that the highest resolution and selectivity occur when DNA length equals the mean size of the constriction. These insights suggest new opportunities to design electrophoretic separation systems that maximize the selectivity of macromolecular analytes.
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23ATOM08: Edward Steers Memorial Award Symposium, Central Pacific A/B/C
Chair: Gerardo Gamez
Co-Chair: Peter Robinson

(ATOM-08.1)**In Memory of Professor Edward Steers – His Achievements and His Legacy**

Peter Robinson; *MassCare Ltd*

Professor Edward Steers spent over sixty years of his life working in Atomic Spectroscopy. This talk will follow his career and share some of its highlights.

In particular, Edward was one of the founding members of the European Working Group for Glow Discharge Spectroscopy (EW-GDS) and we will look at some of his major achievements with the group.

The talk will conclude by describing the awards that are made by EW-GDS in his memory.

(ATOM-08.2)**Investigation of matrix independent calibration of oxygen in GD-OES**

Volker Hoffmann, Gebel Bernhard, Thomas Gemming, Rene Heller,*IFW Dresden, HZDR*

Glow Discharge Optical Emission Spectrometry (GD-OES) is able to obtain depth resolved information about the light elements hydrogen, carbon, nitrogen and oxygen in solid samples, where most of the other analytical techniques fail. However, the interpretation or even quantification of the measured signals sometimes is very challenging.
As GD techniques are direct solid sampling methods they require reference materials for calibration. Unfortunately, the list of available certified reference materials (CRM) suited for calibration of light elements in different matrix is relatively short. Therefore, hot pressed materials doped with the analytes H, O and N were produced at IFW Dresden and applied for calibration of hydrogen, nitrogen and oxygen. Due to the high analyte concentration added, it is very likely that the real concentrations agree well with the added amount of light elements in the corresponding phases.
The performance of GD-OES for matrix independent oxygen determination was investigated using the spectral lines of atomic oxygen at 130 nm and 777 nm and standard conditions for dc discharge with a 4 mm anode (700 V, 20 mA) [1]. Using hot-pressed calibration samples of Cu-, Al- and Mg-powder mixed with their oxides, at 130 nm the dependence of the emission yield on these matrices was

confirmed. However, at 777 nm oxygen has the same emission yield in these matrices. In order to compare the emission yield of oxygen with the emission yield in iron a thick 43 µm FeO-layer was prepared and characterized by Rutherford backscattering spectrometry, X-ray diffraction and glow discharge optical emission spectrometry. At 130 nm, the emission yield of oxygen in FeO is most similar to that in an Al-matrix. At 777 nm, the calibration revealed a higher emission yield of oxygen in FeO in comparison to the common emission yield of oxygen in Cu-, Al- and Mg-matrices.

[1] V. Hoffmann, B. Gebel, R. Heller, T. Gemming, Investigation of matrix independent calibration of oxygen in glow discharge optical emission spectrometry, Journal of Analytical Atomic Spectrometry, (2022) 1223-1228.

(ATOM-08.3)**Glow-Discharge Optical Emission Coded Aperture Spectral Imaging Elemental Mapping**

Harsshit Agrawaal, Rajendra Joshi, Hanuk Kwon, Gerardo Gamez, *Texas Tech University*

Elemental mapping (EM) can reveal the distribution of elements across solid samples to yield necessary insights into mechanisms of interest. Although there are several EM techniques available, long acquisition time is one of the common associated limitations. Glow discharge optical emission spectroscopy elemental mapping (GDOES-EM) is a technique being developed in our laboratory which relies on sputtering and allows direct quantitative elemental analysis with high sensitivity (~ppm) and at very high throughput (~seconds) when operated under pulsed power mode and relatively high pressure. Recently, we successfully demonstrated the sizing and elemental characterization of nanoparticles within a few seconds using GDOES-EM with traditional hyperspectral imaging (HSI). However, sputtering in GDOES consumes the sample, such that traditional HSI techniques can lead to loss of information/resolution due to sequential scanning in at least one dimension, which is compounded for multi-elemental analysis and can be particularly limiting for nanoscale materials characterization. To overcome this limitation, we developed a glow-discharge optical emission coded aperture spectral imaging elemental mapping (GOCAM), which incorporates compressive coded aperture spectral imaging. Here, the entrance slit of a spectrograph is replaced with a pseudo-random binary aperture consisting of many closed and open sections that allow selected light to pass through, which is then dispersed onto an intensified charge-coupled device (iCCD) camera. The convolution of one spatial and the spectral dimensions enables to obtain the full HSI datacube simultaneously with a single snapshot and later deconvoluted in software using convex iterative algorithms. Here, we present the development of the GOCAM technique and its characterization in terms of fidelity as a function of several experimental parameters, including coded aperture properties, spectral complexity, and the type of iterative reconstruction algorithms, including Two-Step Iterative Shrinkage/Thresholding (TwIST), Generalized Alternative Projections Total Variation (GAP-TV), Shearlet Enhanced Snapshot Compressive Imaging (SeSCI), Alternating Direction Multiplier Method Total Variation (ADMM-TV).

(ATOM-08.4)**GDOES investigation on the W/Be erosion/deposition and D retention at the first wall of the nuclear fusion devices**

Eduard Grigore, Cristian Ruset, Flaviu Baiasu, Corneliu Porosnicu, Matej Mayer, Stepan Krat, Anna Widdowson, Jari Likonen, Michael Analytis, Ruediger Meihsner, JET contributors, *National Institute For Laser, Plasma And Radiation Physics, Max-Planck-Institut für Plasmaphysik, Garching, Germany, Euratom/UKAEA, Culham Science Centre, Abingdon, UK*

During the operation of a fusion device, the plasma facing components (PFC) are subjected to a harsh environment caused by high thermal loads and exposure to a high flux of hydrogen isotopes and plasma impurities. This exposure can cause at the surface of the PFCs important phenomena such as erosion, deposition and fuel retention that might affect the operation of the fusion device. In some cases serious safety issues due to increased tritium inventory in the near-surface region of the PFCs might appear. That is why the elemental composition and depth profile investigations at the surface of the exposed tiles are important in assessment of these phenomena. GDOES (Glow Discharge Optical Emission Spectrometry) is a promising investigation technique able to perform depth profile analysis

over large depths (up to 100 μm), with good depth resolution in short analysis time. The GDOES technique was, and it is currently used in our lab as a reliable tool in quality control of the W coating process for about 6,000 tiles for JET (Joint European Torus), ASDEX Upgrade, WEST (W Environment in Steady-state Tokamak) and other fusion devices.
The present presentation is focused on the GDOES analysis performed on JET tiles exposed to fusion plasmas. The path from the production of reference coatings for calibration until the GDOES analysis of exposed tiles is shown. The modifications of the initial structure of the W/Mo coatings deposited on divertor tiles due to the plasma exposure in the JET experimental campaigns are correlated with SEM (Scanning Electron Microscopy) investigations on the GDOES crater flank.

(ATOM-08.5)**Analyte Solution-to-Plasma transfer in the Solution Cathode Glow Discharge**

Jaime Orejas, Yinchenxi Zhang, Cristian Soto-Gancedo, Luis Javier Fernández-Menéndez, Nicholas Hazel, Steven Ray, Jorge Pisonero, Nerea Bordel, *Universidad De Oviedo, SUNY Buffalo Dept of Chemistry,*

The use of atmospheric pressure glow discharges (APGDs) for elemental analysis has gathered attention in recent years as a potential alternative to established techniques such as atomic absorption spectrometry (AAS) or inductively coupled plasma (ICP) coupled to optical emission spectrometry (OES) or mass spectrometry (MS). The miniaturization potential and reduced consumptions of APGDs explain this interest, as it contrasts with the requirements of AAS or ICP, which include pressurized gases or high electrical consumptions. In this sense, APGDs offer new opportunities to develop novel analytical approaches, targeting in-situ applications or fully continuous operation. Their characteristics allow for the development of compact and/or portable instruments that can lead to novel approaches in environmental, clinical, food or material analysis.
Among APGDs, the solution-cathode glow discharge (SCGD) has proved to be a valuable OES source for elemental analysis, presenting competitive limits of detection (middle to low ppb levels), good coverage of the periodic table and simplicity of operation. Its distinctive feature is the use of an aqueous solution electrode, the cathode, which simultaneously acts as the sample. The generated plasma is then in continuous contact with the sample, interacting with the liquid and incorporating the dissolved analytes that are excited to generate the atomic emission used as analytical signal.
Some fundamental characteristics of this interesting plasma-liquid interaction are discussed in the present communication, including the mechanisms by which the analytes are transferred from the aqueous solution into the plasma phase. Information about these mechanisms is obtained by various means and correlated with the SCGD-OES performance in the analysis of solutions with high dissolved concomitant ion contents and with the use of organic additives.

23AWD01: The Coblentz Society Coblentz Award Symposium Honoring Wei Xiong
Chair: Wei Xiong

(AWD-01.1)**Moving towards transient 2D IR: Mapping structure and dynamics with site-specific vibrational probe pairs**

Matthew Tucker, *University Of Nevada Reno*

2D IR vibrational probe pairs provide structural maps for uncovering dynamics associated with active biomolecules, providing insights into the molecular movements leading to their functionality along equilibrium and non-equilibrium pathways. Well-positioned probe pairs can simultaneously detect the dynamics within two different regions and measure distances in places where biological function takes place. We have performed extensive studies on a variety of 2D IR probe pairs, including isotopic labelled amides, selenocyano- modes, cyano- modes, cyanamide modes, ring modes, and azido- modes. These studies have provided structural tools with many different metrics. Now, we utilize these probes with transient 2DIR measurements to track non-equilibrium structural dynamics along the antimicrobial peptide pathway. Applications of these measurements to different chemical ecology problems will also be highlighted.

(AWD-01.2)**Absorber-Specific Dynamics in Vibration-Cavity Polaritons**

Adam Dunkelberger, *Us Naval Research Laboratory*

Strong coupling between an optical mode and an ensemble of molecular vibrations creates new vibrationcavity polariton modes, whose very presence can strongly modify reaction dynamics even without
excitation. The ultrafast nonlinear spectroscopy of the polaritonic system, comprising the polariton modes
and a reservoir of dark states, might serve to elucidate the mechanism of the reaction modification as well
as shed light on novel applications in photonics. Thus far, studies of ultrafast dynamics under strong coupling have largely been limited to hexacarbonyls in solution. Here, we present our recent results from
nitroprusside and thiocyanate ions, which have strong infrared transitions but are distinct from hexacarbonyls in several important respects. We discuss the similarities and differences between the vibration-cavity polariton dynamics in these systems and how the results solidify our understanding of polariton dynamics in general.

(AWD-01.3)**Energy transport control under strong light-matter interactions**

Raphael Ribeiro, Gustavo Aroeira, Enes Suyabatmaz, *Emory University*

Strong interactions between optical cavities and molecular materials induce the formation of hybrid light-matter excitations denoted molecular polaritons with exotic transport and potentially dramatic influence on material properties, including charge conductivity and chemical reactivity. However, there remain many open questions on the mechanisms underlying the polariton control of the properties of molecular materials.

In this talk, I will describe the fundamental properties of energy transport phenomena in disordered polaritonic materials as revealed by our recent work. The focus will be given to (i) optimal conditions for enhanced polariton and weakly coupled exciton transport in photonic devices, (ii) the role of on- and off-resonance cavity modes in polariton-assisted exciton wave packet propagation, and (iii) fundamental differences in the transport phenomena exhibited by electronic exciton and vibrational polaritons. Implications of our findings for future theoretical research and interpretation of experimental data will be emphasized throughout.

(AWD-01.4)**Ultrafast energy relaxation in carbon nanotube exciton-polariton microcavities**

Minjung Son, Michael Arnold, Martin Zanni, *Boston University, University of Wisconsin-Madison*

Coupling between light and matter in an optical cavity creates hybrid states known as polaritons with properties that are not observed in purely molecular systems. We fabricate and investigate polariton microcavities and devices containing semiconducting single-walled carbon nanotubes (sSWCNTs), which are an ideal class of molecules to create polaritons with due to their strong and narrow absorption in the visible and the near-infrared. Applying ultrafast 2D white-light spectroscopy in a donor-acceptor sSWCNT polariton, we find that the interplay between light-matter coupling and molecular parameters, such as interchromophore coupling and disorder in the CNTs, enables a long-range energy transport process across several hundreds of nanometers. Furthermore, we find that long-range energy transport persists even in the presence of weak light-matter coupling, highlighting the active role of polariton light-matter interaction in controlling the dynamics and function of molecular materials.

(AWD-01.5)**New Experimental Platforms for Polariton Reaction Dynamics**

Marissa Weichman, *Princeton University*

Polaritons are hybrid light-matter states with unusual properties that arise from strong interactions between a molecular ensemble and the confined electromagnetic field of an optical cavity. Cavity-coupled molecules can demonstrate energetics, reactivity, and photophysics dramatically distinct from their free-space counterparts, but the mechanisms and scope of these phenomena remain open questions. In this talk, I will discuss two new platforms to investigate the details of molecular reaction dynamics under vibrational strong coupling.

I will first discuss our recent demonstration of gas-phase molecular polaritons. While polaritons are now well-established in solution-phase and solid-state samples, they have not yet been reported in isolated gas-phase molecules, where attaining sufficiently strong light-matter interactions is a challenge. We show that the strong-coupling regime can be accessed for individual rovibrational transitions in cold, gas-phase molecules. We have built an apparatus that combines a cryogenic buffer gas cell with a feedback-stabilized optical cavity to reach this regime.

We are also setting out to survey cavity-altered reactivity in solution phase systems, focusing on radical hydrogen-abstraction processes. These reactions have well-characterized potential energy surfaces, they can be initiated with photolysis and tracked directly on ultrafast timescales, and they are accessible to theory. We are using ultrafast transient absorption measurements to examine intracavity reaction rates with the goal of pinpointing precisely how reactive trajectories may be influenced by strong light-matter interactions.

23CHEM02: Chemometric Opportunities in Forensic Chemistry, Southern Pacific D

Chair: Ruth Smith

(CHEM-02.1)Ignitable Liquid Analysis by DART-MS and Chemometrics

Mengliang Zhang, Shruthi Perna, Ngee Sing Chong, *Middle Tennessee State University*

Ignitable liquids (ILs) are commonly used as accelerants in arson attacks to initiate and intensify fires. The identification and accurate determination of the ignitable liquid residues (ILR) are critical to providing an evidentiary link between the arson and the suspect. In forensic labs, gas chromatography coupled with mass spectrometry (GC/MS) is used for the identification and classification of the unknown ILR from fire debris by following the American Society for Testing and Materials (ASTM) methods (e.g., E1618). Recently, our group has developed direct analysis in real-time mass spectrometry (DART-MS) method for the detection of ILs and found unique less volatile marker compounds in ILs, which are not commonly observed with GC/MS methods. This study aims to investigate the discriminative nature of the DART-MS spectral profiles for ILR analysis and the impact of experimental factors with chemometrics. Analysis of variance-principal component analysis (ANOVA-PCA) was used in this study for multivariate factor analysis. This method separates the variation of the experimental hypothesis from other potentially confounding sources of variation, and it is ideal for investigating the problems with multi-experimental factors such as the weathering degree and temperature effects in the ILR weathering process. ANOVA-PCA was also applied to study the DART-MS profiles for the gasoline samples with different brands. Finally, the partial least squares-discriminant analysis (PLS-DA) models were constructed to classify various ILs, and our results indicate that DART-MS data produce discriminative data profiles for IL classification, and DART-MS can be complementary to the existing GC/MS method for the identification of ILs.

(CHEM-02.2)Elucidation of the Effect of Heat and Sun Exposures on Hair Colored by Permanent and Semi-Permanent Colorants Using Surface Enhanced Raman Spectroscopy (SERS)

Dmitry Kurouski, Aidan Holman, Mackenzi Peterson, *Texas A&M University*

During bloating and active decay, human remains begin to deform and warp their physical identity. After the skin and muscles loosen and detach from their skeletal structuration, everything but bones, teeth, and hair will fully disintegrate into the soil that surrounds the body. Nearly half of people in the world dye their hair with a variety of permanent and semi-permanent colorants. Expanding upon this, we hypothesized that confirmatory analysis of hair colorants can be used to facilitate and advance forensic analysis of human remains. A growing body of evidence suggests that hair colorants can be identified directly on hair using surface-enhanced Raman spectroscopy (SERS). In this talk, I will discuss our most recent results on the accuracy of SERS-based identification of black and blue permanent and semi-permanent dyes on hair exposed to sunlight and heat. Our results showed that although substantial photodegradation of all dyes was observed by week 7, SERS enabled highly accurate detection and identification of hair colorants during all 10 weeks of hair exposure to the sunlight with on average 99.2% accuracy. We also found that SERS could be used to predict fading rates of hair colorants. Similar findings were obtained on colored hair exposed to the heat. This information can shed light on the exposure of human remains to the exterior environment.

(CHEM-02.3)**Expert Algorithm for Substance Identification (EASI) Applied to the Mass Spectra of Seized Drugs**

Glen Jackson, Alexandra Adeoye, Isabel Galvez-Valencia, *West Virginia University*

Since the introduction of the electron ionization (EI) source by Bleakney in the 1930s, mass spectrometrists have strived to understand the relationships between the structures and spectra of organic molecules. The current state-of-the-art in database search algorithms provides probabilities on the order of 80% that the correct identity of a compound will be ranked in the top three in a list of all possible identities (not accounting for GC retention time). By developing a superior algorithm for mass spectral comparisons, we hope to increase the confidence and accuracy of identifying substances from their mass spectra while enabling inter-instrument or interlaboratory comparisons.
Our new algorithm is tied to fundamental concepts of unimolecular fragmentation, which predict that any variation in an instrument's conditions will result in near-linear correlations in fragment ion abundances in replicate spectra. The linear behavior derives from instrument effects on the internal energy distribution of the ions and the apparent observation time of fragmentation kinetics, as described by RRKM or QET theories. Our algorithm uses a general linear regression model (GLM) to enable extrapolation from ion behaviors measured on one instrument to accurately predict ion abundances on other instruments. A partially-supervised binary classifier, trained only on known positives in the training set, then uses collective measures of the accuracy of multiple abundance predictions within a questioned spectrum to decide whether or not to identify the questioned sample as a particular drug. Using external validation spectra of hundreds of replicate spectra, the algorithm predicts abundances with a precision that is typically five times better than models that assume a fixed exemplar, as is the normal approach. The algorithm is sufficiently powerful to accurately distinguish between cocaine and its diasteriomers—allococaine, pseudococaine and allopseudococaine—even when measured on different instruments. The algorithm can also resolve isomers of cathinones with greater than 99% accuracy and fentalogs—like valerylfentanyl and isovalerylfentanyl—with greater than 95% accuracy.

(CHEM-02.4)**The application of Raman spectroscopy to estimate the time since deposition of bloodstains aged under environmental conditions**

Alexis Weber, Igor Lednev, *University at Albany, SUNY*

Blood traces are commonly found at crime scenes and can provide substantial information about the event that occurred and individuals involved. Determining the time of crime is an important goal for crime scene investigations, which can be achieved by estimating the time since deposition (TSD) of bloodstains. If crime scenes contain multiple sets of bloodstains, the calculated TSD should allow for the selection of bloodstains relevant to the crime; and therefore, reduce the number of samples which should be collected, documented, and processed.

Vibrational spectroscopy paired with chemometrics has shown provide reliable, rapid, and non-destructive methodologies to determine the TSD of bloodstains. However, research conducted with these techniques so far have analyzed the aging of bloodstains, specifically the degradation of hemoglobin, in ambient conditions. However, crime scenes are not always in such pristine environments and degradation rate of hemoglobin is commonly affected by the surrounding environment. Therefore, it is necessary to develop a model that is capable of estimating the TSD of bloodstains in different environments.

There are infinite varieties of potential environmental conditions. Our goal is to determine how potentially "extreme" conditions affect the aging mechanism of bloodstains, high temperature in particular. For this purpose, fresh blood samples were collected so that no anticoagulants were present, which potentially can affect the ex vivo aging mechanism of blood. The bloodstains were then aged in a controlled heated environment and tested at numerous time points post deposition. After the spectra were collected, they were loaded into statistical software for preprocessing and modeling. The reproducibility of heated blood analysis and TSD determination model will be discussed.

(CHEM-02.5)**Non-Destructive And Rapid Monitoring Of Cannabinoid Degradation In Hemp Inflorescence During Storage: Kinetic Modeling Using A Time-Based Approach**

Cameron Jordan, Ms. Siyu Yao, Silvia De Lamo Castellvi, Christopher Ball, M. Monica Giusti, Luis Rodriguez-saona, *The Ohio State University*

Current methods for cannabinoid quantification are chromatography based, which are costly, time-consuming, and have a large environmental impact. Fourier Transform Near Infrared (FT-NIR) is a rapid, nondestructive spectroscopic technique. NIR instrumentation costs considerably less than chromatography instrumentation, making it an attractive option for producers of hemp and is substantially easier for untrained operators. More transparency with hemp compliance testing has been of increased importance due to testing inconsistencies. A critical source of variation in product from the initial analysis could be degradation of cannabinoids during distribution. The objective of this study was to design an analysis methodology that can be implemented in real-time, leading to a faster cannabis analysis.

~0.4 grams of homogenized inflorescence were weighed into tubes and placed in different temperature conditions. Three samples were removed from each temperature condition once per week and stored at -40°C until analysis to prevent further degradation. Inflorescence samples were analyzed by ultrahigh-performance liquid chromatography-mass spectrometry (UPLC-MS/MS) to obtain cannabinoid concentrations (CBD, CBG, Δ9-THC, CBN, and acidic forms). Hemp samples were scanned with a handheld FT-NIR Scanner (16 nm resolution, 1350-2500 nm range) with a 10-second exposure. Chemometrics (SIMCA and PLSR) allowed for the classification and correlation of spectral data with reference UPLC data. For kinetics modeling, data were fit to the Weibull distribution and Arrhenius equation to determine the shelf-life rates of degradation.

Partial Least Squares Regression (PLSR) models show the quantification of cannabinoids from inflorescence with low detection (0.021% Δ9-THC) and high quantification (20.7% CBDA) with reproducible and sensitive results (Rcv>0.95). SIMCA models show class separation and classification of the temperature and time points. The kinetics modeling shows the degradation of cannabinoids follows first-order kinetics. These models can be used to determine the appropriate shelf life of hemp inflorescence.

Cannabinoid degradation and quantification are important traits for maintaining the quality control of hemp products. This study provides a nondestructive alternative testing method to UPLC. FT-NIR provides a simple solution for chemically complex matrices, as well as determines the optimal storage conditions of hemp inflorescence. Producers and growers of hemp would benefit greatly from an easy-to-use analytical tool like FT-NIR.

23CTP/EARLY03: Showcasing Career Paths in the Spectroscopic Sciences (Sponsored by SAS Early Career), Sierra 2
Chair: Fay Nicolson
Co-Chair: Samuel Mabbott

(CTP-03.1)**Between two worlds: from researcher to professor**
Andrea Locke, *Vanderbilt University Biophotonics Center*

The transition from postdoctoral researcher to junior faculty can be challenging but exciting. This new role now requires managing a team of students from diverse backgrounds and being responsible for a start-up budget. Along with making your research ideas a reality, junior faculty must juggle teaching, developing coursework, mentoring students, writing and managing grants, and navigating the departmental culture. Or in my case, two different colleges' cultures. This process requires a significant amount of adaptation and grace. In my discussion, I will share my experience transitioning to an academic career, the puzzle pieces that needed to fit together, the lessons I have learned, and the insights I wish I had known before embarking on this journey between two worlds.

(CTP-03.2)**Moving out of academia – Setting up, financing and scaling our university spin out CanSense**

Cerys Mitchell, *Cansense Limited*

After completing a PhD in Raman spectroscopy applications in early cancer diagnosis at Swansea University I completed a year's postdoc in Strathclyde University. I then moved out of academia and worked in health economics and outcomes research as an industry consultant for 18 months prior to starting a spin-out of Swansea University called CanSense. CanSense is built on the research started during my PhD for an early diagnosis of colorectal cancer with a Raman based blood test.

Since launching the company in May 2022 CanSense have set up, closed our seed funding round and grown from 4 people to 11. In this talk I'll discuss the benefits and challenges of leaving academia and discuss the how moving away from academia has helped to shape my career.

(CTP-03.3)**The Experiments Never End: Building a Career in Research Translation and Commercialization**

Steven Asiala, *University Of Notre Dame*

Steve Asiala acts as the Assitant Director for Researcher Engagement for the IDEA Center at the University of Notre Dame. He partners with Notre Dame faculty and researchers to identify research discoveries and innovations which serve as the basis for Invention Disclosures and commercial opportunities. He also acts as the primary point of contact for researchers and is responsible for communicating the IDEA Center's role and processes to the academy. This talk will provide insight into a particular non-traditional career trajectory for a spectroscopist and how scientists and engineers can leverage their skills and experience in the research translation, commercialization, and entrepreneurial spaces, pursuing fulfilling work.

(CTP-03.4)**Harvesting the Benefits and Overcoming the Challenges of a Multi-Cultural Career**

Amandine Calvet, *Boehringer Ingelheim Pharma Gmbh & Co.KG*

In this talk, I will share my personal journey and educational background, highlighting the challenges and benefits of a multi-cultural career. I will discuss how my education in chemistry supports me in my daily work in biologics development. I would also like to spend some time discussing the topics of language and culture as someone who has lived and worked in multiple countries and the transition from academy to industry with a very specialized profile. Finally, I will spare some words on life/work balance which can be especially difficult in an unfamiliar environment. Overall, this talk will

hopefully provide valuable insights and practical tips for anyone looking to harvest the benefits and overcome the challenges of a multi-cultural career.

23IR08: Photothermal IR Spectroscopy and Imaging of Microplastics and Other Materials, Sierra 3
Chair: Curtis Marcott
Co-Chair: Minghe Li

(IR-08.1)**Materials for Inhaled Aerosol Treatment of Disease: PTIR Microscopy for Bioequivalence and Improving Therapeutic Index**

Mark Banaszak Holl, Dipesh Khanal, Sheikh Tanzina Haque, Blessy Joseph, Hak-Kim Chan, *The University of Alabama at Birmingham, University of Sydney*

INTRODUCTION
Dry Powder Inhalers (DPIs) are used every day by millions of people to treat symptoms of asthma, chronic obstructive pulmonary disease (COPD), cystic fibrosis (CF), and other lung diseases. The aerosols emitted by the DPIs into the patient's lungs are highly engineered nano- to microparticles containing a mixture of excipient(s) and drug(s). Improved characterization of these materials offers an opportunity to better understand current formulations.

METHODS
Atomic Force Microscopy – Infrared Spectroscopy (AFM-IR) is a powerful technique that enables assessment of composition, topography, and mechanical properties with a resolution of ~10-50 nm, allowing detailed analysis of dry powder aerosol formulations. Combined with AFM-based nanoThermal analysis to evaluate melting points and nanomechanical analysis, the methods offer a powerful new approach to understanding the complex powder mixtures employed in DPIs. A related infrared spectroscopic method, optical photothermal infrared spectroscopy (O-PTIR) offers a much more rapid IR, Raman, and fluorescence imaging capability with a resolution of ~300-500 nm.

RESULTS
AFM-IR and O-PTIR results provide information on the relative ratios of drug/drug/excipient at the particle level and thus allow an understanding of the distributions of particle composition as a function of particle aerodynamic characteristics for the ~ 1-5 micron size of interest for delivery to the lung. We will present data illustrating how particle composition can vary with formulation conditions and aerodynamic particle size as well as particle level analysis of melting point (Tm) and mechanical data.

CONCLUSIONS
Photothermal infrared spectroscopy combined with nanoThermal and nanoMechanical analysis is a powerful tool for characterization of dry powder inhalers and offer substantial promise for name brand/generic comparisons and optimization of formulations to improve efficacy.

(IR-08.2)**Surely It's Just a Phase: Probing Solid and Semi-Solid Atmospheric Particles from Urban Haze to Algal Blooms to Microplastics with Photothermal Infrared Plus Raman Spectroscopy**

Andrew Ault, *University Of Michigan*

Aerosol particles have large impacts on climate and human health, necessitating detailed measurements of their physicochemical properties. Vibrational spectroscopy is a powerful tool for probing complex samples under ambient temperatures and pressures. However, the optical diffraction limit has limited the application of vibrational spectroscopy, particular infrared spectroscopy, to atmospheric particles, particularly those most abundant in the atmosphere 50 to 2,500 nm. Photothermal infrared (PTIR) spectroscopy detects the expansion of a species when it absorbs infrared radiation from an tunable IR laser at the frequency of a vibrational mode. PTIR is providing a new

approach that can probe particles < 50 nm through the use of atomic force microscopy with infrared spectroscopy (AFM-IR) and the recently introduced mIRage instrument that uses optical detection of the photothermal effect to probe particles down to 500 nm without the need for an AFM tip (contactless). This has recently been coupled with Raman to provide IR and Raman spectra of the same point, which opens numerous doors to probing different modes in atmospheric aerosol species. Initial results will be present of coupled optical PTIR+Raman for model systems, secondary organic aerosol from an atmospheric chamber, microplastics, and ambient samples to demonstrate the potential of this approach. New advances in automation of O-PTIR+Raman will also be previewed. The use for photothermal spectroscopy in the environmental field is enabling new insights into the molecular behavior of key chemical processes.

(IR-08.3)**Simultaneous SERS & SEIRA with Single Molecule Detection – The Application and Characterization of Plasmonically Resonant Structures with Sub-Micron Optical Photothermal Infrared and Simultaneous Raman spectroscopy**

Mustafa Kansiz, Deepthy Kavungal, Felix Richter, Mark Anderson, Hatice Altug, *Photothermal Spectroscopy Corp., EPFL, CALTECH/JPL/NASA*

Optical Photothermal Infrared (O-PTIR) spectroscopy has established itself as a breakthrough vibrational microspectroscopy tool, offering significant advantages over the traditional FTIR/QCL & Raman spectroscopy, providing submicron simultaneous IR+Raman.
In a first, the combined and correlative IR+Raman microspectroscopic approach was used to explore Simultaneous Surface Enhanced Infrared Absorption (SEIRA) and Surface Enhanced Raman Spectroscopy (SERS). Both SERS and SEIRA were simultaneously measured from the same location, at the same time at the same resolution on plasmonically active substrates. The sensitivity of this approach enabled very small quantities of molecules, even to the single molecule level to be interrogated while providing complementary information from both infrared and Raman spectroscopy. This arrangement provides additional improvement of the SEIRA sensitivity through the enhancement of both the optical photothermal detector signal and the infrared absorption. The plasmonic substrates tested were silver nanospheres and a gold coated atomic force microscope tip. The concurrent acquisition of SEIRA and SERS is further demonstrated by nano-sampling material onto an atomic force microscope tip. The analytes, Buckminsterfullerene and 1,2-bis(4-pyridyl) ethylene, were analyzed individually and as mixtures. The concurrent acquisition of SERIA and SERS is a unique approach. It has general applications in trace surface analysis and for the analysis of returned planetary samples.
In a further application of the ultra-high far-field IR spatial resolution properties (<500nm), the newly engineered, counter-propagating mode was utilized to characterize, both spatially via single frequency IR imaging and spectrally, plasmonic structures consisting of patterned gold on 500micron thick CaF2 windows. Exceptional IR images utilizing a 100x, 0.9NA glass objective were collected showing unprecedented spatial detail of photonic structures and their IR resonances <500nm at different IR wavelengths.

(IR-08.4)**Vibrational spectroscopic detection of microplastics in water using diverse microplastics-capturing media**

Yunjung Kim, Sanghoon Cho, Sangjae Kim, Hoeil Chung, *Hanyang University*

Microplastics (MPs) widely present in rivers and oceans become a worldwide environmental concern. MPs are micro and macroscopic plastic particles that could be accumulated in the environment, so they are potential risk to human health. Therefore, qualitative and quantitative analyses of MPs in water are in demand for assessment of risk and various methods have been reported recently. In this study, analytical schemes based on Raman and near-infrared (NIR) spectroscopy with employing perfluorohexane (PFH) as a MPs-capturing medium were demonstrated. PFH is able to extract MPs in water due to its strong hydrophobicity. First, an on-line MPs-capturing configuration was devised for quantification using Raman spectroscopy and polyethylene (PE) particles were adopted as model MPs. Samples of 0.5, 1.0, 2.0, 3.0, and 4.0 mg dispersed PE particles in water were prepared and the

captured PE particle in PFH droplets were directly detected by a wide-area-illumination Raman measurement. Second, since PFH barely absorbs NIR radiation, NIR measurement was feasible and thereby accomplished. A reflective metal disk and a polytetrafluoroethylene (PTFE) disk were used to increase the sensitivity and reproducibility of quantitative analysis. To reflect real field situation, the mixtures of PE, polystyrene (PS), and polypropylene (PP) were prepared and measured by the mentioned Raman and NIR schemes. Third, a nickel foam (NF) was additionally proposed as a MPs-capturing medium and the captured MPs in the NF was measured by the Raman measurement. In final, the pros and cons of each scheme were comparatively discussed.

(IR-08.5)**Overcoming fluorescence in Raman when measuring weathered/oxidized microplastics, using sub-micron optical photothermal infrared**

Jay Anderson, Mustafa Kansiz, Eoghan Dillon, Frank Weston, *Photothermal Spectroscopy Corp,*

The problem of microplastic (MP) contamination is recognized as a major concern around the world. To date, there lacks a robust testing method for smaller microplastic particles. FTIR micro spectroscopy, the workhorse of MP testing, suffers from limited spatial resolution and scatter artifacts. Publications have indicated that both FTIR and QCL direct IR methods like LDIR are limited to MP's >50µm. Raman microscopy offers better spatial resolution, but suffers from auto fluorescence interference, lower chemical specificity, sensitivity, and samples can be burned by high-powered lasers, studies have limited MP characterization using Raman to >20µm.
A new approach to IR micro spectroscopy, called "Optical Photothermal Infrared (O-PTIR)" spectroscopy has demonstrated a unique ability to generate submicron IR spectra without common IR scatter artifacts. O-PTIR uses an infrared pump laser to excite the sample and a visible probe laser to measure the absorbed IR. O-PTIR provides mm to sub-micron size MP characterization, with IR chemical specificity, in a non-contact, reflection geometry, is not affected by fluorescence like Raman and can use 1/10th the visible power of Raman and still collect high SN transmission quality IR spectra for interpretation and searching. When possible, the visible laser can be used for simultaneous acquisition of IR and Raman spectroscopy with submicron resolution. In this report we will use this simultaneous approach, collecting IR and Raman spectra from the same MP at the same time for comparison.
Fluorescence can be caused by additive colors used in MP's manufacturing but can also be a natural effect associated with wear and oxidation/weathering of MP's. In this study we will focus on measuring MP's <50 µm into the 1µm range. We demonstrate the superior IR spatial resolution of O-PTIR but also contrast the measurement of virgin vs oxidized MP's using O-PTIR vs Raman. In all the example spectra whether virgin or oxidized using O-PTIR, the matching exceeded 90%. The measurements of te virgin MPs with Raman were interpretable but the matches were in the 70% range. For the oxidized MP's the Raman spectra were not interpretable and the matches were lower and not meaningful.

23MASS01: Early Career Researchers in Mass Spectrometry, Southern Pacific A/G
Chair: Gabe Nagy
Co-Chair: Chris Chouinard

(MASS-01.1)**Developing a Bioanalytical Toolbox for Human Milk Oligosaccharide Characterization**

Sanaz Habibi, David Williamson, **Gabe Nagy,** *University of Utah,*

Human milk oligosaccharides are one of the most challenging classes of molecules to characterize, largely owing to their high degree of isomeric heterogeneity. Thus, new analytical methodologies are required to improve the confidence of their characterization. Herein, the use of cyclic ion mobility separations coupled with mass spectrometry (cIMS-MS) is presented as a new analytical tool for human milk oligosaccharide analyses. By combining two dimensional cIMS-MS information, stemming from both high-resolution collision cross section values and isotopic shifts, we have

developed a workflow for better resolving key human milk oligosaccharide isomers. The use of tandem mass spectrometry as well as tandem ion mobility spectrometry will also be discussed.

(MASS-01.2)**From Solution to the Gas Phase: An Examination of Protein Charging in Electrospray**

Elyssia Gallagher, Michael Cordes, Alexis Edwards, Madeline Bannon, *Baylor University*

Proteins play many important roles in biology with their structures affecting their function. Native mass spectrometry (MS) has become a valuable tool for analyzing the structures of proteins and protein complexes. However, native MS requires that these biomolecules be transferred from their biological, solvated states to the gas-phase, traditionally using electrospray ionization (ESI) to form positively charged ions. For proteins, the magnitude of a protein's charge in solution is dependent on the amino acid composition. In ESI, basic amino acids accept protons to become positively charged, while acidic amino acids accept protons to become neutral. Thus, protein charge in the gas-phase is dependent on both the amino acid composition and ESI processes. Molecular dynamics (MD) simulations of electrospray have provided molecular insights into the formation of gaseous ions. However, these studies have often utilized Na+ as the charge carrier, rather than modeling proton exchange. This approximation has been utilized because classical MD methods cannot model bond formation or breakage, as required for proton transfer reactions. Here, we present our ongoing efforts to examine charging during ESI by proton exchange using MD simulations. We have developed a modified MD protocol for proton exchange between discrete Grotthuss diffuse H_3O^+ and proteins, allowing for the analysis of the intra-droplet dynamics that lead to charged proteins. Furthermore, we validate the MD protocols using a series of native MS and ion mobility-MS experiments. Together, this work provides novel, molecular insights into protein charging during ESI.

(MASS-01.3)**Coupling Tandem MS and IM^n for Mass and Structure Selective Analysis of Biomolecular Complexes**

Varun Gadkari, Rowan Matney, *University Of Minnesota - Twin Cities*

Structural stability of protein complexes is likely influenced by the properties of their subcomplex and monomeric units. Most current solution-phase biophysical methods are unable to characterize protein complexes at the sub-complex level without disturbing the inherent structural properties of the system. In this work, we demonstrate the utility of combining ion mobility-mass spectrometry, and gas phase activation methods such as surface induced dissociation (SID) and collision induced dissociation (CID) for studying protein complex stability in the gas phase. Data was acquired on a Waters Select Series cyclic ion mobility-mass spectrometer (cIM-MS), equipped with an SID device in the pre-IM region, and the capability to perform IM selective analyses in the cyclic-IM region. Native IM-MS combined with surface induced dissociation has proven effective for analyzing sub-complex protein ions in previous work. It is known that SID activation of native-like compact protein complex ions produces native-like subcomplex ions, enabling the possibility of further gas phase activation of these subcomplex ions to probe them further. In this work, we leverage the capability to IM select fragment ions generated by SID activation of protein complexes for further downstream ion activation. Briefly, protein complex ions will be activated by SID to produce subcomplex ions. The subcomplex ions of interest will be separated from the overall population and sent to the pre-IM "pre-store" region. The ions can then be reinjected into the cIMS, with the possibility of collisionally activating the ions to conduct further CID analysis. The reinjection voltage can be used to unfold protein ions resulting in collision induced unfolding (CIU). CIU has been well established as a gas phase unfolding strategy sensitive to small changes in overall protein structure. Using the cIM-MS platform, we are applying a tandem SID-IM-CIU-IM workflow to study the structural stability of protein subcomplexes. This enables us to determine the contribution of subcomplex stability/instability to the structural stability of the overall protein complex. Early results demonstrate the utility of tandem SID-IM-CIU-IM to perform "Complex-down" gas-phase unfolding.

(MASS-01.4)**Multimodal Mass Spectrometry Imaging Approaches for Probing Complex Biological Systems.**
Elizabeth Neumann, *University of California at Davis*

Organ systems are composed of unique cell types that actively coordinate to enable higher order functions. Even slight deviances in the molecular or cellular states of these systems can result in debilitating disorders whose severity, treatment course, and overall treatment outcome vary widely from patient to patient. This level of complexity likely contributes to promising therapeutics failing within clinical trials and, thus, require further exploration. Thus, the Neumann lab focuses on developing and applying multimodal imaging and profiling techniques to study the molecular and cellular architecture behind complex human diseases, such as renal cell carcinoma, Alzheimer's Disease, and Canavan's Disease. To do this, we combine multiple analytical tools to detect molecules throughout the central dogma of biology, including genes, proteins, and metabolites. Our main instrument is the Bruker timsTOF fleX MALDI MS system, which enables us to detect hundreds to thousands of metabolomic features at <10 μm spatial resolution. In conjunction with immunohistochemistry and spatial transcriptomics, we can connect these metabolomic profiles to specific cell types and cell states. These integrated technologies can be used on a variety of biological samples with high reproducibility and rigor.

(MASS-01.5)**Dissociation Patterns of Ionic Liquid Cations: A Survey of Common Structures and Substituents**

Amanda Patrick, *Mississippi State University*

Ionic liquids are increasingly used or proposed for use in many application areas—from chemical synthesis to battery development to spacecraft propulsion. One of their main strengths is their tunability, meaning that many different scaffolds, substituents, and alterations/functionalizations are either commercially available or synthesized for specific applications and properties. An understanding of the gas-phase dissociation pathways of ionic liquid cations as a function of their structure provides foundational knowledge necessary for analysis of novel syntheses, determination of the ultimate fate of gas-phase species produced from applications such as spacecraft propulsion, and characterization of environmental contaminants that may arise from ionic liquid degradation. We have used energy-resolved collision-induced dissociation, MSn, and complementary computational chemistry to explore the gas-phase dissociation pathways of ionic liquid cations of various skeletal scaffolds with alkyl group substituents, imidazolium-based ionic liquid cations with a wide variety of functional group substituents, and mono- and di-cationic versions of an imidazolium-based cation. These results will be discussed and contextualized with others' works and ongoing and future work.

23PAT02: PAT Pharma/Biotech, Southern Pacific E
Chair: Hossein Hamedi

(PAT-02.1)**In-situ FTIR and Raman in Fermentations of Crop Protection Active Ingredients**
Michael Bishop, David Archer, David Feria-Gervasio, Stefanie Casada, Jeremy McFadden, Bryon Herbert, *Corteva Agriscience*

Corteva Agriscience produces natural products and biologicals through fermentation and aims to increase product volume growth and reduce costs through improved production strains and fermentation process conditions. However, successful transfer of strains and processes to manufacturing requires consideration of scale impacts. Despite ongoing improvements to process models, discrepancies between fermentation scales persist. To help address these challenges, Corteva employs on-line process analytical technologies, including FTIR and Raman, to monitor key fermentation components in real-time. These studies aim to compare the performance of FTIR and Raman partial least squares (PLS) models across multiple process development bioreactors over an extended period.

(PAT-02.2)**Getting more "eyes" on your process: the value proposition of PAT for early-phased development**

Sayyeda Zeenat Razvi, Zhenqi (Pete) Shi, *Genentech*

Since the issuance of PAT guideline by FDA in 2004, PAT has been primarily used in late-phased development, tech transfer to manufacturing and serving either as in-process control (IPC) or real-time release testing (RTRt) on the manufacturing floor. Literature survey also suggests that the three primary intended purposes of PAT for our industry have been process understanding, in-process control and RTRt. On the contrary, the use of PAT for early-phased development is seldom reported. At gRED, we focus on drug discovery and early development with the intention to leverage multi-disciplinary analytical tools to develop a transferrable process with robust process understanding to our late-phased development colleagues at Basel, Switzerland. Real-time spectroscopy techniques are one of many important tools in the department. In this presentation, multiple case studies will be leveraged to illustrate how PAT can play a critical role on improving process understanding in early-phased development, including content uniformity screening via the use of transmission Raman spectroscopy and development of a near infrared spectroscopy-based characterization methodology on blending heterogeneity.

(PAT-02.3)**Optimizing Critical Harvest Process Variables using Real Time Particle and Spectroscopic Measurements**

Jim Cronin, *Mettler Toledo Autochem*

PAT measurements in bioprocessing are becoming more common but generally have been centered around bioreactor monitoring and control. While the benefits of improved bioreactor control have been well documented, there are other common unit operations that lack optimization and would benefit from improved process control. Harvesting a bioreactor, whether from a mammalian or microbial process, currently is based upon off-line measurements or non-specific process variables such as flow rates, turbidity and transmembrane pressures. This presentation will explore the use of in situ particle measurements and infrared spectroscopy to understand critical process parameters in flocculation, cell lysis (chemical and shear), centrifugation control and filter sizing.

(PAT-02.4)**Improving Raman-based Model Transfer and Robustness to Ensure Reliable Process Monitoring in Commercial Manufacturing of Biopharmaceuticals**

Karin Balss, Erin Masucci, Daniel Amchin, *Janssen Pharmaceuticals,*

Raman spectroscopy is a proven process analytical technology (PAT) used to monitor cell culture processes in biopharmaceutical manufacturing. Raman spectra from inline Raman probes provide real-time chemical fingerprints of metabolites and overall cell culture health. However, variations in equipment performance from the same vendor as well as between different commercial vendors can add complexity to method validation and method transfers between products and sites. To build resiliency into the control strategy for Raman-based models, there is a benefit to have flexibility in choosing between multiple Raman vendors. In this work, we examined model robustness considerations when deploying equivalent equipment across multiple sites. The factors influencing model performance were studied at a reduced scale in a design of experiments (DOE). In addition to evaluations with equivalent equipment, a comparison between multiple Raman vendors was also conducted. We will provide examples of the data transformation between Raman spectral data acquired from different vendor equipment during the production bioreactor stage of biopharmaceutical manufacturing. Raman spectral data were successfully converted from vendor B to vendor A using several methodologies. The transformed data accurately predicted using the existing multivariate models developed using vendor A spectra, with minimal deviation. A vendor agnostic model transform approach builds agility into the deployment strategy of sensor-based models across the supply chain network.

(PAT-02.5)**Optimizing Pharmaceutical Process Performance using Advanced Process Control and Process Analytical Technologies (PAT)**

Claudia Corredor, Aparajith Bhaskar, Gregory Lane, Dimuthu Jayawickrama, Sandra Roberts, Brian Breza, *BMS, Applied Materials,*

The implementation of Industry 4.0, Advanced Process Control (APC) and Process Analytical technologies (PAT) continue helping BMS transform its operations. The Enginering Technologies and Drug Product Development groups are developing new capabilities to support our portfolio using advanced manufacturing technologies, such as Continuous Manufacturing and Process Intensification. The implementation of Industry 4.0, APC and PAT continue helping BMS transform its operations. BMS leverages traditional fixed processes within validated design spaces, leveraging standard parametric set-points and process control limits. One of the objectives of this work was to understand how the organization can move from parametric control strategies to Attribute-based control strategies. We present results of a proof-of-concept (PoC) implementation of industry 4.0 concepts, APC and PAT for Fluid Bed Granulation and Fluid Bed Drying.
The PoC consisted of different components:
1. Data infrastructure: powerful environment for computation as well as data and model management with the implementation of PharmaMV. This solution allowed for the implementation of dashboards and increased data visualization 2. PAT for in-line CQA measurements: Two PAT probes were integrated, an in-line NIR probe for water content and drying endpoint and a particle size probe for granule size and distribution.
3. Automation: integration of PAT sensor network into a control system and ability to execute APC algorithms.
4. Data analytics: our team analyzed process dynamics upon parameter change (inlet air flow and inlet air pressure), performed advanced signal integration of PAT sensor and process sensors into the APC modeled and executed advanced control algorithm (feedback loops).
Results of the integration of the different components and the APC control algorithm are presented.

23PMA02: Media Integrity in BioPharma, Southern Pacific D
Chair: Alan Ryder

(PMA-02.1)**Implementation of a control strategy insuring low impact of cell culture media quality on bioprocesses**

Amandine Calvet, *Boehringer Ingelheim Pharma Gmbh & Co.KG*

The presentation will focus on the use of cell culture media fingerprinting to minimize the impact of cell culture media on process performance and product quality. It will present an overarching strategy that can support this goal through implementation in both development and GMP environments, including tracking media quality from raw material and through media preparation. The challenges and rationale of implementing various technologies as well as how the data generated can be used respectively in both environments will also be a point of discussion. Finally, the presentation will address the analytical challenges that cell culture media still face.

(PMA-02.2)**Automated determination of trace metals in biopharmaceutical samples of complex matrix using seaFAST preparation system coupled to inductively coupled plasma mass spectrometry**

Qiang Tu, Chengbei Li, Wendy Zhong, Hillary A. Schuessler, *Merck & Co., Inc,*

Accurate determination of trace metals in biopharmaceutical samples is an important task for quality control, since changes in metal concentrations may impact cell growth, manufacturing yield and product quality in pharmaceutical manufacturing processes. However, this analytical task is often hindered by complex matrices present in many biopharmaceutical samples, especially a high saline matrix would generate a severe matrix effect and spectral interferences for metals detection by ICP-MS. In addition, tedious procedures for the preparation of multiple standards and samples of different

dilution factors require a large expenditure of time and labor. In this work, a commercially available sample introduction system, seaFAST, which was originally designed for seawater samples, was fully integrated with ICP-MS for the preparation of biopharmaceutical samples. The samples pass through a chelation column, where metals of interest are preconcentrated and sample matrices are removed. Elution and detection of the preconcentrated metals lead to a significant improvement in both accuracy and detection limits with ICP-MS detection. The seaFAST also has the capability of autocalibration, auto dilution and auto matrix matching, thus enabling the metals analysis with a new level of automation. Applications of the system to trace metals determination in cell culture media and bioprocess samples will be discussed.

(PMA-02.3)**Comprehensive Metals Mass Balance in CHO Cell Processes**

Cameron Stouffer, Sarah Wysor, R. Kenneth Marcus, *Clemson University,*

It is well established that the metal content in Chinese hamster ovary (CHO) cell growth media greatly affects the media's productivity and critical quality attributes (CQAs). Naturally, it is postulated that the chemical form of those metals (ligated or free ions) affects their uptake and metabolism. Numerous agencies have funded studies exploring the supplementation of metals in media and studies of the bioavailability of the different metal species. While the targeted forms of metals in media are company-specified, their commercial sources, as well as those of the organic media constituents, have been proven to result in concentrations and speciation that differ from the targeted chemical formulas. Metals exist in diverse chemical forms in the process media, however, these forms may change during the monoclonal antibody (mAb) production cycle. Therefore, there is a need for a comprehensive mass balance of metals, their chemical forms, and their physical location (i.e., supernatant vs. intracellular). Given the importance of metal content and speciation in the mAb production process, the ability to speciate starting materials will add value to the overall media production, as well as provide insights into sources of contamination/instability. The development of such a method would provide greater insights into fundamental processes for more informed further refinements. Presented here is a methodology to speciate free-versus-ligated metal species using the Advion Solation ICP-MS and Elemental Scientific prepFast IC platforms in-line to provide enhanced sensitivity, elemental coverage, and the possibility for isotopic tracing of five target metals (Fe, Co, Cu, Zn, and Mn). Initial findings demonstrate an effective quantification method with the use of only a 20 µL injection. It was shown that there was a clear difference in metal uptake versus basal media content. Chromatographic results revealed metal speciation that deviated from reported levels in feed media. The prepFast IC/ICP-MS approach is a straightforward means of assessing CCM composition and speciation. The further development of this method will allow for strides towards quality control, identification of contamination, assessment of media stability/degradation products, and whole cell metal uptake in these growth media.

(PMA-02.4)**Optimal Spectral Resolution for Solids and Liquids Using FTIR and Other Infrared Spectrometers: Results from Database studies**

Timothy Johnson, Brenda Forland, Kendall Hughey, Michael Wilhelm, Olivia Williams, Benjamin Cappello, Connor Gaspar, Tanya Myers, *Pacific Northwest National Laboratory,*

Due to an anachronism stemming from the limited capability of early computers, the spectroscopic resolution used in Fourier transform infrared (FTIR) and other systems has largely been implemented using only powers of 2 for more than fifty years. We investigate debunking the spectroscopic lore of e.g. using only 2, 4, 8 or 16 cm-1 resolution, and determine the optimal resolution in terms of both a) a desired signal-to-noise ratio and b) efficient use of the researcher's and spectrometer's time. The study is facilitated by the availability of newer solids and liquids reference spectral data recorded at 2.0 cm-1 resolution. The study is based on an examination in the 4000 – 400 cm-1 range of 61 liquids spectra (1,765 spectral peaks) and 70 solids spectra (2,472 spectral peaks), with a total examination of 4,237 spectral peaks, each of which was also examined for being singlet/multiplet in nature. Of the 1,765 liquid bands examined, only 27 had widths less than 5 cm-1. Of the 2,472 solid bands examined, only 39 peaks have widths less than 5 cm-1. For both the liquid and solid bands, a skewed distribution of peak widths was observed: For liquids, the mean peak width is 24.7 cm-1 but the median peak width is

13.7 cm-1. Similarly, for solids, the mean peak width is 22.2 cm-1 but the median peak width is 11.2 cm-1. While recognizing specific studies may differ in scope and limiting our study to analysis of only room temperature FTIR data, we have found that a resolution to resolve 95% of all bands is 5.7 cm-1 for liquids and 5.3 cm-1 for solids; such a resolution would capture the native linewidth (no instrumental broadening) of 95% of all the solids and liquid bands, respectively. We suggest that after decades of measuring liquids and solids at 2 or 4 cm-1 resolution, using an optimized resolution of 6.0 cm-1 will capture 91% of all condensed-phase bands leading to instrumental broadening of only 9% of the narrowest of bands with a large gain in signal-to-noise and minimal loss of specificity – only a few barely resolved peaks are blended.

23RAM01: Emerging Raman, Cascade 1
Chair: Pavel Matousek
Co-Chair: Bhavya Sharma

(RAM-01.1)**In Vivo Surface-Enhanced Transmission Raman Spectroscopy: Toward Photosafe Localization of Deep-Seated Lesions**

Li Lin, Jian Ye, *Shanghai Jiao Tong University*

Non-invasive localization of human lesions remains a long-standing pursuit for clinical applications, and its key point lies in the detection and depth prediction of deep-seated lesions in highly heterogeneous tissues. Optical modalities have been widely used for biomedical imaging, diagnosing, and intraoperative guiding. However, due to the strong photon absorption and scattering of biological tissues, it is challenging to realize in vivo non-invasive detection via optical modalities, especially those using safe laser irradiance below the maximum permissible exposure (MPE) threshold. Here, we report in vivo surface-enhanced transmission Raman spectroscopy (SETRS) to achieve non-invasive and photosafe localization of deep lesions hidden in either ex vivo thick porcine tissues or in vivo mouse models. We first synthesize the ultra-bright near-infrared SERS nanotags with single-particle detection sensitivity. Then, we develop a home-built TRS system with an enlarged beam size to reduce the laser power density to 0.264 W/cm2, below the MPE. Using the TRS system, we successfully demonstrate the detection of SERS nanotags through up to 14 cm thick ex vivo porcine tissues, as well as in vivo imaging of SERS "phantom" lesions in an unshaved mouse below the MPE. In addition, we demonstrate a rapid and universal method for predicting lesion depth in highly heterogeneous tissues, with a prediction error of only 8.35%. Our work demonstrates the possibility of non-invasive detection, precise localization, and perioperative guidance of in vivo lesions in the live animal model, thus advancing the translation of SETRS-guided clinical diagnosis to humans.

(RAM-01.2)**Swept Source NIR Raman Spectroscopy for Distributed Multi-Point Sensing Using an Optical Fiber Network**

Nili Persits, Dahlia Dry, Rajeev Ram, *MIT*

NIR Raman spectroscopy is a highly valuable technique for chemical sensing, known for its specificity, non-destructive nature, and compatibility with diverse sample types. However, its use outside of laboratory settings has been limited due to hardware constraints, particularly interferometers or spectrometers with cooled detectors, which offer only a limited number of channels in close proximity. Consequently, Raman spectroscopy faces challenges in applications that demand multiple in-line sensing points or simultaneous operation of multiple sensors, such as large-scale pharmaceutical production or continuous manufacturing, where an additional level of control is required. In order to overcome these limitations, we propose a novel solution: an optical fiber network that connects up to sixteen Raman sensor probes, enabling concurrent operation using readily available datacom switches and fibers.
Our network design builds upon a Swept-Source architecture, replacing the traditional fixed wavelength laser with a tunable laser that serves as a shared resource. By using simple, optically narrow-band silicon photodetectors, we capture the spectrum at each independent sensing location while tuning the laser source. This design not only enables multi-point sensing, but also overcomes the

throughput limitation imposed by standard dispersive spectrometers, resulting in a tenfold increase in light collection efficiency, all while maintaining spectral resolution determined solely by the narrowband optical filter.
Preliminary results showcase the effectiveness of our design, demonstrating its comparability to standard bench-top systems for solid and liquid samples alike. We achieve a glucose limit of detection under 5 mmol/L with a mere 8mW of excitation power. Furthermore, we prove the ability to deploy rugged sensors hundreds of meters away from the laser source, maintaining equivalent performance. Through distributed chemical sensing, our proposed approach unlocks new possibilities for the practical integration of Raman spectroscopy in material characterization, large-scale pharmaceutical production, and the food and beverage industries. This is particularly relevant in scenarios where multiple in-line sensing points or simultaneous sensor operation are required, enabling enhanced process visibility and control.

(RAM-01.3)**Efficient Optical Plasmonic Tweezer-Controlled Surface-Enhanced Raman Spectroscopy For Single-Molecule Studies In Solution**

Jinqing Huang, *The Hong Kong University Of Science And Technology*

By removing ensemble averaging, single-molecule techniques discern the signal of individual molecules to unveil hidden details and revolutionize our understandings of physics, chemistry, and biology. Of particular interest is the characterization of single or a few biomolecules, such as intrinsically disordered proteins (IDPs), in an aqueous milieu containing hydrogen-bonding, electrostatic, and hydrophilic-hydrophobic interactions during complex biological processes. Although significant advancements have been achieved by single-molecule fluorescence methods, the structural determination is limited by fluorophore labeling, and the single-molecule scheme is restricted to ultra-dilution and/or molecular immobilization because the diffraction-limited detection volume cannot be further reduced.

Here, we developed a convenient optical plasmonic tweezer-coupled SERS platform for efficient single-molecule SERS characterization of biomolecules in aqueous milieus. Specifically, we utilized optical plasmonic trapping to construct a dynamic nanocavity, which reduces the diffraction-limited detection volume and generates reproducible SERS enhancements for efficient single-molecule characterizations in solution. By switching the trapping laser between on and off states, an Ag nanoparticle was trapped and released at the plasmonic junction of an Ag nanoparticle-coated silica microbead dimer, respectively, which enables efficiently and continuously high-throughput detections at the well-defined location under microscopic visualization. Since both optical plasmonic trapping and SERS techniques are surface-sensitive relying on nanostructured substrates, the integration overcomes the optical diffraction limit to confine the position of the plasmonic nanocavity and reduce the SERS active-detection volume for consistently high SERS enhancements. Hence, this platform can detect the freely diffusing analytes at the single-molecule level without molecular immobilization or solution dilution. After the verification of its single-molecule sensitivity by the bi-analytes SERS approach (BiASERS), we studied the pH-dependent structural transition of Tyrosine and the conformational features of hIAPP species under two physiological pH conditions (pH 5.5 and pH 7.4) in microfluidic flow. With high-throughput capability, this platform holds the promise to advance the solution-phase single-molecule vibrational characterization method developments to probe a single biomolecule among heterogeneous mixtures during complex biological processes.

(RAM-01.4)**Wearable Microfluidic Devices for Sweat Analysis**

Limei Tian, *Texas A&M University*

Wearable sweat sensors have the potential to provide valuable information related to the health and disease states of individuals. Existing sweat sensors mainly rely on biomacromolecules, such as enzymes and antibodies, as biorecognition elements to achieve specific quantification of metabolites and hormones. However, these biomacromolecules tend to degrade over time, limiting the sensors' shelf life and compromising the sensor performance upon environmental changes, such as varying temperature and humidity. Here, we introduce a wearable plasmonic paper-based microfluidic system

to continuously and simultaneously quantify sweat loss, sweat rate, and metabolites in sweat. Plasmonic sensors based on surface-enhanced Raman spectroscopy (SERS) are label-free and can identify the analytes of interest via the chemical "fingerprint" information. We show that simple and low-cost plasmonic papers allow for detecting and quantifying uric acid in sweat at a low concentration of 1 μM. The well-defined flow kinetics of paper microfluidic devices enable accurate quantification of sweat loss and sweat rate in real time. Reliable quantification can be achieved when the devices are under strain and at high temperatures. We demonstrated two operation modes for continuously monitoring biochemicals at changing concentrations that are physiologically and pathologically relevant in human sweat. The wearable plasmonic device is soft, flexible, and stretchable, providing a robust interface with the skin without inducing chemical or physical irritation.

23RAM07: ECR Raman, Cascade 3
Chair: Bhavya Sharma
Co-Chair: Sian Sloan-Dennison

(RAM-07.1)**Investigating the structure of non-crystalline macromolecular assemblies and aggregates using Raman Spectroscopy**

David Punihaole, *University Of Vermont*

Developing bioanalytical tools that can interrogate the structures of macromolecular assembles and aggregates is of great importance to the biomedical sciences. Examples include understanding how polymer nanoparticles used in gene therapy applications complex to their nucleic acid cargo, how proteins aggregate to form amyloid fibrils in neurodegenerative diseases such as Alzheimer's, and how proteins and complex carbohydrates in the extracellular matrix become disordered in cystic fibrosis. Studying these systems can be difficult using conventional methods such as X-ray crystallography and NMR spectroscopy because of their non-crystalline and insoluble nature. In this talk, I describe recent work by my group to develop detailed structural models of polymer-DNA complexes, amyloid fibrils, and collagen found in the extracellular matrix using recently developed methods that rely on Raman spectroscopy.

(RAM-07.2)**Imaging Though – Advances in Transmission Raman for Non-Invasive Whole Animal Imaging**

Benjamin Gardner, Alexandra Vaideanu, Ryan Mellor, Ijeoma Uchegbu, Andreas Schatzlein, Pavel Matousek, Nick Stone, *University Of Exeter*

Raman spectroscopy has gained much interest in recent years for its potential application within in vivo medical diagnosis. This is especially driven by advances in "deep Raman", where large volumes can be probed using spatially offset Raman spectroscopy (SORS) or transmission Raman spectroscopy (TRS) approaches. When these approaches are coupled with nanomaterials i.e. surface enhanced Raman spectroscopy (SERS), this provides a set of tools that can rapidly probe the chemical environment in real time in a multiplex fashion i.e. informing on concentration, pH, redox or biodistribution.
Here we discuss the latest work and limitations in developing transmission Raman spectroscopy as a tool for rapidly monitoring the biodistribution of Raman nanotheranostics (RaNT) nano-constructs in whole animals. This work is corroborated with secondary techniques, including micro-CT and ICP-MS, to answer the question how quantitative can Raman be?

(RAM-07.3)**Developing Surface-Enhanced Raman Spectroscopy Methods For Quantification Of Complex Analyte Mixtures In Plant-Based Extracts**

Stephanie Zaleski, *California State University East Bay*

Analytes such as polyphenolic compounds, which are an important metabolic product associated with plant adaptation to stressful conditions. Anthraquinones are an important class of analytes that are found in solutions of natural red dye extracts of plants. Plant-based extracts are complex mixtures;

their composition largely depends on plant species, location, or its biochemical expression. SERS is an ideal method to analyte these mixtures due to its inherent chemical sensitivity and specificity. However, despite its relative popularity, SERS is most often used for the detection of 1-2 analytes. The goal of this research is to develop a more quantitative model of SERS for the identification and quantification of complex mixtures of analytes found in plant-based extracts, such as polyphenolic compounds in pistachio nut and leaf extracts or anthraquinone compounds found in madder root extracts. In addition, this work will allow us to develop a deeper fundamental understanding of analyte-nanoparticle competitive adsorption and surface interactions, which will help improve the use of SERS for quantitative measurements. Our group's approach involves determining the optimal conditions for solution-based SERS using standard solutions representing the main classes of polyphenolics and anthraquinones found in plant extracts. Using the optimized conditions, we have quantified binding affinities of target analytes by fitting concentration-dependent SERS intensities to a linearized Langmuir adsorption isotherm model. We have found that molecular binding affinity is a more important factor in analyte detection than an analyte's inherent Raman scattering cross section. With this data, we have begun to classify molecular analytes with common features that yield various intensities of SERS signals. We are now exploring the application of our results to actual plant-based extracts.

(RAM-07.4)**Multiplex Cellular Imaging Using Stimulated Raman Scattering Microscopy and Spectral Phasor Analysis**

William Tipping, Ewan Hislop, Liam Wilson, Henry Braddick, Nicholas Tomkinson, Karen Faulds, Duncan Graham, *The University Of Strathclyde*

Rapid advances in the field of Raman imaging, particularly stimulated Raman scattering (SRS) microscopy are opening up many new avenues for imaging and quantification of drugs and small molecules in living systems.[1] As a result, images of small molecules within cells might be acquired without the use of "bulky" fluorescent labels, (which may be as big as the small molecule under observation) or the use of nanoparticle sensors (which might perturb cellular biology). Additionally, spectroscopically bio-orthogonal Raman labels, including alkynes and nitriles, which produce spectrally isolated peaks in the cell-silent region of the Raman spectrum (1800–2800 cm-1) can thus be used for imaging intracellular processes. By combining Raman tags with SRS microscopy and chemometric analysis, including spectral phasor analysis,[2] a powerful methodology for cellular imaging applications is created.
Statins have displayed significant, although heterogeneous, anti-tumour activity in breast cancer disease progression and recurrence.[3] Hyperspectral SRS imaging with spectral phasor analysis is reported for the investigation of statins as repurposed drugs in breast cancer treatment.[4] A spectral phasor analysis of the hyperspectral dataset enables rapid differentiation of discrete cellular compartments based on their intrinsic SRS characteristics. Applying spectral phasor analysis to studying statin-treated cells identified a lipid accumulating phenotype in cell populations which displayed the lowest sensitivity to statin treatment, whilst a weaker lipid accumulating phenotype was associated with a potent reduction in cell viability. The application of spectral phasor analysis is then extended to visualise a ratiometric Raman sensor for esterase activity,[5] and for multiplex detection within the cell-silent region of the Raman spectrum. SRS imaging provides a novel and innovative technique for phenotypic assessment drug–cell interactions at the subcellular scale.

[1] K. Dodo, K. Fujita and M. Sodeoka, J. am. Chem. Soc., 2022, 144, 19651.
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(RAM-07.5)**Scanning Ion-Conductance Microscopy for Tip-Enhanced Raman Spectroscopy**

Naihao Chiang, *University of Houston*

Tip-enhanced Raman spectroscopy (TERS) combines the nanoscale spatial resolution of scanning probe microscopy with the single-molecule chemical sensitivities of surface-enhanced Raman spectroscopy. TERS has achieved Ångström-scale spatial resolution in chemical imaging under ultrahigh vacuum conditions. We are developing a custom-built laser-coupled scanning ion-conductance microscope (SICM) for TERS, which enables the translation of this unprecedented spatial resolution in vibrational spectroscopy to liquid environments. SICM is an SPM technique that is particularly suitable for topological imaging of surfaces in controlled liquid environments. The proof-of-concept experiments have already demonstrated the potential of SICM-TERS on two-dimensional transition metal dichalcogenides. This new chemical imaging method opens a new avenue for researchers to obtain more detailed chemical information of surfaces in electrolyte solutions.

23SPR02: 50 Years of Plasmonics, Cascade 4
Chair: Igor Lednev

(SPR-02.1)**Plasmonics as a strategy for the design and sensitive readout of immunodiagnostic platforms by surface-enhanced Raman scattering: It's not only about limits of detection**

Marc Porter, *University Of Utah*

The drive for early disease detection, the growing threat of bioterrorism, and a vast range of other innovations across biotechnology have markedly amplified the demand for ultra sensitive, high-speed diagnostic tests. This presentation will describe efforts to develop platforms and readout methodologies that potentially address these needs by coupling plasmonic labeling concepts with surface enhanced Raman scattering. Strategies will be detailed for both the fabrication and readout of chip-scale platforms, along with examining fundamental dictates for optimal performance. Examples will focus on the use of immunoassays for the rapid, sensitive detection of cancer markers and viral pathogens. Challenges related to sample preparation, sensitivity, speed, and nonspecific adsorption will be discussed.

(SPR-02.2)**A plasmonic tongue to predict maple syrup quality from sap**

Jean-Francois Masson, Simon Forest, Juklien Coutu, Issraa Beniani, Zhe Si Yu, Morgan Craig, *Universite de Montreal*

Remote sensing presents many opportunities and challenges. This presentation will address both through the development of sensors for maple syrup quality testing in the maple sugar shacks of Quebec. We will present the development of a testing strategy for maple sap and syrup based on the aggregation of gold nanoparticles in the presence of off-flavor molecules. Validation of the test with approximately 30,000 maple syrups revealed that the test responds differently to different off-flavor profiles. Adaptation of the test using a titration-based nanoparticle aggregation assay on sap can predict the quality profile of maple syrup with an ordinal mixed effects model. Changes in sap were correlated with changes in amino acid concentrations and other physical factors of the sap. Seasonal trends were observed for a number of producers, demonstrating the potential for them to better manage their harvest season.

(SPR-02.3)**Development of SERS Sensing Platform for TSH Detection: From Buffer Solution to Patient Samples**

Piyush Raj, Peng Zheng, Lintong Wu, Takayuki Mizutani, Miklos Szabo, William Hanson, Ishan Barman, *Johns Hopkins University*

We present a common Surface-Enhanced Raman Scattering (SERS) sensing platform for the detection of Thyroid Stimulating Hormone (TSH) in buffer solution, serum, and patient samples. SERS spectra exhibit significant variations in intensity since nanometric changes in molecular position, orientation, and nanoparticle geometry result in very large changes in the scattering response, making them

challenging to use for concentration prediction. While this can be partially addressed with digital analysis, the number of spectra needed for that analysis is high which is a major roadblock when we are constrained by scanning time in a clinical setting. To address this, we first developed a sensing platform that operates based on peak shift, which is caused by nanomechanical perturbations to Raman molecules This perturbation is caused by interaction between antibody and antigen, which ends up transducing a robust signal in the form of frequency shift instead of the peak intensity variation, making the spectral signal more reliable and reproducible. Subsequently, we devised a spectral processing pipeline that incorporates outlier detection before model training and validation. To detect outliers, we employed the robust Principal Component Analysis (ROBPCA) algorithm, and we performed model training and validation using Partial Least Squares Regression (PLS). The analysis pipeline was evaluated for TSH detection in buffer solution, serum samples, and patient samples, demonstrating high accuracy and reproducibility. Moreover, we demonstrated that the pipeline could be applied to patient samples measured on different days and different SERS chips, making it suitable for clinical applications. Our work highlights the potential of SERS-based TSH detection and provides a reliable and robust approach for clinical diagnosis.

(SPR-02.4)**Janus MOF-based Micromotors as Sensors**

Eric Languirand, Matthew Collins, Errie Gibson Parrilla, *Languirand*

Micromotors are an active material that have been used in drug delivery systems, pollution mitigation, and self- assembly applications. More recently, Janus metal organic framework (MOF) based micromotors have begun to be explored as a potential active decontamination technique for chemical warfare agents due to MOF-chemical interactions. Chemical-MOF interactions provide the mechanism for decontamination but also provides an avenue for sensing both the analyte and the breakdown products from an interaction of the MOF and a target compound. In this work, we utilize a UiO-66 based Janus micromotor providing Ag@UiO-66@SiO2.

In addition to potential decontamination benefits, the external growth of MOFs naturally creates a roughened nanoscale surface. While MOF-based structures have been explored via the chemical mechanism of surface enhanced Raman spectroscopy (SERS) due to their internal metal structures, micromotors inherently have a hemispherical coating of a metal that may provide electromagnetic enhancement. This presentation explores the plasmonic capabilities of Janus MOF-based micromotors for surface enhanced sensing.

23AES03: Innovations in Device Fabrication and Applications, Southern Pacific F
Chair: Alex Hyler
Co-Chair: Olivia Ernst

(AES-03.1)**Advanced 3D Printing for High Resolution Microfluidic Devices**

Greg Nordin, Dallin Miner, Mawla Boaks, Matthew Viglione, *Brigham Young University*

While there is great interest in 3D printing for microfluidic device fabrication, a main challenge has been to achieve feature sizes that are in the truly microfluidic regime (<100 μm). A key issue is that microfluidic devices are comprised primarily of negative space features, which therefore dominate 3D printing resolution requirements, as compared to positive space features that are typical for many other 3D printing applications. Consequently, we have developed our own stereolithographic 3D printers and materials that are specifically tailored to meet these needs. In 2017 we showed that flow channels as small as 18 μm x 20 μm can be reliably fabricated, as well as compact active elements such as valves and pumps. In this talk we introduce a new multi-resolution 3D printing method in which we can now print channels as small as 1.9 μm x 2.0 μm, and yet preserve fast print times. These advances open the door to 3D printing as a replacement for expensive cleanroom fabrication processes, with the additional advantage of fast, parallel fabrication of many devices in a single print run due to their small size.

(AES-03.2)**Enhancing Accessibility and Reproducibility in Zeta Potential Measurements: A Novel Approach with Commercial Microfluidic Chips and Open-Source Workflows**

Jonathan Cottet, Josephine Oshodi, Ada Erus, Ariel Furst, Cullen Buie, *MIT*

Surface charge is a critical feature of microbial cells that affects their interactions with other cells and their environment. Because bacterial surface charge is difficult to measure directly, it is indirectly inferred through zeta potential measurements. Existing tools to perform such characterization are either costly and ill-suited for non-spherical samples, or they rely on microfluidic techniques which require costly fabrication equipment or specialized facilities. Here, we report the application of commercially available PMMA microfluidic chips and open-source data analysis workflows for facile electrokinetic characterization of particles and cells. Our workflows eliminate the need for microchannel microfabrication, increases measurement reproducibility, and make zeta potential measurements more accessible. This novel methodology was tested with functionalized 1 μm and 2 μm polystyrene beads as well as Escherichia coli MG1655 strain and Shewanella oneidensis MR-1. Measured zeta potentials for these samples were in agreement with literature values obtained by conventional measurement methods. Taken together our data demonstrate the power of this workflow to broadly enable critical measurements of particle and bacterial zeta potential for numerous applications.

23ATOM05: High-end ICP-MS Applications, Central Pacific A/B/C
Chair: Frank Vanhaecke
Co-Chair: Thibaut Van Acker

(ATOM-05.1)**Evaluation of fs-LA-ICP-TOFMS for multi-matrix analysis with high spatial resolution**

Jorge Pisonero, Cristian Soto, Ana Mendez, Jaime Orejas, Nerea Bordel, Ines García, Alex Calón, Jennifer Linares, Esteban Avigliano, Antonia Cepedal, *University Of Oviedo*

Laser Ablation Inductive Coupled Plasma Mass Spectrometry (LA-ICP-MS) is rapidly evolving into a well-established technique for direct, highly sensitive and high lateral resolution analysis in numerous fields such as geology, biology, metallurgy, or environmental sciences [1]. Significant research and advances continue to thrive for achieving the fastest, most accurate and efficient analysis, such as those focused on improvements such as low dispersion setups and cell geometries for the finest control of aerosol trajectories [2]. Additionally, ultra-short pulsed lasers, such as femtosecond laser ablation, are employed to reduce melting effects around the ablated area as well as fractionation effects. Therefore, the combination of a fast-response femtosecond laser ablation-based units and novel ICP-TOFMS technology provides one of the top-most interesting analytical methods for high spatial resolution determination -mapping- in samples of different matrices and nature.

In the present work, the analytical capabilities of a NWRfemto laser coupled to a Nu Vitesse ICP-TOFMS, which includes a collision/reaction cell and a controlled ion beam attenuation, are critically evaluated for high spatially resolved analysis of challenging samples, including:

- Determination of multielemental diffusion in metallurgical samples after welding processes and after interaction with aggressive environments.
- Determination of multielemental distribution in cancer cells after chemotherapeutic treatments with Pt-based compounds.

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(ATOM-05.2)**The Power of a Multi-Modal Platform Based on ICP-MS to Provide New Insights into the Fractionation of Titania Particles in Food Samples**

David Ojeda, Malvinder Singh, Dorota Bartczak, Paul Hancock, Heidi Goenaga-Infante, *LGC*

Titanium dioxide (TiO2) E171 is a food additive widely used in a range of foods. Recently, after a study underlining the potential for TiO2 particles to cause carcinogenesis in animal studies [1], its use as food additive has been banned in Europe [2]. However, in the UK, its use is still under review due to the lack of robust data to enable a comprehensive risk assessment. In this vein, there is a need for metrological methods for the quantification and sizing of TiO2 particles in food samples.

In this study, a multi-modal ICP-based platform was developed to obtain information about the fractionation of titania particles in food samples. The determination of total Ti in commercial products purchased from local supermarkets was performed by ICP-OES, finding concentration levels of 60-1419 mg kg-1. The representative test material NM-100, with similar properties to E171, and three different food samples were analysed by spICP-MS to determine number concentration and size. Two size fractions were detected in all samples: a fraction with sizes between 15 nm (size limit of detection) and 35 nm, and a larger population of particles of up to 400 nm. The latter is the one typically reported for E171 in the literature. The application of an orthogonal method based on the use of AF4 coupled to MALS and in combination with ICP-MS, suggested the presence of the two Ti-containing size fractions for NM-100. Results obtained with this multi-modal platform for NM-100 were also in agreement with the TEM data from the material provider [3].

Size distributions obtained for food samples by spICP-MS showed that the smaller size NP population is the most relevant in terms of number concentration, whereas the fraction with larger particles is significant in terms of Ti mass. Following European Commission recommendations for the assessment of nanomaterials [4], all food samples analysed in this study were found to meet the definition of nanomaterial, with 73-80% of the particles in number concentration below 100 nm. The importance of these results to inform decision making with regards to the potential hazard of titania nanoparticles in food products will be discussed.

(ATOM-05.3)**Stretching the boundaries of high-precision isotopic analysis using multi-collector ICP-MS**

Frank Vanhaecke, Lana Abou-Zeid, Eduardo Bolea-Fernandez, Marta Costas-Rodriguez, José Ignacio Garcia-Alonso, Steven Goderis, Kasper Hobin, Sara Lauwens, Björn Meermann, Katerina Rodiouchkina, Ana Rua Ibarz, Laura Suarez-Criado, Ir. Thibaut Van Acker, *Ghent University*

The introduction of multi-collector ICP-mass spectrometry (MC-ICP-MS) has revolutionized the use of high-precision isotopic analysis, not only in geo- and cosmochemistry, but also in many other research areas. As a result of the high ionization power of the ICP ion source, a large suite of elements can be targeted, including high-IE metals, such as Hg, and even non-metals. Although the sample preparation is demanding as it traditionally consists of chromatographic target element isolation, the sample throughput is still sufficiently high for analyzing larger collections of samples, and the ICP ion source is operated under atmospheric pressure and shows a robustness enabling a variety of sample introduction strategies to be used.

The application range is continuously being expanded as a result of commercially available instrumentation showing improved capabilities, e.g., in terms of mass resolving power or sensitivity, and of analytical scientists developing new methodologies. Novel approaches often rely on a variety of alternative sample introduction systems.

In this presentation, it will be shown how as a result of the above: (i) isotopic analysis can be carried out successfully at ever decreasing concentration levels and of ever smaller sample sizes, (ii) the collection of elements for which MC-ICP-MS can be used is continuously expanded, (iii) sample preparation can be simplified or avoided altogether, and (iv) an additional level of information is obtained by addressing specific species, rather than carrying out bulk isotopic analysis. The relevance of such analytical methodology will be demonstrated using real-world applications carried out at the A&MS lab at Ghent University.

(ATOM-05.4)**Data processing tool for automated calculation of isotope ratios from transient signals - IsoCor**

Dariya Tukhmetova, Jan Lisec, Jochen Vogl, Björn Meermann, *Federal Institute for Materials Research and Testing (BAM)*

Hyphenation of chromatography and electrokinetic separation methods with multicollector (MC) ICP-MS for isotope analysis is opening new insights into the species-specific behavior of elements. Hyphenated systems enable performing isotope analysis within a shorter time, less consumption of reagents, and minimized "human error" compared to conventional sample introduction on a species-specific basis. However, the main drawback of such systems is the generation of short transient signals which leads to high uncertainty. Therefore, along with the optimization of measurement parameters, a robust data processing strategy is highly needed. The lack of a universal tool for the processing of transient signals motivated us to build a versatile application to facilitate the data treatment step for isotope ratio measurement.
Our data processing application, IsoCor, was developed using R open-source language (https://www.r-project.org/). The application is available online via https://bam.de/IsoCor, works as a standalone tool, and does not require knowledge of programming language to use. The application performs steps such as baseline correction, peak detection, isotope ratio calculation, mass bias correction, and delta calculation. The feasibility and reliability of the application were tested using three datasets generated with Gas Chromatography, Liquid Chromatography, and Capillary Electrophoresis coupled on-line with MC-ICP-MS. The results from IsoCor showed an agreement of one standard deviation with the results from the original publication of the datasets.
As a further development of our application, we recently added a new functionality to carry out mass flow calculations for quantitative elemental speciation performed with isotope dilution analysis.
IsoCor is a data processing solution to the gap in species-specific isotope analysis with hyphenated systems. IsoCor improves reproducibility and trackability of the results, thus it is helpful for validation and quality control.

(ATOM-05.5)**Single-cell ICP-TOF-MS as a powerful tool to investigate the elemental chemical profile of snow algae and their responses to phosphorus starvation**

Silvana Ruella Oliveira, Helen Feord, Cícero A. Lopes Júnior, Ravi S. Peters, Liane G. Benning, Björn Meermann, *Federal Institute For Materials Research And Testing - BAM, Division 1.1, Inorganic Trace Analysis*

Cryophilic algae, and specifically "snow algae", bloom on oligotrophic snow fields worldwide. Due to their intracellular pigments (red, orange, or green), these algae decrease the albedo of the snow they live on, consequently increasing melting and contributing to sea level rise. Little is known about the environmental factors which impact algae growth, and how nutrient-poor conditions affect cell morphology, growth rates, or life cycle stages. The elemental composition of snow algae, particularly at a single cell level, has not been investigated, although knowledge about nutrient requirements for these species is crucial for bloom modelling. In this framework, we have developed a novel multielemental single cell-ICP-ToF-MS method to quantify the intrinsic elements - Ca, P, Mg, Cu, Fe, Mn, and Zn - in single cells of cultures of three snow algae strains - Microglena sp., Raphidonema sempervirens, and Deuterostichococus sp. GUPI-18. We quantified the total single-cell contents of these fingerprint elements under standard growth conditions and assessed how these contents changed under nutrient starvation. Overall, our results revealed a significant elemental cell-to-cell variation in all analyzed species and for all elements, highlighting the relevance of such single-cell measurements to contrast with traditional bulk ICP-MS analysis results. In standard growth conditions, the three snow algae species separated well when a multivariate analysis (discriminant analysis) was employed based on the defined fingerprint elemental profile distributions. In addition, when exposed to five days of phosphorus starvation, Microglena sp. revealed a halving of intracellular P and Mg in single cells, while Raphidonema sempervirens showed no significant effect for any elements. These differences in

responses to nutrient limitation point to diverse adaptations to nutrient limitation, such as P, among snow algae species. Furthermore, FlowCam measurements were carried out to match elemental concentration distribution to biovolume distributions within a given sample. Ongoing work includes testing this single-cell method with environmental samples of snow algae collected from various polar regions.

23AWD02: The Coblentz Society Clara Craver Award Symposium Honoring Ishan Barman, Sierra 5
Chair: Ishan Barman

(AWD-02.1)**Chemical imaging: fit for purpose instruments**

Rohit Bhargava, Sudipta Mukherjee, Kianoush Falahkheirkhah, Yamuna Phal, Anirudha Rao, Ruo-Jing Ho, Kevin Yeh, Seth Kenkel, *University of Illinois at Urbana-Champaign*

Infrared (IR) spectroscopy is a long-established tool for molecular analyses. The past decades have seen improvements that explore the limits of performance in terms of signal to noise ratio (SNR), speed of data recording, molecular content and spatial localization. Here, we explore each of the axes to show how emerging techniques can provide new insights that suit particular characterization challenges. First, we address the challenge of recording high fidelity data in reasonable times to scan large tissue samples with high spectral content. The use of integrated machine learning can provide new insights and well as improve performance, allowing an analysis of tissues for research and clinical diagnoses. We describe a new microscope design for increased speed and rapid coverage that is useful for biomedical and clinical tissue imaging. Second, combining recent advances in photothermal spectroscopy with wide field of view imaging, we show how tissue structure can be rapidly imaged with microscopy optics. Third, we describe a configuration to measure chirality in samples that promises higher spectral information that present methods. Finally, we present a new approach to nanoscale IR imaging that explores the limits of spatial localization with high signal to noise ratio. For each instrumentation advance, examples of use cases will be presented.

(AWD-02.2)**Innovation of far-ultraviolet spectroscopy in condensed phase**

Yukihiro Ozaki, *School Of Biological And Environmental Science*

This talk is concerned with the recent progress of ATR-far-ultraviolet (ATR-FUV) spectroscopy. ATR-FUV spectroscopy was established about 15 years ago by our group and has been used extensively in various fields of chemistry. In this lecture I report two topics; the first one is ATR-FUV and quantum chemical calculation studies of electronic structures of cyclic alkanes such as cyclohexane, methyl- and dimethyl cyclohexane, and decalin. Methyl cyclohexane shows a stronger ATR spectrum than cyclohexane. ATR-FUV spectra of equatorial and axial conformations of methyl cyclohexane are significantly different from each other probably because its HOMO-2 orbit destabilizes by 0.16eV in the axial conformations. The second topic is ATR-FUV spectroscopy and ultraviolet-resonance Raman spectroscopy studies on the electronic structure and structure of four kinds of saccharides. ATR-FUV spectroscopy and ultraviolet resonance Raman spectroscopy have been nicely combined, showing synergetic effects. It was found that amide I, II, and III bands of N-acetyl-D-glucosamine and N-acetyl-D-galactosamine are strongly resonance enhanced with their amide π-π^* transitions. Quantum chemical calculations of FUV spectra N-acetyl-D-glucosamine and N-acetyl-D-galactosamine were also carried out to interprete the results of ATR-FUV and resonance Raman spectra.

(AWD-02.3)**Spectroscopic Marker vs. Biomarker for Disease Diagnostics and Forensics**

Igor Lednev, *University at Albany, SUNY*

It has been well over a decade since artificial intelligence, machine learning, chemometrics and other forms of advanced statistics became a valuable part of spectral data analysis. Researchers came to the

realization that the golden rule of spectroscopy, "if you do not see a change in the spectrum by the naked eye, then you are chasing a ghost," is no longer applicable. [doi.org/10.1021/cr900152h] Various statistical methods have been developed for processing spectral data and extracting essential information about the composition and evolution of the system. The combination of analytical spectroscopy and artificial intelligence has been most valuable for the analysis of complex biological systems including tissue and bodily fluids. In many cases, the analysis is complicated by significant variation within the "same class." The concept of a multidimensional spectroscopic signature can be utilized in these situations. [doi.org/10.1366/11-06455] In addition, the target of the analysis is no longer the detection or quantitation of a specific biomarker, but rather probing the changes of the total biochemical composition through spectroscopy. As a result, statistically significant changes are characterized by a nuanced spectroscopic marker, often latent and unable to be easily assigned to specific biochemistry without further study. The advantage is that this spectroscopic marker can potentially have contributions from multiple biomarkers, vastly increasing its probative value. [doi.10.1039/d0cs01019g] The application of Raman spectroscopic markers for disease diagnostics and forensic purposes including phenotype profiling will be discussed.

(AWD-02.4)**SERS Immunoassay quantifications at ultra-low concentrations**

Alexandre Brolo

Surface-enhanced Raman scattering (SERS) was first observed ~50 years ago and it promised to revolutionize analytical chemistry . The initial enthusiasm was motivated by the recognition that the SERS method not only detects species at very low concentration, but also provides a (vibrational) fingerprint that uniquely characterizes the molecule of interest. However, several challenges have limited the analytical application of SERS in the "real world", and, historically, the technique has been dubbed "unreproducible" and "unreliable" by the wide community of analytical/bioanalytical chemists. On the other hand, a better understanding of the effect has revealed the sources of intensity variations that must be taken into consideration for analytical applications and quantification .
In this presentation, we will show that by recognizing fundamental aspects of the effect, including the role of SERS hotspots and the existence of time-dependent fluctuations in SERS intensities, it is possible to devise methods for quantification even at very low concentrations of the analyte. We hope to demonstrate that SERS has a lot to offer in terms of analytical applications, as long as the advantages of the technique are explored while fully appreciating the fundamental characteristics of the effect

(AWD-02.5)**Bridging the Gap from Whole Body to Subcellular Level using Fluorinated Probes for MRI and Raman Microscopy**

Renzo Vanna, Cristina Chirizzi, Carlo Morasso, Matteo Tommasini, Fabio Corsi, Linda Chaabane, Francesca Baldelli Bombelli, Pierangelo Metrangolo, *IFN- CNR / Politecnico di Milano*

In the fields of clinical practice and biomedical research, various imaging tools have been successful in localizing specific probes at different imaging scales, ranging from subcellular to whole body sizes, using different imaging techniques such as fluorescence, MRI, or PET imaging, along with their corresponding specific probes. However, visualizing a single probe with multiple imaging approaches, focusing on different imaging scales, remains a challenge. Common strategies employed to detect specific MRI probes from whole body to cellular or tissue levels often involve combining MRI contrast agents and fluorescence molecules, which are limited by issues of stability, durability, accuracy, and technical complexity.
In this study, we demonstrate the ability to detect fluorinated (19F) nanoprobes in vivo using 19F-MRI across the entire body, as well as at the tissue and cellular levels using confocal Raman imaging. The unique features of perfluorinated molecules enable clear visualization of both MRI and Raman signals. Initially, we show the detection of these bimodal nanoprobes at the subcellular level and in multiplexing modalities using Raman imaging. Subsequently, we employed a mouse model with multifocal neural inflammation in the spinal cord, resembling human multiple sclerosis, to track inflammation sites throughout the whole body using in vivo MRI after treatment with fluorinated nanoprobes. Fresh frozen sections of the spinal cord were then directly analyzed by Raman

microscopy without the need for additional labelling or staining. Raman analysis not only facilitated the precise localization of fluorinated nanoprobes in the tissue at high resolution but also provided insights into the biomolecular composition of the surrounding tissue, leveraging its intrinsic label-free features.
These findings pave the way for potential research and clinical applications of such probes. The chemical versatility of fluorinated nanoprobes allows for further chemical functionalization and subsequent targeting. For instance, one possible application could involve the detection of tumors by MRI, followed by their intraoperative or ex vivo localization using non-invasive Raman imaging.
Reference:
Chirizzi et al. Journal of the American Chemical Society (2021) 143.31: 12253-12260."

23BIM01: Machine and Deep Learning for Biomedical Diagnostics, Sierra 2
Chair: Thomas Bocklitz
Co-Chair: Oleg Ryabchykov

(BIM-01.1)**Machine learning for pathology detection along with macromolecular orientation using FT-IR bioimaging**

Tomasz Wrobel, *Jagiellonian University*

Machine learning (ML) is paving its way in almost every field of research. Advancements in FT-IR imaging instrumentation allowed recently to properly utilize the benefits of ML in histopathologic examination thanks to the increased dimensionality of measured datasets. The other way around has also become true since classification models with proper feature selection pushed the design of optimized Quantum Cascade Laser microscopes, acquiring only a small percentage of the original datasets in terms of spectral content. These advancements will be presented along with a recent development in macromolecular orientation determination using IR imaging and its prospects in pathologic examination.

(BIM-01.2)**Using machine learning for the processing and modeling of vibrational spectral data**

Oleg Ryabchykov, MSc Azadeh Mokari, Rola Houhou, Ute Neugebauer, Juergen Popp, Thomas Bocklitz, *Leibniz Institute of Photonic Technology (IPHT)*

Raman and IR spectroscopic techniques provide rich information data from biological samples. The non-destructive nature of these photonic methods makes it possible to combine them with other measurement techniques and to design the probes for the in-vivo application. One of the main obstacles for in-vivo applications is the long acquisition time required to obtain high-quality spectra with low noise levels. Besides random noise, non-additive artifacts and non-uniform intensity response may corrupt the measured spectra. Our research focused on developing computational methods to enhance the quality of spectral measurements, suppress various artifacts, and reduce the time needed for data acquisition. These techniques aimed to remove both noise and baseline components from the spectra at the same time.
Another way to speed up the Raman measurements is to use non-linear modifications of the technique that increase the intensity of collected light. Coherent anti-stokes Raman spectroscopy (CARS) can increase the signal by orders of magnitude, leading to a significant acquisition time decrease. Unfortunately, measured CARS spectra contain intense non-resonant background. Although various computational approaches for reconstructing the CARS resonance part exist, they typically require setting parameters based on the knowledge of the measured sample. Therefore, we implemented a deep-learning approach for the first time and showed its outperformance of the standard methods.
The computational methods for retrieving high-quality spectra from measurements with a short acquisition time or from non-linear measurement techniques can improve the performance of the vibrational spectroscopic techniques and make them more suitable for spectral imaging in clinical applications.

Acknowledgment

Financial support from the EU, the TMWWDG, the TAB, the BMBF, the DFG, the Carl-Zeiss Foundation, and the Leibniz Association is greatly acknowledged. This work is supported by the BMBF, funding program Photonics Research Germany (FKZ: 13N15466, 13N15706, 13N15708, and 13N15713) and is integrated into the Leibniz Center for Photonics in Infection Research (LPI).

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(BIM-01.3)**Standardizing Raman Line Illumination Microscopy Data to Investigate Hydrogel-Mediated Reprogramming of Cancer Stem Cells**

Jean-emmanuel Clément, Zannatul Ferdous, Masumi Tsuda, Shinya Tanaka, Jian Ping Gong, Thomas Bocklitz, Katsumasa Fujita, Tamiki Komatsuzaki, *Hokkaido University*

Cancer stem cells (CSCs) are a subpopulation of cancer cells thought to be key players in cancer metastasis, recurrence, and therapy resistance. Recently, we demonstrated that differentiated cancer cells cultured on hydrogels exhibit stem-like characteristics, suggesting that hydrogels may provide an effective platform for investigating the properties of CSCs in order to develop new therapeutic strategies. In this study, we combined hydrogel-based cell culture systems with a high-speed line-illumination Raman microscope , aiming to enhance the identification and characterization of CSCs across different cancer types.

This presentation emphasizes the standardization of Raman data for hydrogel-based substrates. While Raman data provide a rich cellular spectral signature, they are often corrupted by experimental biases including laser fluctuations, optics, and sample preparation. We introduce a standardization workflow that mitigates these challenges, enhancing the reliability and accuracy of Raman data interpretation for hydrogel-based substrates.

Our standardization technique assumes that the local environment around single cells carries experimental artifacts close to the one present in single cells. Briefly, the first step of our method involves segmentation of Raman images into two distinct groups: single cell and background regions (hydrogel zone without cells), using an advanced hyperspectral image denoising framework. Second, we reduce each pixel of a Raman image belonging to single cells by subtracting a local weighted Gaussian average background estimated in the pure hydrogel zone. The weights of the Gaussian function are optimized with a Bayesian optimization framework so that the average spectral distance between single cells in a Raman image is minimized. We show that this Raman image preprocessing workflow allows us to reduce the spectral variability between different types of substrates and different biological replicate experiments.

In conclusion, our local background correction approach enhances the quantitative analysis of CSCs on hydrogel substrates, offering significant potential for advancing our understanding of CSCs.

(BIM-01.4)**Volterra based Explainable Artificial intelligence (XAI) methods for spectroscopy and imaging.**

Msc Jhonatan Contreras, **Thomas Bocklitz,** *Leibniz-ipht*

Deep learning techniques like convolutional neural networks (CNN) have shown remarkable results in several fields in the analysis and modeling of spectroscopic and microscopic measurements. Usual used performance metrics such as accuracy quantifies the model performance but fail to detect biases in the trained models. Explainable artificial intelligence (XAI) methods aim to evaluate models, extract the basis of model decisions, and thereby identify biases [1].

In the contribution, we introduce an agnostic XAI method based on the Volterra series that approximates models [2]. Our model architecture is composed of three second order Volterra layers. With the help of this approximating model, an interpretation of the original model can be gained, and a relevance map can be generated. Our Volterra-XAI learns its Volterra kernels comprehensively and is trained using the output of a trained network. Therefore, no labels are required, and even when training data is unavailable, it is still possible to generate an approximating Volterra model utilizing similar data. We evaluated our model against a standard Taylor based approach. We tested our XAI method for two classification tasks, e.g., a 1D Raman spectral classification task and a 2D images classification task, which were tackled by two common CNN architectures without hyperparameter tuning.

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Acknowledgment
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23CHEM03: Chemometrics Something Borrowed, Something New, Southern Pacific E
Chair: Federico Marini

(CHEM-03.1)DIAGNOSIS AND CORRECTION METHODS FOR SPECTRAL INTERFERENCE IN THE FRAMEWORK OF LIBS IMAGING

Ludovic Duponchel, Vincent Motto-Ros, *University Of Lille*

Laser-Induced Breakdown Spectroscopy (LIBS) has become a powerful imaging technique for elemental characterization in analytical chemistry due to its advantages over other techniques. Major, minor, and trace elements are detected with high measurement dynamic, a low limit of detection and a high acquisition rate, allowing for the quick analysis of large sample surfaces. Today, chemometric tools are commonly used to ensure the most compre-hensive and unbiased exploration of such spectroscopic data. However, the integration of the signal from a wavelength assumed to be specific to the element of interest remains the basic tool for generating a chemical distribution map from a hyperspectral dataset. This classical approach is based on a strong assumption, the specificity of the chemical information on the spectral domain being considered. Any spectral interference inevitably result in the genera-tion of a biased distribution image. In this publication, we demonstrate how Principal Com-ponent Analysis (PCA) can diagnose the potential presence of a spectral interference and how Multivariate Curve Resolution-Alternating Least Squares (MCR-ALS) can ultimately correct it if necessary using a LIBS imaging dataset obtained from the analysis of a complex rock sample. The proposed approach combines the simplicity and effectiveness of the inte-gration method with the diagnostic and correction capabilities of chemometric tools, provid-ing a comprehensive solution for spectral interference in LIBS imaging.

(CHEM-03.2)Screening Trans Fatty Acids in Butter Using a Portable MIR Spectrometer Combined with Multivariate Analysis.

Celeste Aurora Matos Gonzalez, Luis Rodriguez-Saona, *The Ohio State University*

Production of trans fatty acids in dairy products occurs naturally from bio-hydrogenation of dietary cis unsaturated fatty acids by gut rumen microorganisms. Typically, butter contains around 2 to 4% of

trans vaccenic acid (18:1 trans 11), which is produced from linolenic (18:3) and linoleic acids (18:2). Consumption of trans fatty acids has been linked as a risk factor for coronary heart diseases, and also trans fatty acids report correlation with increased levels of LDL (Low-density lipoprotein) cholesterol. Currently, approved techniques to estimate trans fatty acids are time-consuming, require personal training, and also produce chemical waste. This study aims to develop a fast and accurate predictive algorithm to quantify total trans fatty acids and other major fatty acids using a portable mid-infrared spectrometer for butter products. Butter samples (n=77) were collected from local grocery stores, and their fatty acid content was analyzed as fatty acid methyl esters (prepared by transesterification) using gas chromatography with flame ionization detection (GC-FID). Ten microliters of each butter sample at 40°C was placed onto a triple-refection diamond attenuated total reflectance (ATR) crystal, and its spectra were collected by using a portable Fourier Transform mid-infrared (FT-MIR) spectrometer. Spectral data were combined with GC-FID reference data and analyzed using partial least squares regression (PLSR) model. According to the reference GC-FID technique, the total trans-fatty acid level in butter ranged from 1.12-5.58%. The PLSR model showed a strong correlation (R Pre ≥0.92), a low standard error of prediction (SEP~0.3%), and its predictive performance is suitable for quality assurance applications. Portable mid-infrared spectroscopy provided excellent characterization of butter samples, allowing for real-time and in situ answers to the dairy industry for actionable decisions.

(CHEM-03.3)**Labeled Baseline Correction of NIR Spectra via Regularized Least Squares**

Erik Andries, Ramin Nikzad-Langerodi, *Central New Mexico Community College*

Near infrared (NIR) instrumentation provides measurements consisting of two components: specular (mirror-like light that does not penetrate the sample) and diffuse. In the context of NIR spectroscopy, the diffuse component depends on the physical nature of the sample (e.g., particle size). The specular component provides no information on sample composition, so measurements are usually conducted to minimize its contribution. Moreover, spectra from highly sensitive instruments can also be influenced by subtle changes within a device, or across devices, or by changes in environmental conditions such as temperature or pH. As a result, one naturally assumes that spectra are contaminated by uninformative sample-specific signal ranging from broadband background to high-frequency jitter. The existence of this contamination manifests itself as non-constant and low-frequency (or smooth) curves or baselines, and can negatively affect qualitative or quantitative analytical results. These baselines should be fitted and subtracted out from the spectra to mitigate the negative influence.

Historically, a common method of adjusting for a baseline is to fit a quadratic (or polynomial) function to each spectrum and use the difference as a normalized spectrum. Alternatively, the baseline can also be achieved via derivative spectra. Current state-of-the-art baseline correction methods do not consider analyte concentrations across samples. For example, biological samples contain considerable moisture content, and water absorbance often dominates the observed spectral variability across multiple bands in the NIR spectra. However, this moisture information is not considered for baseline correction purposes even when reference measurements for moisture are available. In short, current baseline correction methods are unsupervised in that they are agnostic with respect to analyte concentration. We propose how current baseline correction methods can be augmented to accommodate reference measurements associated with strongly absorbing analytes in both a supervised and semi-supervised manner. At its core, the proposed mechanism involves a penalized least squares approach via Generalized Tikhonov Regularization.

(CHEM-03.4)**Class modeling: something old, something new…**

Federico Marini, *University Of Rome "la Sapienza"*

Many chemometric applications in the field of analytical chemistry involve some sort of classification, i.e., the prediction of one or more qualitative attributes of samples, based on the measured data. In this context, class-modeling techniques, which aim at describing one particular category at a time, are, in principle, best suited to deal with situations such as food authentication or process control, where the

classification problem is almost always asymmetric, with one category of interest being well characterized and representatively sampled (e.g., a food product with PDO origin or, in the case of process data, the so-called Normal Operating Conditions), while the alternative (made of everything that is not that specific group) is almost always under-represented by definition and highly heterogeneous. Despite this, such approaches are not as popular as the discriminant ones and largely underused.
In this communication, the fundamentals of class modeling [1] will be illustrated, through discussion of well established methods, like SIMCA or UNEQ, revival of almost unused techniques (e.g., Non-Parametric class modeling) and proposition of new approaches based on potential functions, together with some possible extensions to the multi-blòck context, where multiple data matrices are used to characterize the analyzed samples. Representative examples will help understanding the potential and the limitations of the different approaches.

23FORENS03: Forensic Analysis in the Lab and at the Crime Scene, Southern Pacific A/G
Chair: Igor Lednev
Co-Chair: Marisia Fikiet

(FORENS-03.1)**Forensic Science R&D Funding Programs at the National Institute of Justice**

Gregory Dutton, *National Institute Of Justice*

The National Institute of Justice (NIJ) — the research agency of the U.S. Department of Justice (DOJ) — is a leading federal funder of research and development in the forensic sciences. NIJ maintains an external grant funding program ranging from applied research to the evaluation of novel methods to the development of prototype devices. Continued R&D in the forensic sciences helps ensure that the true perpetrators of crime are identified and convicted, increasing public safety and promoting the fair administration of justice for all Americans.

Forensic science is a collection of applied disciplines that draws from all branches of science. Nevertheless, practicing forensic scientists most often tend to be concerned with the detection, collection, separation, and analysis of chemical and biological samples. Due to the unique circumstances of forensic evidence, there is an ongoing need for these analyses to be done on ever smaller, degraded, or mixed samples. At the same time, increased backlogs in operational forensic laboratories create pressure to increase the speed and decrease the cost of analysis. Balancing this is the need to ensure that these methods are rigorously validated and defensible in a courtroom context. These needs drive NIJ's continuing R&D investments in analytical chemistry and applied spectroscopy for forensic application.

An overview of NIJ's R&D portfolio will be presented, highlighting relevant examples in Trace Evidence (fibers, glass, paint, geological, etc.); Seized Drugs and Toxicology; and Forensic Biology. The scope and scale of NIJ's R&D portfolio will be discussed, including measures of program impact and examples of notable projects. NIJ anticipates continued interest in advancing the practice of forensic science through analytical chemistry research. In this effort, NIJ strives to engage the research community to bring novel perspectives to solving forensic problems. Information on the funding cycle and anticipated funding opportunities will be presented.

(FORENS-03.2)**Validation of the Fast Blue BB (FBBB) Colorimetric Test for the Detection of delta-9- tetrahydrocannabinol (Δ9-THC) in Oral Fluid**

Jose Almirall, Roberta Gorziza, Nicole Valdes, *Florida International University*

Marijuana-type cannabis is one of the most abused substances in the world and remains classified as a schedule I controlled substance at the federal level in the United States. As of May 2023, twenty-two (22) states and the District of Columbia have legalized marijuana for both recreational and medicinal use and an additional 18 states have legalized marijuana for medicinal use. Marijuana-type cannabis is

differentiated from hemp-type cannabis by the delta-9-tetrahydrocannabinol (Δ9-THC) content with plants containing more than 0.3% (w/w) of Δ9-THC classified as marijuana. The Fast Blue BB (FBBB) colorimetric test is a simple, fast, and affordable presumptive test that has been previously validated for the differentiation between marijuana and hemp cannabis plants. The FBBB reaction produces a red color and fluorescence in the presence of high concentrations of Δ9-THC where high concentrations of CBD produce an orange color and no fluorescence. This presentation reports, for the first time, the use of the FBBB color/fluorescence field test to determine [THC] in plants and the development of a field test to detect THC in an oral fluid (OF) matrix. The FBBB test has been miniaturized on a spot of glass fiber filter paper (6 mm x 6 mm) providing a limit of detection of 0.5 μg/mL of Δ9-THC in OF, sufficient to detect the amount expected for an individual that recently smoked marijuana. We also report on the potential impact of interferences including other cannabinoids, colored drinks and substances that could be in the oral cavity. We also report on the stability of using pre-loaded reagents on a prototype device and color/fluorescence persistence over time. Analytical figures of merit including the linear dynamic range (0.5–10 μg/mL), precision and accuracy for the analysis of plant and OF samples with known [THC] are also reported. Future studies include an OF extraction method coupled to a robust prototype that could be deployed for field use.

(FORENS-03.3)**Probing Phototransformation of Saliva Stain with Steady State Fluorescence Spectroscopy**

Entesar Al-hetlani, Igor Lednev, *Kuwait University*

Photostability and aging of saliva stains was investigated using steady state fluorescence spectroscopy keeping in mind a potential forensic application. In this preliminary study, detection of endogenous fluorophores in saliva was explored, which revealed the presence of tryptophan (Trp) and nicotinamide adenine dinucleotide (NADH). Analysis of Trp resulted in a decrease of the fluorescence signal with time, indicating the high sensitivity of the fluorophore to radiation. On the other hand, an increase in NADH fluorescence signal was observed after irradiating the saliva sample for Trp detection. The increase in NADH fluorescence signal can be assigned to the phototransformation of Trp to NADH through kynurenine pathway. These results were further validated by performing control experiments; (1) saliva stain was dried under dark conditions for four hours, then Trp emission spectra was collected, (2) saliva stain was dried under dark conditions for one hour and emission spectrum at λ_{exc1} = 287 nm was collect every minute for an hour these experiments showed rapid decrease in Trp fluorescence signal and strongly suggested its limited photostability. To understand the connection between Trp phototransformation and NADH, Trp was photobleached for one hour, then NADH fluorescence signal was measured, this demonstrated a decrease in Trp fluorescence signal whereas NADH fluorescence increased. This preliminary investigation discovered a complex behaviour of endogenous fluorophores in saliva. A tentative mechanism of Trp phototransformation to NADH is proposed.

(FORENS-03.4)**Streamlining Decision-making Processes at the Crime Scene and the Laboratory by Incorporating Fast Screening Tools into Current Gunshot Residue Workflows.**

Tatiana Trejos, Luis Arroyo, Kourtney Dalzell, Leah Thomas, Madison Lindung, Thomas Ledergerber, *West Virginia University*

With over three hundred thousand shooting incidents a year in the U.S, research advances in this field directly impact how safety and justice are maintained in our communities. Of utmost importance to these gun violence cases is assessing relationships between an individual of interest and the case's activities and circumstances. The identification of gunshot residues can provide valuable leads to this matter. However, despite existing reliable and standardized methods for GSR analysis, the field still faces several challenges. For instance, the turnaround time required to produce a forensic report is lengthy (~2 months). Also, unlike most forensic disciplines, there are no screening methods routinely used for rapid field and laboratory GSR testing. As a result, this research focused on building capacity through the development of innovative methods that are faster, more objective, informative, and applicable to laboratory and on-site crime scene settings for more effective decision-making processes.

The proposed screening approach combines the advantages of LIBS and electrochemical sensors. They are compatible with universal collection protocols used in current practice, can generate results in under 5 minutes as opposed to 2-8 hours per sample (i.e., SEM-EDS), and detects both IGSR and OGSR simultaneously while leaving the sample nearly intact for further testing.

The performance of the screening methods was compared to confirmatory methods (SEM-EDS and LC-MSMS) using characterized custom-made IGSR/OGSR standards and authentic specimens. We created one of the most extensive datasets of IGSR and OGSR profiles derived from over 3,300 hands' samples from hundreds of individuals. This population's analysis has generated a unique collection of nearly 90,000 LIBS, electrochemical, LC-MS-MS, and SEM-EDS datafiles from populations that represent various background, transfer, and persistence complexities. Statistical methods using neural networks were applied to assess and interpret the evidence. LIBS and EC sensor performance rates were evaluated for portable and lab-based configurations, with low misclassification rates and accuracy better than 90%.

The technology applied here provides reliable screening tools that can process more significant numbers of GSR samples, lower the GSR processing costs, and assist with better decisions at the laboratory and the scene, allowing a more efficient process in firearm-related cases.

(FORENS-03.5)**Multi-Element Analysis of Inorganic Gunshot Residues via Single Particle Inductively Coupled Plasma Mass Spectrometry**

Sarah Szakas, Alexander Gundlach-Graham, *Iowa State University*

Inorganic gunshot residue (IGSR) is trace evidence created when a firearm is discharged. Firing a gun results in a high temperature and pressure combustion event that causes metals in the ammunition primer, bullet, and bullet casing to condense into particles, which can land on surrounding surfaces. In conventional IGSR analysis, particles are collected off these surfaces for examination of particle morphologies and elemental compositions with Scanning Electron Microscopy-Energy Dispersive X-Ray Spectroscopy (SEM-EDS). Characteristic IGSR particles are spherical and have a composition consisting of Pb, Ba, and Sb. While SEM-EDS provides individual particle information without sample destruction, it is a low throughput technique, and sensitivity is dependent on particle size. Here, we use single particle inductively coupled plasma time-of-flight mass spectrometry (spICP-TOFMS) to examine IGSR collected from IGSR primer standards, collected IGSR, and reported sources of particle interferences.

spICP-TOFMS provides a highly sensitive, high-throughput technique to detect particles from ~30 nm to 2 μm in diameter. With spICP-TOFMS, thousands of particles can be detected per minute, and multiple elements per particle can be quantified. spICP-TOFMS is especially useful for measuring elements not typically analyzed in routine IGSR SEM-EDS analysis, such as lower atomic number elements found in lead-free primers. We characterized two IGSR primer particle standards by spICP-TOFMS: leaded Winchester ammunition with characteristic particle compositions of Pb, Ba, and Sb, and lead-free Inceptor ammunition composed primarily of Ti and Zn. With spICP-TOFMS, we found that some additional minor elements, such as Cu and Bi, improved IGSR classification accuracy. We also used spICP-TOFMS to distinguish IGSR in 'real' samples through analysis of IGSR in mixtures of particles previously shown to have elemental compositions similar to that of IGSR. We demonstrate that IGSR can be differentiated from particles associated with brake pads and fireworks based on population-level particle characteristics, as well as the major and minor elements detected within individual particles.

23IR03: 140 Years of the Coblentz Society and the Infrared and Raman Discussion Group (IRDG), Sierra 3
Chair: John Waslyk
Co-Chair: Ashley Love

(IR-03.1)**The Interplay Between Spectrometer Development (Lab And Portable) And Professional Spectroscopic Societies**

Richard Crocombe, *Crocombe Spectroscopic Consulting*

In the UK, The Infrared and Raman Discussion Group (IRDG) was formed in 1950 by spectroscopists from academia, industry and instrument developers, and is one of the oldest independent spectroscopy groups in the UK. This organisation caters for all who are interested in the theory, practice and teaching of infrared and Raman spectroscopy. In the USA, the Coblentz Society was formed in 1954 by a similar wide-ranging group "To foster the understanding and application of infrared spectroscopy". The Society for Applied Spectroscopy (SAS) has roots dating to 1954, with a forerunning formally constituted in 1956, and SAS itself, in its present form, founded in 1958. SAS then took over the New York area's journal (Arcs and Sparks) and it became Applied Spectroscopy. In its early years, SAS and Applied Spectroscopy had its majority focus in atomic spectroscopy.
What did all these events have in common? The answer is the development and availability of commercial spectrometers, both atomic and elemental, reaching a new generation of customers, and the desire to ensure that those customers understood the instrumentation and techniques, became part of a community, and had access to the latest subject matter knowledge.
Are we now at a similar turning point? Portable spectroscopic instruments, both atomic/elemental (XRF, LIBS) and molecular (mid-infrared, near-infrared, uv-visible and Raman) are now widely available and operated by non-scientists. Simultaneously, university libraries are deaccessioning classic spectroscopic texts from their collections. Is there a new role for these professional societies to educate the new generations of spectroscopists and operators of portable spectrometers?

(IR-03.2)**"You Ought to be in Pictures": The Emergence of Infrared Chemical Imaging**

Linda Kidder, E. Neil Lewis, *HORIBA Scientific*

The development and maturation of chemical (spectroscopic) imaging as a valuable problem-solving tool in the life sciences, pharmaceutical, and polymer industries is well documented. In particular, near-infrared (NIR) and mid-infrared chemical imaging using array detectors have proven to be highly versatile implementations when rapid industrial or even process analytical measurements are desired. This is due in part to the rugged measurement characteristics inherited from their conventional single-point or 'spatial-averaging' counterparts. Additionally, infrared imaging is especially flexible with respect to the range of sample sizes that can be evaluated, and as such it is able to interrogate the micro and macro characteristics of many different types of spatially and chemically complex manufactured, or naturally occurring materials. In many cases, images can be recorded and analyzed with video frame-rate refresh speeds and can track evolving or reactive chemical processes. Specifically, chemical imaging delivers information beyond the identification and concentration of a specific chemical species by simultaneously quantifying its localization and distribution. This spatial component adds statistically robust and powerful insights into the factors that may determine or control structure/function relationships and/or product quality and performance.

In this presentation, I will reflect on and partially summarize the history of the development of laboratory infrared chemical imaging with an emphasis on the important insights and applications that continue to drive interest in the techniques(s). In summary, not just how we measure chemical images, but just as importantly, why?

(IR-03.3)**Closing The Loop In The Pulp And Paper Industry - A Process Intensification Approach For Chemical Recovery**

Karin Wieland, Miranda Eisenköck, Barbara Weiß, Anna Katharina Schwaiger, Wolfgang Anton Fuchs, Bernhard Lendl, Michael Harasek, Martin Kraft, *Competence Center Chase GmbH*

The recuperation of chemicals employed in the solubilization of cellulose from wood is a paramount process technology in the pulp and paper industry, contributing to both ecological and economic benefits. The primary constituent of the "cooking liquor," which is utilized in this solubilization process, is Mg(HSO3)2, along with substantial concentrations of SO2*H2O and other cations, such as

Ca2+ and Na+. To efficiently recover SO2, the utilized cooking liquor - containing high concentrations of organics - undergoes incineration. The SO2 is subsequently retrieved from the hot flue gas through a cascade of Venturi scrubbers, upon exposure to a Mg(OH)2 slurry. The magnesium bisulphite cooking liquor is formed following a two-step reaction of Mg(OH)2 + 2 SO2 -> MgSO3 + SO2 + H2O -> Mg(HSO3)2. However, the scrubbers' operating conditions are challenging, characterized by temperatures exceeding 60 °C, a pH range of 4-7, and high ionic strength. Alongside intricate interactions between gas and liquid phases, these circumstances culminate in the formation of insoluble salts, thereby diminishing the recovery efficacy, inducing unscheduled downtimes, and increasing maintenance expenses. As such, an enhanced process understanding is essential to counteract these precipitations. However, the hostile environment presents a challenge, as the majority of available chemical process probes degrade quickly in industrial practice.
We demonstrate the effective use of Raman spectroscopy as a valuable tool in the chemical recovery of the pulp and paper industry. We show that Raman is fit for purpose by establishing a multivariate regression model based on several hundred at-line reference spectra to predict multiple target variables – parameters that are crucial for process control, and presently determined by sampling and titration. Effects on the chemical system due to a change in the process control strategy may be corrected or averted by early countermeasures due to timely measurements within seconds instead of hours. Hence, long downtimes and loss of valuable chemicals due to unwanted precipitation may be mitigated.

(IR-03.4)**A retrospect of spectroscopic studies on dipyridophenazine - from Queens University Belfast to Los Lamos to the University of Otago New Zealand**

Keith Gordon, *University Of Otago*

The talk will discuss some aspects of the decades of work done on dipyridophenazine (dppz) and its complexes; the connection with researchers in IRDG and across three continents. Specifically, the development of time-resolved techniques to expose the complexity of the dppz system in which their is an interplay between ligand-centred and two types of metal-to-ligand charge-transfer (MLCT) states. The elegant use of time-resolved infrared in characterizing these will be discussed. The further development of these ligands to incorporate intra-ligand charge-transfer (ILCT) states will also be described.

23LIBS05: LIBS for Nuclear Applications, Southern Pacific B/C
Chair: Kyle Hartig
Co-Chair: Hunter Andrews

(LIBS-05.1)**Recent advances in laser-based sensing for nuclear safety and security applications**
Milos Burger, *University Of Michigan*

Nuclear security stands as one of the pivotal challenges in our era. The spectrum of nuclear threats encompasses intentional dispersal of radioactive materials to contaminate critical infrastructure, as well as the illicit diversion and smuggling of special nuclear materials for clandestine nuclear programs. Consequently, there is an urgent need to foster and maintain nuclear forensics capabilities, which can be enhanced through a deep comprehension of the intricate processes occurring in plasmas containing nuclear materials.

Within the realm of nuclear safety, there has been a resurgence of public interest. Safeguarding used nuclear fuel and identifying structural material failures in nuclear power systems, particularly in innovative reactor designs envisioned for future implementation, have become paramount. Laser-produced plasmas, as complex and extreme environments, hold the potential to generate intense and highly distinctive signatures of nuclear and radiological materials. These signatures can be harnessed for various applications, including interdicting and swiftly detecting nuclear materials, determining their isotopic composition, enabling long-distance detection, simulating weapons effects in laboratories, monitoring the condition of structural materials in dry cask storage containers, and developing novel instrumentation for nuclear power systems.

We will discuss recent representative examples that demonstrate the application of laser spectroscopy, specifically laser-induced breakdown spectroscopy, to address nuclear safety and security challenges. We argue that spectroscopic techniques based on laser-produced plasmas offer complementary, and at times unparalleled, capabilities that justify further exploration of their efficient production and broader understanding of the signatures they generate.

(LIBS-05.2)**Effects of atmospheric turbulence on remote isotope sensing for nuclear security applications**

Changmin Kim, Boyu Zhang, Jose Chirinos, Xianglei Mao, **Vassilia Zorba,** *Lawrence Berkeley National Lab & Uc Berkelely*

Controlled long-range pulsed laser beam propagation under turbulence is essential in advancing current capabilities for the detection of special nuclear material. Long-range propagation using femtosecond filaments stands out as the only viable approach for the delivery of pulsed laser energy at remote distances for plasma-based elemental and isotopic analysis of solid samples. In this work we study in detail the effects of atmospheric turbulence on filament properties such as onset distance and length, as well as spectrochemical signatures. We correlate turbulence with ablated mass per plasma formation event and quantify the effects of turbulence of different strengths on the Laser-Induced Breakdown (LIBS) signals. These finding lay the foundation for controlling ultrafast laser beam propagation, remote plasma formation, and the development of predictive capabilities for long-distance propagation under atmospheric turbulence conditions.

(LIBS-05.3)**Bulk Aerosol and Single Particle Dynamics in Femtosecond Laser Filaments for Aerosol Sensing**

Kyle Latty, Kyle Hartig, *University Of Florida*

Laser-induced breakdown spectroscopy (LIBS) is a technique capable of performing rapid quantitative elemental analysis without sample preparation or contact by measuring emissions derived from a laser-produced plasma (LPP). The robust measurement capabilities of LIBS have been leveraged in the past to conduct measurements of radiological materials in nuclear applications where prolonged exposure can be avoided through remote-sensing or standoff propagation. With advancements in fission reactors and ongoing efforts to mitigate the effects of potential nuclear release events, an interest in applying LIBS in the detection and characterization of radiological aerosols has emerged for real-time monitoring and atmospheric plume tracking. Through mechanisms such as energy reservoir regeneration and fog-clearing hysteresis, fs laser filaments have been demonstrated to be tolerant to instabilities from atmospheric turbulence and attenuation in aerosolized environments, making them promising for standoff characterization of airborne particles. Our previous efforts have demonstrated that dilute Na aerosols can be measured through single-shot conditional LIBS analysis but reported extreme changes in emission strengths, plasma lifetimes, and single-shot particle sampling probabilities given variances stemming from single particle interactions.
In this study, we investigate the effects of aerosols on filaments using time-resolved plasma imaging and emission spectroscopy for both bulk aerosols and isolated single particles. Filaments are used to measure radioactive Sr aerosol surrogates, employing several plasma diagnostics tools to determine axial emission profiles, plasma temperatures, electron densities, and self-absorption effects uniquely present in aerosol measurements using filaments. The emissions resulting from particles are demonstrated to be strongly influenced by hysteresis effects resulting from preceding filament channels at higher repetition rates and pulse energies where several highly emissive regions begin to localize along the filament. To better examine the dynamics of filament-particle interactions, single particles are levitated in-proximity to the filaments with high spatial fidelity using hollow beam optical trapping of monodisperse particles. The position of single particles is precisely manipulated relative to the filament core and spatiotemporally resolved plasma images are used to characterize the fundamental filament-particle interactions pervasive to bulk aerosol measurements.

(LIBS-05.4)**Detection and Analysis of Light Isotopes in Nuclear Materials using Laser Induced Breakdown Spectroscopy**

Elizabeth Kautz, Annie Xu, Ajay Harilal, Mathew Polek, Arun Devaraj, Andrew Casella, David Senor, Sivanandan Harilal, *North Carolina State University*

Laser induced breakdown spectroscopy (LIBS) is a promising rapid, standoff analysis method for the detection and quantification of light isotopes (e.g., ^{1}H, ^{2}H, ^{3}H, ^{6}Li, ^{7}Li). The detection of these light isotopes is critical to several nuclear applications including forensics, safeguards, and tritium production, retention, and transport in advanced reactor systems and components of tritium producing burnable absorber rods. However, isotopic detection via LIBS can be challenging due to spectral broadening, presence of fine and hyperfine structures, and line distortion effects (e.g., self-absorption and self-reversal) possible in emission spectra of laser produced plasmas (LPPs). Linewidth and line shape influence the ability to detect finely spaced isotopic shifts such as those for ^{6}Li and ^{7}Li ($\simeq$15.8 pm), ^{1}H and ^{2}H (180 pm), and ^{1}H and ^{3}H (240 pm). Here, two case studies on isotopic detection of light isotopes will be presented for: (1) ^{1}H and ^{2}H (as a proxy for ^{3}H) in Zircaloy-4 substrates, and (2) ^{6}Li and ^{7}Li in $LiAlO_2$ target materials. Optical diagnostics including optical emission spectroscopy, 2D spectral imaging, monochromatic and self-emission imaging, were used in order to evaluate the spatial/temporal evolution and dynamics of species in LPPs generated from target materials of interest. The influence of multiple parameters on line broadening, line shape distortion mechanisms, and hence isotopic detection capabilities were investigated. Specifically, the role of ambient gas and pressure, and plasma generation conditions will be presented. Lastly, challenges and opportunities for LIBS in light isotope detection will be discussed.

(LIBS-05.5)**Calibration, chemometrics, and mapping of rare earth elements with laser-induced breakdown spectroscopy**

Daniel Diaz, Amir Fayyaz, Tyler M Wilson, David Hahn, *University of Arizona.*

The rare earth elements lanthanum, neodymium, and praseodymium were detected and quantified in certified reference materials developed for the mining industry. Univariable calibration curves, limits of detection and quantification, principal component analysis, and chemical mapping are reported. Powder samples were prepared as pressed pellets and analyzed with a LIBS system consisting of a 1064-nm laser, and a Czerny-Turner spectrograph coupled to an intensified CCD camera. The analyte concentrations varied approximately between 16 and 290 mg/kg for La, 13 and 32 mg/kg for Nd, and 3 and 13 mg/kg Pr. Highly linear calibration curves were obtained when the normalized net LIBS intensity was utilized. Limits of detection below 5 mg/kg and limits of quantification below 20 mg/kg were obtained. The use of principal component analysis provided sample classification according to the sample matrix. Finally, LIBS mapping indicated that sample chemical composition was homogeneously distributed due to the sample preparation.

23PMA01: Vibrational Spectroscopy to Support Pharmaceutical Manufacturing, Southern Pacific D

Chair: Patrick Wray
Co-Chair: Sergei Kazarian

(PMA-01.1)**Studying Monoclonal Antibody Aggregation Under Flow Using Attenuated Total Reflection Fourier Transform Infrared Spectroscopic Imaging**

Céline van Haaren, Bernadette Byrne, Sergei Kazarian, *Imperial College London*

Studying Monoclonal Antibody Aggregation Under Flow Using ATR-FTIR Spectroscopic Imaging

Céline van Haaren[1], Bernadette Byrne[2], Sergei G Kazarian[1]
[1] Department of Chemical Engineering, Imperial College London, UK
Email: s.kazarian@imperial.ac.uk

[2] Department of Life Sciences, Imperial College London, UK
Email: b.byrne@imperial.ac.uk

Biopharmaceuticals such as therapeutic monoclonal antibodies (mAbs) are highly effective drugs for the treatment of a range of diseases, including various types of cancer. During the production, purification, formulation, transportation, storage and clinical use, mAbs are exposed to different types of physical and chemical stress conditions. These may result in undesirable changes to the protein structure, increasing the probability of protein unfolding and subsequent aggregation. Since these phenomena directly impact the efficacy and safety of the drug, it is of vital importance that the structural integrity of mAbs is investigated under a range of conditions and monitored during manufacturing. Fourier-transform infrared (FTIR) spectroscopy is a powerful tool to study protein secondary structures based on the absorption of infrared light. It is a fast and non-destructive method which does not require complex sample preparation or labelling. By applying FTIR spectroscopic imaging, where a focal plane array detector is used, chemical images are generated which provide both spatial and chemical information on the sample, allowing for the study of dynamic and multi-component systems. In this study, macro-ATR FTIR spectroscopic imaging is combined with microfluidic technology to study mAbs under flow. The mAbs were exposed to increased temperatures and/or air-liquid interfaces in the form of air bubbles, while flowing through a PDMS channel. Analysis of the chemical images and the Amide I band in the resulting absorption spectra indicated that protein aggregation is induced under such conditions, specifically near the air-liquid interface of the injected air bubbles. Measurements were taken at multiple time points, in order to follow the aggregation process and associated secondary structural changes over time. These findings suggest that the presence of air-liquid interfaces under flow reduces the stability of the mAb product.

(PMA-01.2)**Process Related Particulate Analysis In Pharmaceutical Manufacture**

Don Clark, *Pfizer Ltd,*

Particulate matter in parenteral formulations is often assumed to be product contamination. Often these visible particles originate from the manufacturing process or ingoing active pharmaceutical ingredient (API) and formulation excipients. Confirming their identity also points to their origin in the manufacturing process and from that information strategies to eliminate then from drug product can be implemented. In this case study, Infrared and Raman spectroscopies were key techniques in identifying three different process related particulates. These were identified as originating from the API, the manufacturing equipment, and the packaging material.
The complementary nature of IR and Raman was used to explicitly identify the source of a polymer particulate from several very similar packaging components. Subsequent multivariate analysis of the spectroscopic data enabled the complexity of the composition of the particulates to be fully understood and sources identified. The versatility of sample preparations available for vibrational spectroscopy was the key to identifying the manufacturing process particulate and the API related material where mass and nuclear magnetic resonance spectroscopies had failed.
With the identity and origin of all three particulates confirmed, processes were put in place to eliminate particulate formation or to prevent them entering the drug product process stream ensuring drug product supply to patients was not impacted.

(PMA-01.3)**Spectroscopic Applications for Pharmaceutical Development**

John Wasylyk, Robert Wethman, Ming Huang, *Bristol Myers Squibb*

The application of in-line spectroscopy as a PAT (Process Analytical Technology) tool to monitor various processes during the development cycle provides insight into real time kinetic behavior. In-line studies involving vibrational techniques has been utilized to follow reaction and reagent stability, as well as crystallization processes. The crystallization processes often include following polymorph transformations. Raman technology, including low frequency Raman, is a valuable tool in distinguishing different polymorphs, and can also be used in the study of solvate forms, as well as the kinetics of multiple polymorphic transitions during crystallization and storage. Near Infrared has also been applied as an option to Raman Spectroscopy. Sometimes overlooked, is the advantage that in-

line spectroscopy brings to the table, namely sustainability. Benefits often include real time analysis with no sample preparation. Likewise, grab samples can also be analyzed via the sample spectroscopy-based techniques. Once integrated in an appropriate control strategy, PAT tools can enable enhanced process step control and help achieve improved product purity and yield. This presentation will cover a range of studies covering in-line and off-line polymorph transformations as well as reagent stability studies, both of which are key to driving sustainability in analytical analyses.

(PMA-01.4)**Spectroscopic Imaging Of Multi-Material 3D Printed Pharmaceutical Dosage Forms**

Zoë Whalley, *The University Of Birmingham*

3D printing of pharmaceuticals is an emerging field, which will have many exciting applications in the sphere of personalised medicine. The inherent advantages are that it offers manufacturing flexibility and platform versatility. The technique offers the possibility to print multiple drug formulations in one dosage form and control their individual release based upon their geometries.

This research focuses on a type of 3D Printing known as Fused Deposition Modelling. A filament is produced by combining a polymer with an active pharmaceutical ingredient in a hot melt extruder. The printer then melts the filament and deposits it onto a print bed layer-by-layer to form the 3D printed tablet. 3D printing tablet designs provides access to different, novel geometries which would be otherwise inaccessible using traditional compaction methods. The mechanics of drug release and water ingress, however, will be different to that of compacted tablets.

In this work, multiple drug and placebo filaments were used to produce differently shaped novel dosage forms, with their geometries used to control the release of the individual drugs. Chemical imaging was used to provide a series of global images of the drug release from the tablets, as a function of time. These images gave us spatially-resolved data which facilitated the tracking of water ingress, polymer swelling and drug release in real time, throughout the dissolution process. Near Infrared and Raman mapping were employed as complementary techniques to perform this work on multi-material formulated tablets and to understand how the interplay of different geometries affect the drug release. This data will help to advise the future of the controlled release of multi-material formulated 3D printed tablets.

(PMA-01.5)**Machine Learning and Data Rich Process Analytical Technology (PAT) for Enhancing Biocatalysis**

Joseph Smith, *Merck & Co.*

Biocatalysis has rapidly become an essential tool in the scientific and pharmaceutical communities for the development of efficient, safe, and sustainable chemical syntheses. Immobilization of the biocatalyst, typically an engineered enzyme, offers significant advantages that include increased enzyme stability, improved resistance to environmental change, and enhanced reusability. Determination of the spatial distribution of the immobilized enzyme of interest is key for ensuring proper functionality; however, currently available analytical tools are frequently inadequate. Machine learning with Raman hyperspectral imaging is presented herein as a process analytical technology (PAT) based methodology for investigating the spatial and chemical distribution of evolved pantothenate kinase immobilized onto two diverse microporous resins. An exhaustive analysis indicates that this proposed technology can successfully resolve, both spatially and spectrally, all chemical species involved in enzyme immobilization, including the enzyme of interest, resin, and other key components. Quantitation of the spatial coverage of immobilized enzyme, a key parameter used for biocatalytic process development, was successfully accomplished. Optimal analytical parameters were elucidated, including evaluation of different excitation wavelengths. Overall, the totality of this information can now be utilized for an enhanced understanding of enzyme immobilization processes and help enable improved implementation of biocatalysis within the scientific community.

23RAM03: SERS 2, Cascade 3
Chair: Zac Schultz
Co-Chair: Sian Sloan-Dennison
Co-Chair: Roy Goodacre

(RAM-03.1)**Applications of Surface-Enhanced Raman Spectroscopy in Environmental Pollutant Detection**
Huiyuan Guo, *Binghamton University*

As the human population grows, the anthropogenic impacts from various agricultural and industrial processes produce unwanted contaminants in the environment. The accurate, sensitive, and rapid detection of such contaminants is vital for human health and safety. Surface-enhanced Raman spectroscopy (SERS) is a valuable analytical tool with wide applications in environmental contaminant monitoring. The aim of this talk is to showcase recent advancements within SERS research as it applies to environmental detection, as well as inform and encourage further development of SERS techniques in protecting environmental quality and safety. Specifically, we highlight SERS methods for the detection of long-existing and emerging pollutants, such as pesticides, microplastics, nanoplastics, and pathogens. We also discuss the current limitations of SERS technologies in environmental detection and propose several avenues for future investigation. We encourage researchers to fill in the identified gaps so that SERS can be implemented in a real-world environment more effectively and efficiently, ultimately providing reliable and timely data to help and make science-based strategies and policies to protect environmental safety and public health.

(RAM-03.2)**Detection of Multiple Foodborne Pathogens by SERS**

Hayleigh May, Royston Goodacre, Duncan Graham, Karen Faulds, *University Of Strathclyde*

For many common pathogens antibiotic resistance rates are dramatically rising, putting pressure on healthcare providers to rapidly and accurately diagnose the cause of bacterial infections. Within the food manufacturing industry, it is crucial that bacterial pathogens are rapidly isolated and detected to ensure the health of the general public. Food contaminated with bacterial pathogens can lead to foodborne illness, hospitalisation and in many cases, even death. Hence, there is an urgent demand to develop highly efficient strategies for isolating and detecting these pathogens and in particular, platforms that can detect bacterial pathogens at the point of use (POU - deployable on-site) are essential. Surface enhanced Raman scattering (SERS) is a versatile technique for the detection of biomolecules with high sensitivity, specificity and multiplexing capabilities. In recent years, we have shown that SERS using functionalised nanoparticles tagged with Raman reporters has proven ideal for detecting multiple bacterial pathogens. Herein, the novel assay involves using biomolecule (lectin or polyclonal antibody) functionalised magnetic nanoparticles for capture and isolation of bacteria from the sample matrix followed by specifically detecting bacterial pathogens using SERS active nanoparticles functionalised with antibodies which are strain specific. Three bacterial pathogens, Escherichia coli, Salmonella typhimurium and Staphylococcus aureus, were successfully isolated and detected, with the lowest concentration for each of the strains detected at just 10 bacteria per mL (CFU/mL). In addition to single pathogen detection, a mixture of all three bacterial strains were isolated and identified within the same sample matrix using SERS, with the triplex detection also being confirmed using principal component analysis. Furthermore, we have demonstrated the transferability of our approach from a bench-top, lab based system to a portable Raman device. Since then further improvements have been made to the bionanosensor and the approach has been adapted for the rapid detection of Listeria species. Due to the sensitive nature of the test, it is now possible to detect down to just 4 CFU/mL, hence demonstrating it's potential as a rapid 'on-site' detection platform for bacterial pathogens.

(RAM-03.3)**Revealing the Multiplexing Potential of SERS Nanoparticles for Molecular Imaging of Cancer**

Olga Eremina, Alexander Czaja, Augusta Fernando, Cristina Zavaleta, *University Of Southern California*

The outstanding multiplexing capability combined with high sensitivity of surface-enhanced Raman spectroscopy (SERS) have greatly accelerated the adoption of this rapidly developing spectroscopic tool. The combination of modern hyperspectral imaging instrumentation and cutting-edge nano-technology enables fast, efficient, and precise Raman analysis. Utilizing reporter-carrying SERS gold nanoparticles (AuNPs) as biological contrast agents and functionalizing their surface with protein-targeting moieties has brought Raman spectroscopy into the realm of molecular imaging.[1] Previously, the largest reported number of SERS NPs multiplexed using Raman imaging was achieved with 10 unique SERS flavors. We are now discovering that far more SERS NPs can be simultaneously present and identified using a single Raman spectral acquisition.
Qualitative and quantitative aspects: molecular fingerprints and enhancement factors, are all important features to evaluate when developing a new set of SERS NPs. Therefore, we propose an approach to building a library of SERS flavors that integrates density functional theory (DFT) modeling of Raman scattering with experimental guidance and validation.[2] The ability to reliably calculate and assess SERS spectra enables wise selection of Raman reporters with the adequately different molecular fingerprints.
To demonstrate the potential of SERS NPs as highly specific molecular targeting imaging agents, we functionalize NPs by chemically conjugating them to moieties which recognize prototypical cancer biomarkers. We validate their targeting efficiency on cultured cancer cells with well-known biomarker expression profiles.[3] Herein, we present an expansive library of SERS nanoparticles, each bearing a unique Raman fingerprint, and reveal the potential of SERS nanoparticles to specifically target biomarkers and gain an unparalleled understanding of the spatial relationships between a multitude of cell types using Raman imaging. Our ability to deconvolve a mixture of more than 20 SERS NPs in a single imaging pixel both in vitro and in vivo has the potential to enable efficient interrogation of heterogeneous molecular expression found within and across patient samples.

References:
[1] Zavaleta, C.L., et al. Proc. Natl. Acad. Sci. U. S. A. (2009); 106, 13511.
[2] Eremina, O.E., et al. J. Phys. Chem. Lett. (2021); 12, 5564.
[3] Eremina, O.E., et al. ACS Nano (2022); 16, 10341.

(RAM-03.4)**Raman and SERS of liquid biopsy biofluids for cancer diagnostics: focus on extracellular vesicles (EVs)**

Randy Carney, *UC Davis*

Aim: To improve patient outcomes, there remains a critical need to develop faster, less invasive platforms capable of identifying biomarkers. Multi-omics approaches, while promising, are high in cost and complexity, low throughput, slow, and require large sample volumes, thus are impractical for many stages of clinical care. Raman spectroscopy (RS) addresses many of these needs: it requires little to no sample preparation, is non-destructive, does not need exogenous dyes or labelling agents, and can be performed directly in aqueous solutions. Surface enhanced Raman spectroscopy (SERS) offers further improvements in terms of sensitivity but increases complexity. In this study we carried out comprehensive RS and SERS measurements on a >100-person cohort of blood and saliva from head and neck cancer (HNC) patients and benign controls. Analyses were carried out on whole biofluids, in addition to extracellular vesicles (EVs) isolated from the biofluids to investigate the added value. EVs are emerging biomarkers of interest found in high numbers in circulating biofluids with great promise to increase the specificity of diagnostic platforms.

Results: We analyzed a robust clinical dataset of HNC patient saliva and plasma to build a diagnostic model. We found distinct Raman and SERS signatures of metabolites in all cancer samples uniquely present compared to non-cancer controls. Raman spectral measurements fused together for EVs and whole biofluids across saliva and plasma delivered accuracies of >95% using a novel machine learning

module based on convolutional neural networks. Our platform features an automated method to isolate EVs amenable to rapid clinical use.

Conclusions: The results of this study indicate an exciting step in validating Raman as a robust diagnostic tool revealing that complementary chemical information spread out across biofluids and biomarkers of interest can strongly improve simple, quick Raman based diagnostics of whole biofluids and EVs.

23RAM13: Nano Raman 1, Cascade 1
Char: Andrew Whitely
Co-Chair: Andrey Krayev

(RAM-13.1)**TERS Origins and the Next Generation**

Volker Deckert, *Friedrich Schiller University Jena*

In this contribution, a short and personal overview on the beginnings of tip-enhanced Raman scattering (TERS) will be given before concluding with recent developments that might possibly boost the performance of "ordinary" TERS without the everlasting search for better tips.

The quality of the tips was and still is a major part of the research and huge improvements were made regarding the theoretical understanding of tip-sample interaction and the associated lateral resolution. This theoretical and technical improvement was also directly linked towards TERS applications to more and more complex samples. While in the early stages TERS was mostly used on dye films and carbon nanotubes, it was quickly adapted to chemistry and biology and essentially wherever a lateral resolution beyond the diffraction limit was required and only the intrinsic molecular fingerprint was available. Examples from the different fields will provide an overview how the capabilities and the understanding of TERS evolved over the years.

The recent development of near-field IR techniques clearly provides complementary tools for high lateral resolution and TERS is not the only possibility if high resolution and direct structural information is required. Most likely this will push the development of both methods and high-resolution spectroscopy remains an exciting field with lots of surprises yet to come.

Consequently, the second part of the presentation will deal with recent developments of our group aiming towards more sensitive TERS experiments and a better physical understanding of the involved mechanisms. Specifically, automated alignment procedures allow an optimized beam profile adaption that increases the following TERS experiments intensities generally by a factor of ten and more. With respect to theory, we will show first attempts on how to include the field gradients over the molecule, allowing a better prediction of ultra-high spatial resolution. If time permits, we will provide a brief outlook on the "boring" aspects of novel TERS tip development.

(RAM-13.2)**Nanoimprinted pyramid scanning probe for nanoscale optical mapping**

Junze Zhou, Edward Barnard, Keiko Munechika, Adam Schwartzberg, Alexander Weber-Bargioni, *Lawrence Berkeley National Laboratory*

Tip-enhanced Photoluminescence (TEPL) enables the simultaneous collection of emission spectra and topographic information with sub-diffraction spatial resolution, providing valuable insights into the correlation between local material properties, structure, and macroscopic functionality of a material. In this study, we focus on the development of a novel near-field probe using a cost-effective and efficient nanoimprinting technique for investigating the optical properties of low-dimensional quantum materials. Through extensive experimental demonstrations, we showcase the performance of the nanoimprinted probe in terms of high-precision height sensing and high-resolution optical mapping on a 2-dimensional (2D) semiconductor. Additionally, we introduce advanced plasmonic designs and

nanofabrication strategies based on the pyramidal probe, which facilitate the observation of nanoscale dark states and plexciton emission in 2D materials.

(RAM-13.3)**TERS/TEPL imaging of the moiré domains in transition metal dichalcogenide bilayers**

Thomas Darlington, Yinjie Guo, Emanuil Yanev, Kevin Kwock, Cory Dean, P. James Schuck, *Columbia University*

Stacking and twisting of 2D materials has opened up a new paradigm to materials engineering by allowing atomistic control of neighboring atomic interfaces. A fundamental effect of mismatched adjoining lattices is the moiré potential that modifies the optical and electronic properties of the host materials, where the size can be continuously tuned by choice of the twist angle. At low twist angles, strong layer interactions can lead to dramatic lattice reconstruction, as the stacked layers adjust to minimize the stacking energy. This results in the formation of domain walls between moiré cells, which possess high amounts of strain.
In case of twisted monolayer transition metal dichalcogenides (TMDs), such domains have been the subject of extensive scanning probe investigations including conductive atomic force microscopy and scanning tunneling microscopy, showing strong modifications of the electronic landscape. The strong light matter interactions of TMDs would suggest that Raman and photoluminescence measurements would likewise be significantly modified. However, optical characterization of the moiré domains is challenging owing to their nanoscopic size and the difficulty in achieving high uniformity across reconstructed moiré lattice at the scale of conventional optical probes.
Here we present nano-Raman and nano-photoluminescence of reconstructed moiré domains in twisted WSe2. We show that the domain shows enhanced Raman scattering compared to flat regions, as well as enhanced PL emission. High resolution nano Raman spectroscopy of the domain walls shows the WSe2 bilayer to be under significant shear strains containing signatures of both tensile and compressive Raman shifts, with an apparent mode splitting of the 1st order Raman peaks of 5 cm-1. Using electrically biased semi-contact AFM we confirm the moiré origin of the domain wall, and uncover signs of alternating charge puddling in the moiré lattice. To our knowledge, these results are the first direct measurement of the modified Raman modes in twisted WSe2.

23SPSJ01: Higher Energy UV Spectroscopy, Cascade 4
Chair: Yusuke Morisawa
Co-Chair: Ichiro Tanabe

(SPSJ-01.1)**A study on electronic structure and transition of saccharides by ATR-FUV and UVRR spectroscopy**

Kosuke Hashimoto, Fatima Matroodi, Mariagrazia Tortora, Barbara Rossi, Yusuke Morisawa, Yukihiro Ozaki, Hidetoshi Sato, *Kwansei Gakuin University*

We are aiming to develop a new technique for analyzing biological molecules locating on cellular membrane with a non-labelled and non-destructive manner using far and deep ultraviolet light. Far-ultraviolet (FUV) spectroscopy is the spectroscopy in the region of 120–200 nm where one can measure electronic spectra of various kinds of molecules. Attenuated total reflection (ATR)-far-ultraviolet (FUV) absorption spectroscopy is possible to explore the electronic states and transitions of almost all types of molecules in the condensed phase, such as water and aqueous solutions, polymers, and organic liquids and solids because electronic transitions from n and s orbitals in the FUV region can be studied for intermolecular interaction in the condensed phase. Because of the short penetration depth (～100 nm) , ATR-FUV spectroscopy has high potential to analyze biomolecules on surface of living cells. However, FUV spectroscopy has not been applied to biomolecules because it is difficult to assign spectra to complex biological samples containing many molecules. In order to overcome this problem, we employed UV resonance Raman spectroscopy. As a first step, we demonstrated to measure ATR-FUV spectra and multi-wavelength UVRR spectra for four kinds of saccharides (D-glucose, D-galactose, N-acetyl-D-glucosamine (GlcNAc), and N-acetyl-D-galactosamine (GalNAc))

to study of electronic states and structure. Those saccharides are major components of carbohydrate chain localizing on the surface of cells. According to ATR-FUV measurement, a difference of the broadness of the band at 190 nm assignable to π-π* transition of amide between GlcNAc and GalNAc is observed. This may reflect susceptibility for the difference in the orientation of -OH at C-4. The SR-UVRR spectroscopy revealed the excitation wavelength dependent susceptibilities were different among a variation of electronic structures and transitions of amide I(1645 cm-1), II(1565 cm-1), and III(1326 cm-1). The combined use of SR-UVRR and ATR-FUV spectroscopy provides new insight into localized electronic and vibrational states of a specific functional group in biomolecules.

(SPSJ-01.2)**SPR sensing in far and- deep-ultraviolet regions**

Ichiro Tanabe, *Rikkyo University*

Plasmonics in the ultraviolet region has been widely focused
because of the higher energy and the abundant electronic resonances compared to the conventional visible plasmonics. Recently, we have investigated the surface plasmon resonance (SPR) properties of the Al film, aiming for the application as refractive index sensors. Utilizing the ultraviolet lights, we expect three advantages: high sensitivity, material selectivity, and surface selectivity. By using an original attenuated total reflectance spectroscopic instrument, Al-SPR angle and wavelength were investigated with changing environments on the Al film.

(SPSJ-01.3)**Enabling Label Free Biosensing With Ultra Violet Plasmonics Engineered Native Fluorescence.**

Yunshan Wang, *University Of Utah*

UV plasmonics has shown promises in label-free sensing of biomolecules. However, applications of UV plasmonics to the sensing of biomolecules in real biological samples have been hindered by two factors. Firstly, the native fluorescence of biomolecules undergoes rapid photobleaching under UV illumination. To address this issue, UV plasmonics nanostructures has been proposed to improve the photostability of molecules. In addition, surface passivation strategies and oxygen scavenger have been used to improve the stability of plasmonic metals and molecules in solutions. However, the enhancement factor of photostability by UV plasmonics remains small. In this talk, I will discuss our efforts in designing better plasmonic geometries to enhance the photostability of molecules. Secondly, in real biological samples, multiple molecules are present in the solution. Differentiating molecules in a mixture is a challenging task as most biomolecules absorb and emit in the UV range. I will discuss a new mechanism to differentiate molecules with similar structures. We experimentally measured the native fluorescence of several monoamine neurotransmitters on different plasmonic substrates. We observed enhanced fluorescence yields of neurotransmitters on plasmonic substrates. In addition, we conducted proof-of-concepts experiments to differentiate molecules with similar structures using UV plasmonics engineered native fluorescence.

(SPSJ-01.4)**Electronic states of water in “Water-in-Salts” and “Hydrate-melts” electrorytes.**

Yusuke Morisawa, *Kindai University*

A new high-concentration salt aqueous electrolyte that is non-flammable, non-toxic, and inexpensive, called a “water-in-salt” (WIS) and a "hydrate-melt" (HM), is attracting attention. For instance, Suo et al. discovered that the potential window could be significantly expanded by using an ultra-highly concentrated aqueous electrolyte solution (> 21 mol/kg) called water-in-salt (WIS). Independently, Yamada et al. developed a more concentrated aqueous salt solution by combining two types of lithium salts in a molar ratio of LiTFSI:LiBETI:H_2O = 0.7:0.3:2, which can yield a salt aqueous solution with a high concentration of 7 mol L-1. In our experiments with Li hydrate-melts, we observed the electronic state of water using attenuated total reflection far-ultraviolet spectroscopy (ATR-FUV), and demonstrated that the electronic excitation state of water is significantly blue-shifted due to coordination with Li ions in high-concentration saltwater solutions.[1] To investigate whether similar

results can be obtained in Na hydrate melts, which have been discovered in recent years, we first observed the electronic state of water in high-concentration sodium salt using ATR-FUV.

The ATR-FUV spectrum of 0-5.0 mol L^-1 NaOTf aqueous solution shows only the absorption of water. The 163 and 157 nm bands observed in pure water decreased monotonically as the salt concentration increased. On the other hand, a peak remained at 153 nm at 5.0 mol L^-1. This change is similar to that observed in LiOTf aqueous solution at the same concentration, but the center wavelength of the remaining band was on the longer wavelength side. To further increase the concentration of sodium, we measured a solution containing 1 mol L^-1 of NaTFSI added. While further blue-shift was observed in Li-hydrate melts in this concentration region, it was not observed in Na-hydrate melts. This difference is thought to represent the difference in the coordination state of water due to the difference in the Lewis acidity of Li and Na.
[1] Nami Ueno, Masato Takegoshi, Anna Zaitseva, Yukihiro Ozaki, Yusuke Morisawa, J. Chem. Phys., 2022, 156(7) 074705.

Poster Presentations

Monday Poster Sessions

(Mon-P2)**The Analysis of Major and Trace Elements in Plant-Based Foods Using the NexION ICP-MS**

Aaron Hineman, Liyan Xing, Tamas Ugrai, **Chady Stephan,** *PerkinElmer*

Plant-based foods have become popular due to their nutritional, environmental, sustainability, and ethical benefits. However, plant-based foods present challenges in formulation, nutrition, and safety. Micronutrients such as iron, zinc, and calcium are not found in the same levels as animal-based foods. Heavy metals can be taken up and sequestered by plants if grown in contaminated soils, necessitating periodic testing. ICP-MS is a powerful analytical technique that can be used for environmental monitoring, food and beverage analysis, and forensic science. However, polyatomic interferences generated by matrix ions and plasma-based ions can lead to the formation of unwanted ions. PerkinElmer's NexION® ICP-MS series is equipped with Universal Cell Technology™ (UCT) that can run in Collision mode with kinetic energy discrimination (KED) and Reaction mode with dynamic bandpass tuning (DBT) to reduce these interferences. This work described a procedure for the determination of major and trace elements in various plant-based protein products using the NexION 2000 series ICP-MS. PerkinElmer's MPS 320™ microwave digestion system was used for sample preparation.

(Mon-P3)**Gold nanoparticles influence on accumulation and translocation of essential elements in hydroponic common bean sprouts (Phaseolus vulgaris L.)**

Aline Pereira de Oliveira, Juliana Naozuka, Cassiana Nomura, *University of Sao Paulo*

The global production and applications of nanomaterials in various areas for biological and industrial purposes escalate their release into the environment, raising environmental and public health concerns. Nanomaterials, such as silver (AgNPs) and gold (AuNPs) nanoparticles (NPs), found in the growth medium are potentially absorbed by plant roots and translocated to edible parts. In this case, antagonistic and synergistic effects between NPs and essential elements in plant metabolism must also be studied, because it can modify the chemical composition of agricultural products. In this study, common bean sprouts (Phaseolus vulgaris L.) were submitted to hydroponic cultivation in nutrient solution containing Au(III) or AuNPs. After that, it was evaluated the gold uptake, accumulation, and translocation and the effect on distribution of essential elements (Cu, Ca, Fe, Mg, Mn, P, and Zn) in roots and aerial part. Sprouts growth was carried out for 8 days and 12-hour photoperiod in which the roots of each seed (n=7) were kept submerged in 7 mL of deionized water or nutrient solution containing different Au concentrations (0; 0.5; 2.5; 5.0; and 10.0 mg/L at pH = 5.5 - 6.5) from AuNPs (60 nm) or $HAuCl_4$. After microwave-assisted acid digestion, elemental determination was carried out

by inductively coupled plasma optical emission spectroscopy (ICP OES). It was found that in plants exposed to Au(III) or AuNPs in water or nutrient solution there was no translocation of Au (< LOQ: 11.5 μg/g). However, Au accumulation in roots was 5.2 (H_2O, Au 10 mg/L) to 3.6 (nutrient solution, Au 10 mg/L) times greater in plants grown on NPs-supplemented medium. Plants grown in nutrient solution had a higher concentration of essential elements, however, supplementation of the nutrient solution with AuNPs (10 mg/L) increased Mg concentration in the aerial part by 52 and 123%, compared to the control group and Au(III) supplementation, respectively. On the other hand, nutrient solution supplementation with AuNPs reduced the P concentration in aerial part, and mainly in the roots, up to 27% compared to the control group, highlighting possible competitions between P and AuNPs accumulation and translocation. ACKNOWLEDGMENT: FAPESP (2022/02167-9 and 2021/14125-6).

(Mon-P4)**Spectra Classification on Pharmaceutical Product by Machine Learning Assisted Chemometrics**

Yechan Hwang, Ziyi Cao, Garth Simpson, *Purdue University*

Generative adversarial linear discriminant analysis (GALDA) was developed for spectral classification applied to pharmaceutical products. A theoretical foundation for implementing GALDA for spectral dimension reduction and classification was derived, and simulations with known ground truth spectral classes supported the assessment of GALDA. Application of GALDA improved classification accuracy in polymorph discrimination by conventional Raman spectroscopy. In analysis of aspirin tablet constituents, pixel-wise application of GALDA produced composition maps in agreement with preliminary assignments based on crystal habits.

(Mon-P5)**Propagating highly-correlated error structures through multivariate classification models to optimize class-differentiation thresholds**

Helder V. Carneiro, Caelin Celani, Karl S. Booksh, *University Of Delaware*

Presented is a novel approach for determining optimal threshold levels in classification models based on error propagation. In classification model applications, the threshold is crucial for distinguishing the target class from non-target classes. Current state-of-the-art methods, such as Bayesian joint probabilities assuming a normal distribution of sample scores and empirical tests like the K-S test / ROC curve, aim to maximize sensitivity and selectivity of analyses.

The proposed method, however, takes a finer route by calculating the propagation of measurement errors through the model to establish a statistical threshold. Unlike traditional statistical tests that assume independent, identically distributed Gaussian errors with a diagonal covariance matrix, this strategy employs the error covariance matrix to generate a statistical error distribution using Monte Carlo simulations. By building a regression using the noise extracted from each sample, the boundary is set at the noise score mean plus two times the standard deviation. This results in a classification limit of detection with a 95% confidence level. One of the remarkable aspects of this approach is its ability to handle non-normally distributed data, which marks a significant advancement in the field.

The efficacy of the new algorithm was tested using simulated data, ICP data, and LIBS, with promising results. This approach opens up new possibilities for calculating boundaries based on chemical information. The ultimate objective is to extend this innovative method to hierarchical and non-parametric classification techniques, thereby paving the way for a broader range of applications in the future.

(Mon-P6)**Multivariate Classification of Geospatial Origin of Ash Analyzed by Inductively Coupled Plasma Mass Spectrometry**

Maria Delgado-Cornelio, Collin White, James A. Jordan, Michael E. Ketterer, Helder V. Carneiro, Caelin Celani, Barry Lavine, Karl S. Booksh, *University Of Delaware*

We can identify native soil type from ashed plants by multivariate classification. Analyzing tree ashes can help predict long term forests' recovery and nutrient redistribution following a fire. A pilot study of 140 Pinus Ponderosa samples from eight geologically distinct locations and six soil types in Northern Arizona and Southern Colorado were collected to determine if geolocation is feasible. The samples were ashed using hydrogen flame, and subsequently analyzed by ICP-MS. Preliminary results show that Partial Least Squares Discriminant Analysis (PLS-DA) and Support Vector Machines (SVM) were able to differentiate among the eight locations with greater than 0.85 and 0.9 accuracy, respectively.

(Mon-P7)**Making the Process of Multivariate Analysis Accessible to the Data Science Novice: A Workflow for Getting Answers From Fluorescence A-TEEMs**

Karen Gall, Eunah Lee, Jeffrey Julien, Linda Kidder, Brad Swarbrick, Rajani Davuluri, Joonsup Lee, *Horiba Scientific*

Multivariate analysis (MVA) is used to reduce large data sets down into meaningful and useful answers, but for some data sets, the workflow is not always an easy road. While automation is the wish of some for the future in MVA, software interfaces should consider the users' knowledge of the data to which they are applying chemometrics. Automating prediction from quantification or classification models takes much work and many samples to include variances in experimental setup and decisions by the scientist(s) as to which results are important to a decision-making process. Automation is not so easy here, but informative guidance is possible. Variations in data formats, data dimension, preprocessing and the different models available can make the decision tree for a MVA workflow overwhelming for beginners to chemometric methods. When working with data that does not fit traditional 1D spectra of intensity versus wavelength or energy, the steps to analyze the data tends to become tedious and more prone to human error. We take a different approach to MVA on a type of multidimensional data: the Absorbance-Transmittance Excitation Emission Matrix (A-TEEM). We approach the use of MVA from an end-user's perspective in both an applications-driven and guided stepwise manner. The Guided Workflow for A-TEEM data in a MVA package called A-TEEM Direktor incorporates a step-by-step guide for data import, visualization, pre-processing, modeling, evaluation, prediction, and reporting steps. The Guided Workflow will be demonstrated on two different A-TEEM data sets of biologically relevant samples. This new take on a Guided Workflow process is described in detail and includes a recommended decision tree for approaching A-TEEM data analysis with MVA methods. We hope this novel user interface will drive MVA into a more global scientific audience and unlock these methods to more types of spectroscopic analysis going forward.

(Mon-P8)**Multivariate Analysis of Granule Size Distributions: A Tool for Process Understanding in Continuous Granulation Systems**

Samuel Henson, Jacob Guess, Md. Nahid Hasan, James Drennen, Carl Anderson, *Duquesne University*

The transition from batch to continuous wet granulation (cWG) has been a challenge to continuous manufacturing (CM) implementation. Intense development efforts are required to gain adequate process understanding for novel cWG processes. Granule size is the primary indicator of success for granulation processes. Granule size distributions (GSD) visually represent the granule size data, and the metrics used to describe the data are d-values. These metrics were intended for use with normal or log-normal distributions but are used to define percentiles for GSDs. Deviation from a normal distribution shape is an expected result of cWG processes but reduces the relevancy of d-values since the GSD shape information is not captured and the interpretation of percentile values is reliant on distribution shape.
Multivariate approaches such as principal component analysis (PCA) seek to extract the variance between samples. Treating GSDs as multivariate data is logical in the development stage of cWG systems since the GSD shape is often uncharacterized. The goal of process development is to provide process understanding leading to implementation. The current work demonstrates the applicability of PCA for GSD analysis, highlighting its relevancy to cWG process development and increasing process

understanding. Principal component (PC) loadings capture the shape of the variance in the GSDs, and the PC scores identify GSD shape change associated with parameter settings. Comparing PCA and d-value results highlights the deficiency of d-values for describing GSD shape change.
Overall, PCA is shown as a viable data analysis technique for GSDs in the context of process development. PC loading shapes capture key regions of the GSD which guides the interpretation of the PC score plot trends. The scores plots are used to guide the interpretation of parameter effects in a screening study. Additionally, PC score trends are used to confirm significant process parameters. These results are compared with d-values to highlight the additional information provided by PCA. Multivariate analysis of GSDs is demonstrated to be a practical and effective approach for process development of cWG systems.

(Mon-P9)**Automated Pipelines for Deployment of Web-based Tools in Chemometrics, Machine Learning & Research Data Management**

Julian Hniopek, Jonas Eichhorn, Rodrigo Escobar, Nazar Stefaniuk, Thomas Bocklitz, *Leibniz Institute of Photonic Technology Jena*

Methods of data science, especially those dealing with machine learning and chemometrics are indispensable tools in the field of analytical sciences today. Without these methods, most experimental observations could not be transferred to useful scientific results. Furthermore, as experimental data is often composed of multiple measurements, acquired on different instrumentation, consistent management of data and metadata is imperative to allow the application of these methods.
However, in many cases the researchers developing data science methods are not the end-users of them. Especially for today's complex models, such as in the field of deep-learning, highly specialized researchers are necessary to develop and implement appropriate methods for a specific analysis task. Commonly, these methods are developed in script form in popular data science languages like GNU R or Python. However, the analytical scientist domain experts performing the collection of data and interpretation of the results obtained using data science usually are not experts in programming or interacting with non-GUI interfaces to run programs. This necessitates a quick and easy way to deliver user-friendly, GUI based data science tools to the end-user.
To enable this, we have developed workflows combining tools for web-based GUI development and tools for automatic provisioning of these tools to researchers with none or minimal need for system administrator intervention. Using Flask, Django and Shiny it is possible to easily create responsive, web-based GUIs as a frontend for access to new algorithms or models tailored to a specific task. Using a Docker based development workflow, data science researchers can use git templates to integrate their algorithms into these GUIs. Using these templates, the resulting applications are automatically built using continuous integration/continuous delivery (CI/CD) pipelines and deployed to a Kubernetes based cluster. These workflows include testing, building and packaging the application, deploying it to a Kubernetes Cluster as well as setting up appropriate encrypted networking to the application for easy and secure web-access.
Together, this workflow allows to deploy finished data science applications to the end user in a few minutes and facilitates rapid updating and changing of data science methods to adjust to a specific task.

(Mon-P10)**Effects of Crystalline Micro-Structures on the Application of Multivariate Curve Resolution to Hyperspectral Raman Images**

Elizabeth Licht, Rachel McCormick, Joseph Smith, Karl S. Booksh, *University Of Delaware Graduate Student*

Hyperspectral Raman imaging is a popular method for determining the spatial distribution of chemical compounds across a solid sample that can be improved using multivariate data analysis. Multivariate curve resolution (MCR) can separate discriminative Raman signals from obfuscating baselines, fluorescence backgrounds, and other Raman bands. This research investigated the use and benefits of multivariate curve resolution via alternating least squares (MCR-ALS) analysis and modeling on hyperspectral Raman images of TiO_2 and Mannitol polymorphs. The RRUFF database was used as reference data for the TiO_2 polymorphs for impact spherule analysis, while

mannitol spectra for models were taken and analyzed using D-Mannitol converted into the three anhydrous crystal forms, alpha, beta, and gamma. This research found that reference data of expected chemical species containing orientation information can be used to determine the optimal number of factors while building an MCR-ALS model and extracting relevant predicted spectral profiles. When existing reference spectra are unavailable, "pure" spectra can be generated by building a model based on data collected using a Raman microscope, even when polarization of the laser and crystal faces are unknown. Changes in relative intensities due to crystal orientation leads to predicting multiple spectra of the same chemical species in a model, but this can be overcome by comparing the model components to reference spectra.

(Mon-P11)**Integration of Immersive Virtual Reality with Machine Learning for Calibration Model Selection and Classification**

Jordan Peper, John Kalivas, Rajiv Khadka, *Idaho State University*

As technology continues to improve exponentially, the efficacy of numerous autonomous computer algorithms that eliminate human supervision entirely has come under increasing scrutiny. For example, autonomous algorithms can exclude invaluable expert human insight and pattern recognition when making decisions that may be pivotal in many circumstances. The utilization of hybrid algorithms, combining real-time human insight with computational efficiency, accuracy, and scalability, proves highly advantageous in certain scenarios of intricate data analysis or interpretation. Hybrid approaches that maximize human senses and leverage our innate ability to recognize patterns or outliers is crucial to reducing erroneous outcomes and capitalizing on our instinctual responses to the physical environment. The capacity of virtual reality (VR) environments to render comprehensible three-dimensional space and simulate real-world encounters suggests their hybrid suitability for difficult data analysis tasks, such as calibration model selection or classification. In this study, we examine an integrated immersive VR real- time model selection algorithm for both semi-supervised and unsupervised model updating methods, as well as a complex multi-class classification approach that embeds advanced target and source sample similarity measurements into visually, sonically, and haptically unambiguous and distinguishable features. Immersive VR model selection and classification results using NIR data are reported, demonstrating the strengths of VR as a human data analysis interface.

(Mon-P12)**Application Of A Spectral Window Angle Mapper For Variable Selection To Improve Iterative Optimization Technology Algorithm Prediction Robustness**

Adam Rish, Natasha Velez-Silva, Samuel Henson, Md. Nahid Hasan, James Drennen, Carl Anderson, *Duquesne University: Graduate School Of Pharmaceutical Sciences*

Near-infrared (NIR) spectroscopy is one of the most popular process analytical technology (PAT) methods for monitoring active pharmaceutical ingredient (API) concentrations in powder blends. However, advanced multivariate models are required to extract the relevant chemical information from NIR spectra. Calibration-free models such as iterative optimization technology (IOT) algorithms have become attractive to the industry, primarily due to their reduced material and time burdens. A particular challenge to these methods is prediction robustness when sample spectra exhibit non-chemical variability. Wavelength/variable selection as a preprocessing treatment has been shown to improve both prediction performance and robustness for IOT. Within this work, we propose a novel wavelength selection method based on measuring the difference between spectral regions of a mixture spectra and the net analyte signal (derived from the pure components) for the analyte of interest via a spectral window angle mapper (SWAM). The spectral regions are compared using the spectral angle measurement. The SWAM method requires two parameters: window size and selection threshold. These parameters can be optimized utilizing a small training set of potency steps. When setting the window size parameter to a single wavelength, the SWAM method becomes a true calibration-free wavelength selection approach referred to as the wavelength angle mapper (WAM) method. The minimal calibration burden and use of pure components for wavelength selection make the WAM/SWAM methods appealing over alternative wavelength selection methods.

The WAM/SWAM methods were developed on a series of NIR spectra from pharmaceutical powder blends with chemical variation. When combined with IOT, the WAM/SWAM methods showed improved performance over base IOT and comparable performance to a partial least squares model. Robustness enhancement by wavelength selection was demonstrated using NIR spectra from a different pharmaceutical powder formulation collected under various density conditions. The prediction performance of base IOT and the WAM/SWAM methods across different densities were compared to assess prediction robustness. The WAM and SWAM methods demonstrated more consistent prediction performances compared to the base IOT, thus showing enhanced prediction robustness.

(Mon-P13)**Metrics for Quantifying Overfitting in Chemometric Analyses**

Garth Simpson, Yechan Hwang, *Purdue*

An intuitive analytical resolution-based metric is proposed for quantification of overfitting through comparisons between testing and training data during cross-validation analyses of spectrochemical data. Common metrics based on classification accuracy depend not only on the "loading plots" used in the initial dimension reduction operation, but also on the subsequent selection of decision boundaries within the reduced-dimensional space. Metrics dependent only on dimension reduction alone are desirable to enable disentanglement of these two contributions. In analysis of supervised datasets, the eigenvalues recovered in linear discriminant analysis (LDA) for dimension reduction provide a direct metric for the resolution between the data classes. However, testing data does not conform to the same eigenvector/eigenvalue relationships. We demonstrate an analytical solution to the eigenvector/eigenvalue problem that recovers the identical eigenvalues for training data but can also be directly applied for testing data. The utility of this approach is demonstrated in quantifying the degree to which overfitting is suppressed through iterations of generative adversarial linear discriminant analysis (GALDA) of Raman spectra and handwriting images.

(Mon-P14)**Exploration of Acoustic-Based Ion Optics for the Control of Gaseous Ions**

Julia Danischewski, Yi You, Jens Riedel, Jacob Shelley, *Rensselaer Polytechnic Institute*

Ion optics are a critical part of ion-based spectroscopies, such as mass spectrometry (MS) and ion mobility spectrometry (IMS). Analogous to traditional light optics, ion optics control the path of ions through electric and/or magnetic fields to minimize losses from the source to the detector. A wide variety of ion optic devices exist to reflect, focus, separate, and filter ions based on physical properties such as mass-to-charge ratio (m/z) and collisional cross section. Under reduced pressure conditions, ion motion is more directly related to the applied forcefield due to decreased collision rates. As such, ion optics are quite efficient at low pressures. At higher pressures, such as atmospheric pressure (AP), collisions of ions with background gas molecules hinders the intended function and thus require high field strengths, complex geometries, and large device footprints. A recently discovered phenomenon in our lab, termed acoustic ion manipulation (AIM) has shown that weak acoustic fields are sufficiently capable of altering trajectories of AP ions. The AIM phenomenon could provide unique insights to develop novel ion optics.
Here, we explore the possibility of AIM-based ion optics, specifically gating and focusing gaseous ions at AP. Experimentally, ion beams are directed towards a standing acoustic wave, formed through the resonance of two opposing ultrasonic speakers, resulting in the formation of pressure nodes and antinodes. In the gating mode, greater than 90% of ions were blocked when an antinode was positioned between an ion source and detector. In contrast, a 2-mm wide ion beam was compressed by roughly 1 mm after passing through a node. The impact of various ion-source and acoustic-field parameters on efficiency of gating and focusing was characterized to better understand the AIM phenomenon. Three detection approaches, mass spectrometry, Faraday-plate collector, and IonCCD ion-current profiles, were used to account for possible instrumental artifacts, such as vacuum pull or the introduction of external electric fields. This work lays a foundation for acoustic-based ion optics at atmospheric pressure.

(Mon-P15)**Analysis of Industrial Gasses, Sulfur Hexafluoride (SF6) and Novec™ 4710, Using Mass Spectrometry, Gas Chromatography, and Raman Spectroscopy**

Dawson Dodd, Theresa Evans-Nguyen, *University Of South Florida*

The removal of impurities in insulating gasses is required to maintain functional circuit breakers. Sulfur hexafluoride (SF6) is the most popular insulating gas, due to its excellent arc quenching ability. When SF6 quenches an electrical arc in the presence of water, byproducts, such as SO2, are produced. These byproducts can corrode the inside of the breaker, leading to expensive repairs. It is therefore important to have the ability to detect such byproducts. However, SF6 is one of the most potent greenhouse gasses, and is being phased out by 2025, thus, alternatives are being created. A relatively new alternative gas, Novec™ 4710, has properties similar to SF6 when used in CO2 mixtures. Novec™ 4710 has the possibility to create byproducts harmful to your health, meaning the verification of its purity is also of importance. The goal of this project was to detect the presence of any undesired byproducts in SF6 gasses and Novec™ 4710 / CO2 mixtures. This was achieved using a quadrupole time of flight mass spectrometer and gas chromatography with an electron capture detector. We also employed the use of a home-built Raman spectrometer designed to enhance the analysis of gasses.

(Mon-P17)**Design and Optimization of a new microwave microreactor for mass spectrometry**

Buddhika Kumara, Steven Ray, *The State University Of New York at Buffalo*

Microwave energy has recently been shown to be an effective mechanism for accelerating the rate of verity of chemical and biological reactions, including enzymatic processes. Microwave dielectric heating uses the interaction of electromagnetic radiation (2.45 GHz) with specific materials in order to deposit energy into experiments at specific locations and times, and with a focused power density. The controlled deposition of thermal energy can be used to control the rate of chemical reactions, and can be used in a wide range of advantageous way such as to decrease the time that it takes for proteolytic digestion of proteins prior to analysis by mass spectrometry. In this presentation, the development of a new microwave microreactor system for use in sample treatment prior to mass spectrometric analysis will be presented. The use of a dedicated microwave reactor to accomplish sample preparation decreases the sample preparation time, and also allows for inline reactions immediately prior to mass spectrometry. Here, we discuss the design and optimization of a novel custom-designed microwave resonator that are uses microwave microstrip techniques to focus microwave energy into a capillary microreactor contained within a electrospray ionization nanospray emitter. Thus, samples can be loaded into an electrospray nanoemitter tip, and chemically treated before analysis in a single step. One specific advantage of microwave accelerated enzymatic reactions can be kinetically modulated, and indeed chemical modifications can be turned on- and off prior to mass spectrometry simply by controlling the microwave field. Here, we explore different designs and optimize the parameters of the resonator using a 3D High Frequency Simulation Software (Ansys HFSS) and experimental performance.

(Mon-P18)**Monitoring long-term chemical exposome by characterizing the hair metabolome using a high-resolution mass spectrometry-based suspect screening approach**

Pao-Chi Liao, *National Cheng Kung University*

Hair has recently emerged as a biospecimen for characterizing the long-term chemical exposome in biomonitoring investigations spanning several months, as chemical compounds circulating in the bloodstream accumulate in hair. Although there has been interest in using human hair as a biospecimen for exposome studies, it has yet to be widely adopted compared to blood and urine. Here, we applied a high-resolution mass spectrometry (HRMS)-based suspect screening strategy to characterize the long-term chemical exposome in human hair. Hair samples were collected from 70 subjects and cut into 3 cm segments, which were then mixed to prepare pooled samples. The pooled hair samples underwent a sample preparation procedure, and the hair extracts were further analyzed using an HRMS-based suspect screening approach. An in-house chemical suspect list containing 1227 chemical entries from National Report on Human Exposure to Environmental Chemicals (Report)

published by the U.S. CDC and the Exposome-Explorer 3.0 database developed by the WHO was subsequently used to screen and filter the suspect features against the HRMS dataset. Overall, we matched 587 suspect features in the HRMS dataset to 246 unique chemical formulas in the suspect list, and the structures of 167 chemicals were further identified through a fragmentation analysis. Among these, chemicals such as mono-2-ethylhexyl phthalate, methyl paraben, and 1-naphthol, which have been detected in the urine or blood for exposure assessment, were also identified in human hair. This suggests that hair reflects the accumulation of environmental compounds to which an individual is exposed. Exposure to exogenous chemicals may exert adverse effects on cognitive function, and we discovered 15 chemicals in human hair that may contribute to the pathogenesis of Alzheimer's disease. This finding suggests that human hair may be a promising biospecimen for monitoring long-term exposure to multiple environmental chemicals and perturbations in endogenous chemicals in biomonitoring investigations.

(Mon-P21)**Understanding Harmful Algal Blooms in Chautauqua Lake, New York through Toxin Detection and Genetic Analysis**

Abigail Ross, Vincent Moriarty, Manuel Castro-Berman, Jonathan Dordick, Jacob Shelley, *Rensselaer Polytechnic Institute*

Harmful algal blooms (HABs), containing toxin-producing cyanobacteria, pose a threat to human and environmental health. The number of reported HABs in freshwater lakes has increased dramatically over the past decade, potentially due to climate change and increased nutrient pollution. One class of toxins commonly found in freshwater lake HABs are microcystins, which are cyclic heptapeptides with several congeners that act as hepatotoxins. Microcystin congeners have varying toxicity levels, with microcystin-LR being the most commonly measured due to its global distribution, ubiquity, and toxicity. The EPA recommended concentration limits of microcystin-LR is 8 µg/L for recreational waters and 1.5 µg/L for drinking water. Due to cyanotoxin-associated human health risks, there is a need for fast and accurate cyanotoxin quantification methods for evaluating lake water quality. Additionally, very little is known about the factors that lead to HAB formation and proliferation. Chautauqua Lake (CL), in western New York state, supports both large fishing and recreational communities, but suffers from frequent HABs in the southern basin. CL's northern basin has far fewer algal blooms and lower nutrient levels, making CL an ideal location to study HAB dynamics. Here, we describe the use of liquid chromatography coupled with mass spectrometry (LC-MS) to detect, identify, and quantify microcystin toxins from CL samples collected throughout 2022 and 2023. An LC-MS procedure was devised based on EPA Method 544 to quantify and identify three microcystin congeners (microcystin-RR, -YR, and -LR) and nodularin-R. Sample analysis time for the optimized method is six minutes with detection limits in the nanogram per liter level. This analysis approach is faster, more sensitive, and more selective than the standard ELISA method. Results from this method revealed microcystin toxins were present throughout the summer and fall in both basins of CL. Toxin quantities from the lake samples were compared with water chemistry data, such as chlorophyll concentration and total phosphates, as well as cyanobacterial genetics in order to gain a deeper understanding of HAB formation, growth, and proliferations in CL.

(Mon-P22)**Investigation of potential anthropogenic pollution of rare metals in urban river water and sewage treatment effluent**

Akane Yaida, Akitoshi Okino, Kazuhiko Nakano, Akihide Itoh, *First Tokyo Institute Of Technology*

Abstract

Rare metals are essential to high technology industries. The use of these rare metals as industrial materials and pharmaceuticals has rapidly increased. We have been continuously investigating potential anthropogenic pollution of rare metals in river water such as Tama River. We have established a method for the comprehensive determination of multi-element ranging from major-to-ultratrace elements in river water and sewage treatment effluent. In this study, river water and sewage treatment effluent from Tama River were regularly sampled for a period of 5 years. The methods established by we were applied to the sampled water to determine the current status of potential

anthropogenic pollution. In addition, we investigated the contamination of tap water for the elements for which a significant increase in concentration was observed. For all samples, determination of 60 elements including rare metals in the concentration range of 0.16 ng/ L for Lu to 38 mg/ L for Na was possible with analytical precision of approximately RSD 2-10%. 5 years of monitoring (up to 12 water samples) at the same site for rare metals in Tama River water showed a steady increase in the concentration of 21 elements (Li, B, Mo, Mn, Co, Ni, Ge, Rb, Cs, Ba, Sc, Y, Se, Dy, Gd, Tm, Ho, Er, Yb, Lu, Pt) due to the influence of sewage treatment effluent. In particular, the concentrations of Mo, Co, Ni, Ge, Rb, Cs, Gd and Pt were 10 times higher than in clean river water. Of these, Gd was detected in the sewage treatment effluent from all 5 sampled sites. At times, its concentration exceeded 150 ng/ L. So, we are currently investigating the effect of Gd on tap water sourced from subsoil water in the downstream of Tama River.

References

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(Mon-P23)**Quantitative screening of Tuberculosis targeting isoniazid tablets using handheld Raman spectrometer.**

Ed Bethea, Melissa Growney, Jonelle Carson, Matthew Eady, David Jenkins, *FHI360*

Tuberculosis is a communicable disease and a leading cause of death from a single infectious agent. The World Health Organization has reports that one in ten medical products in low- and middle-income countries is either substandard or falsified. Portable Raman spectrometers have the capacity to rapidly screen a greater number of samples per lot, opposed to more traditional analysis methods like high-performance liquid chromatography. Our previous handheld Raman work has shown the ability to qualitatively distinguish between five manufacturers of isoniazid tablets each at different isoniazid percentage label claims. As a next step in the quality screening process, here, we are evaluating the ability of a handheld Raman (143 – 2495 cm-1) to quantify the percentage isoniazid from five manufacturers with varied isoniazid percentage label claims. Tablets were pressed in-house using a universal excipient mixture and varying the isoniazid content in 10% increments between 40 and 100% of tablet weight. The dataset was made up of 108 in-house pressed tablets and 84 tablets obtained from five manufacturers, resulting in N = 192. A calibration dataset containing 134 tablets was constructed, while 58 tablets were reserved as an independent validation dataset, both spanning the in-house pressed tablets and five manufacturing sources. Preprocessing consisted of standard normal variant normalization to compensate for potential scaler differences in the dataset. A partial least squares regression model was applied to the calibration data set resulting in an R2 of approximately 0.98 as compared to 0.93 for the validation data set. The independent test data set was predicted using the calibration model and resulted in an R2 of approximately 0.88 and a root mean squared error of prediction of approximately 0.045. The results for the handheld Raman are promising and suggest the possibility for rapid and low-cost spectral analysis for quality screening in resource limited settings.

(Mon-P24)**International supply chain implementation logistics of multiple handheld spectrometers for rapid screening of public health commodities**

Matthew Eady, Christopher Harmon, Chayanee Changpim, Mohammed Jinnah, Jonelle Carson, Melissa Growney, Noah Peters, David Jenkins, *Fhi 360*

Global public health initiatives work to supply safe and efficacious essential medications and public health commodities to resource limited areas. Substandard and falsified medications are an ongoing issue, globally. Handheld spectrometers offer a cost-effective screening method for quality compliance but come with challenges in global screening implementation. Understanding their value and limitations are keys in building low-cost screening methods for finished pharmaceuticals. Here, five handheld spectrometers (900 – 1700 nm) of the same make are implemented in the United States, South Africa, and Thailand for screening purposes. A variety of products are considered, including oral contraceptives, depot-medroxyprogesterone acetate, tuberculosis medications, and long-lasting

insecticidal nets. Sensors first go through an internal qualification and operational procedure. Next, a daily instrumental qualification procedure is performed for assuring consistency in data collection. Tablets used in treating tuberculosis infections were scanned in the U.S. as well as South Africa. A qualitative brand discrimination method was established, showing that there was statistically significant bias between the two sensors, but that a qualitative model could be established with 100% accuracy (N=482) that was robustness enough to account for the bias. Handheld spectrometers showed that they were able to detect oral contraceptives that were stored under increased temperature and humidity settings with noticeable changes in water associated spectral regions. Qualitative and quantitative regression models were established for discriminating between insecticides and predicting insecticide amounts on long-lasting nets with an RMSEP % as low as 5.75 within the WHO acceptability limits. Brand discrimination models for injectable contraceptives were also established with 100% classification accuracy. There are challenges ahead in evaluating environmental impacts to sensitivity. Adding multiple instruments to a global screening model imparts a range of variances. Handheld spectrometers show promise for use as a global screening application with essential medicines, where a rapid general screening process is necessary, with traditional methods reserved for more in-depth analysis on a case-by-case basis.

(Mon-P26)**A Top-Down Spectroscopic Approach to Correlating Coating Thickness Distributions with the Dissolution Profiles of Enterically Coated Drug Pellets**

Daniel Willett, Huzeyfe Yilmaz, Wenjing Xi, Zongming Gao, Jason Rodriguez, *US Food & Drug Administration*

Pharmaceutical dosage forms such as tablets and capsules are often coated with a functional polymer to modify the drug release. To obtain the drug release profiles, ensure quality control and predict in-vivo performance, dissolution studies are performed. However, dissolution tests are time-consuming, sample destructive and do not readily allow for at-line or in-line characterization. Since the coating material and thickness are critical quality attributes that have a direct effect on the drug release kinetics, these parameters can be utilized to indicate if a product will meet quality specification. For conventional dosage forms these parameters can be rapidly assessed by both traditional methods such as height-, weight- or diameter gains or by spectroscopic approaches. However, these approaches aren't readily applied to very small dosage forms. Rapid assessment of functional coatings of drug products is essential for products where a single capsule is comprised of hundreds of functionally-coated pellets and the collective drug release kinetics of the entire capsule depends on contributions from each pellet.

First, Raman mapping coupled with multivariate curve resolution (MCR) modeling was performed to characterize the different components and the MCR scores indicated that each pellet consisted of a sucrose core, an active pharmaceutical ingredient (API) layer and a coating layer with polymer and talc. A spectroscopic approach was then developed in which the integrated area of a polymer Raman band to an API Raman band from single point measurement for each pellet was reported and used to represent the local polymer coating thickness. This data was confirmed by Raman mapping, SEM and EDS. Next, this approach was applied to test ~100 pellets per product and the results were analyzed by both the supervised approach of taking ratios described above, as well as by an unsupervised, multivariate analysis approach that allows for the entire spectrum to be used. Overall, the thicker coating led to a slower release rate and a combination of coating thicknesses for each layer resulted in several drug release stages. In addition, the standard deviation in the pellet coating distribution represented by the polymer to API ratio could be used to access the coating uniformity.

(Mon-P28)**Using Ultra-High Resolution to Overcome Isobaric Interferences with the Liquid Sampling-Atmospheric Pressure Glow Discharge / Orbitrap Coupling**

Joseph Goodwin, Benjamin Manard, Brian Ticknor, Paula Cable-Dunlap, R. Kenneth Marcus, *Goodwin*

Isobaric interferences, interferences with the same mass as the analyte of interest, are a common problem across inorganic mass spectrometry techniques. These interferences could be directly from

other elements in the sample matrix or polyatomic complexes with other sample matrix components. Two approaches to overcoming isobaric interferences are commonly employed in inorganic mass spectrometry. The first is to separate the analyte of interest from potential interferences before analysis by complex sample manipulations, including solid phase extraction, before mass spectrometric analysis. While this technique has been largely successful, it does require additional sample preparation time and incurs higher operational overhead costs. The second approach is to use online gas-filled cells, in which reactive gases are introduced post-ionization to interact with either the analyte of interest or the interfering ions or to use kinetic energy discrimination where collisions with inert fill gases reduce the kinetic energy of polyatomic species below a potential barrier so that these polyatomic species are not transmitted further along the ion path. While this approach is extremely powerful and dramatically reduces sample preparation, it may result in loss of analyte signal due to incomplete reactions or analyte ions being unable to surmount the potential energy barrier. A third, often not explored, approach is to use ultra-high mass resolution to fully resolve the analyte of interest from the offending isobaric species. To this end, Marcus and coworkers at Clemson University have developed the liquid sampling-atmospheric pressure glow discharge (LS-APGD) microplasma ionization source. Unique among elemental ionization sources, the LS-APGD can directly mount to mass spectrometers typically reserved for "organic" mass spectrometers such as the ultra-high resolution Orbitrap mass spectrometer (mass resolution of 70K at 200 m/z). In addition, the resolution of the Orbitrap can be further expanded by using an external data acquisition system such as the Spectroswiss FTMS Booster X2. To this end, overcoming isobaric interferences from several different sampling systems, including direct sampling from cotton swipes for uranium isotope ratio determinations, will be presented.

(Mon-P29)**Development of a Spectral Reaction Library for 78 Elements with a Variety of Reaction Gases**

Aaron Hineman, Ewa Pruszkowski, Karl Andreas Jensen, Valeriia Morozova, *PerkinElmer*

This work presents a library of product ion scans acquired using the NexION® 5000 Multi-Quadrupole ICP-MS in the mass range of 2 to 285 amu, six reaction gases, and 78 elements including the radionuclides ^{99}Tc, ^{226}Ra, ^{237}Np, ^{241}Pu and ^{243}Am. The library contains 1,300 scans. Each element was scanned at a concentration of 1 µg/L and the corresponding blank. Additional scans of potential isobaric-, polyatomic-, or doubly charged interferences in higher concentrations expand the library. The scans can be overlaid and subtracted in the Syngistix™ for ICP-MS software.

The first result of this library are 6 colour-coded periodic tables for the individual gases, pre-informing the user about the suitability of the reaction gas for interference correction. In most cases, several alternatives are available. If the analyte reacts strongly with the reaction gas but the interferent does not, the Mass Shift mode, where detection occurs at a higher mass, is the method of choice. In the opposite case, where only the interferent but not the analyte reacts with the gas, the MS/MS mode will be chosen. Here, the original mass is used for detection, while the interferent is either removed from the ion beam by a charge transfer reaction or shifted to a higher mass by a mass shift reaction.

(Mon-P30)**Speciation of Selenium and Chromium in Drinking Waters using an Inert HPLC and DRC-ICP-MS**

Aaron Hineman, *PerkinElmer*

In this talk, a method for measuring five selenium species and a method for determining hexavalent chromium in drinking water will be presented. Chromium III (Cr 3+) and selenium (Se) are essential elements at trace levels and toxic at higher levels. Selenium exists in several oxidation states in a variety of inorganic and organic compounds and chromium VI has carcinogenic properties. Consequently, ensuring that we are consuming only essential forms and levels of these two elements is important.

To assess trace concentrations of selenium and chromium in drinking water, it is necessary to have an instrument capable of measuring down to ppt concentrations and that the chromatographic baselines

are also low to be able to accurately characterize and quantify the various species. With chromium, it is especially important to consider the fluid path of the HPLC being used as metal flow paths can contribute to the background. The benefits of the inert fluid path of the PerkinElmer NexSAR™ HPLC allow for single-digit ppt-level detection of chromium VI in waters. The formation of polyatomic ions in the argon plasma causes interferences with both selenium and chromium isotopes. The major interference on selenium is the formation of the dimer $^{40}Ar^{40}Ar+$ which overlaps with the most abundant selenium isotope, ^{80}Se (49.8% abundance). A challenge in the analysis of chromium is all mobile phases have some level of carbon, where $^{40}Ar^{12}C+$, in addition to $^{35}Cl^{16}OH+$ poses as a potential interferent on ^{52}C, significantly increasing the chromatographic baseline.

An effective way to eliminate interferences in ICP-MS is through collision/reaction cell technology. Though helium is often used as an inert collision gas for many interferences, it is not ideally suited to larger polyatomic interferences. Pure ammonia or methane, in contrast, can be used as an effective reaction gas to remove these argon- and chloride-based interferences. For example, in the chromium work we will present limits of detection with improvements in the range of two orders of magnitude improvement with the use of a pure reaction gas, and a cell design which allows the control of reactions inside the cell, versus using helium as a collision gas.

(Mon-P31)**Microplastic Particle Quantification in Seawater Using Single-particle ICP-TOFMS with Online Microdroplet Calibration**

Stasia Harycki, Alexander Gundlach-Graham, *Iowa State University*

Single particle inductively coupled plasma time-of-flight mass spectrometry (spICP-TOFMS) is a powerful analytical technique for counting and quantifying elements in nanoparticles and microparticles. This technique can be used to measure the (multi-)elemental compositions of particles in liquid samples at rates up to thousands of particles per minute. However, ICP-MS is highly susceptible to matrix effects, which makes quantifying particles in diverse matrices challenging. To ensure accurate measurements of particle size and concentration, calibration is required. Most often, this calibration is achieved with dissolved element standards and a well-characterized particle reference material to estimate sample transport efficiency and absolute detection sensitivities for a range of elements. However, this conventional approach does not account for matrix effects from the sample being analyzed. To overcome matrix effects, we use online microdroplet calibration.[1] In this setup, particle-containing samples are introduced to the plasma along with monodisperse microdroplets that contain known element mass amounts. Microdroplet signals are used to calibrate absolute element sensitivities and determine accurate element mass amounts in particles, regardless of the sample matrix.

The matrix tolerance of spICP-TOFMS with online microdroplet calibration was demonstrated by characterizing rare-earth element (REE) doped polystyrene microplastic beads in artificial seawater. The polystyrene beads were sized by quantifying the mass of carbon. In pure water, the sensitivity for carbon (measuring ¹²C⁺) is 27 cts/pg; however, this sensitivity decreases to 7 cts/pg in 80% seawater. The decreased sensitivity as a function of seawater concentration leads to systematic under-sizing of the polystyrene beads without matrix-matched calibration. With online microdroplet calibration, matrix matching is accomplished automatically without preparing new calibration solutions or modifying the calibration system. Our results demonstrate that polystyrene beads are sized accurately and consistently at a diameter of 3.4 µm in matrices from ultrapure water to 80% seawater. In addition, REEs can be quantified accurately and simultaneously in these matrices. We will discuss the operation of online microdroplet calibration combined with spICP-TOFMS and its application for the analysis of microparticle analytes, such as microplastics.

1. Mehrabi, K.; Günther, D.; Gundlach-Graham, A., Environmental Science: Nano 2019, 6 (11), 3349-3358.

(Mon-P32)**Determination of silicon in biological samples by ICP-MS: Stabilization of volatile silicon species during evaporative removal of hydrofluoric acid from digests**

Hydrofluoric acid (HF) is essential for dissolution of silicates, but excess HF either needs to be neutralized or removed to avoid its deleterious effects and formation of insoluble fluorides. Traditional boric acid addition often provides inadequate neutralization. Further, evaporation of HF is not feasible as it results in loss of volatile hexafluorosilicate species (H2SiF6) in digests. In this work, a new approach is described for stabilization of volatile silicon species as M2SiF6 (M+: Li, Na, K, Rb and Cs) to remove excess HF via evaporation. Appropriate volumes of 10% LiCl, NaCl, KCl, RbNO3 and CsCl solutions were added to respective test solutions at a molar ratio of 10 for each metal ion ([M+]/[Si]) that contained 0.1 mL of 10 mg/mL Si (as H2SiF6) in 2 mL HNO3 and 0.25 mL HF. The test solutions were heated to incipient dryness at 130 oC on a hot block to evaporate HF. The residue was dissolved in 4 mL of 10% HNO3 and analyzed by ICP-MS. Results indicated that LiCl, NaCl and KCl improved stability of silicon whereas RbNO3 and CsCl showed no improvement. Recoveries were about 52%, 104% and 65% for LiCl, NaCl and KCl, respectively. Adding methanol or propanol to reduce solubility of M2SiF6 species provided stabilization with lower NaCl levels (e.g., [M+]/[Si]=2.5). A volume of 80 µL of 10% NaCl, [Na+]/[Si]=4, was found to be optimum in the presence of 1 mL propanol to retain all silicon quantitatively. Limit of detection (LOD) varied between 60 and 150 µg/L for blank solutions prepared from evaporation 1 mL HNO3, 0.5 mL HF, 1 mL propanol and 80 µL of 10% NaCl. Several plant and tissue certified reference materials (SRMs) are digested by closed-vessel microwave-assisted digestion in 4 mL HNO3, 1 mL HCl and 0.5 mL HF. Portions of digests will be treated with developed method for removal of HF. Results for Si will be presented comparatively for untreated and treated SRMs.

(Mon-P33)**Improving the Lithium Battery Supply Chain: from Raw Materials Testing to Recycling**

Aaron Hineman, **Chady Stephan,** *PerkinElmer*

Lithium (Li) holds significant importance in our daily lives and is widely present in various aspects of modern living. Notably, lithium finds extensive use in batteries that power cell phones, computers, electric vehicles, and numerous portable electronic devices. The continuous pursuit of longer battery life and faster charging capabilities by consumers has driven advancements in lithium battery technology. Furthermore, the growing trends of electrification, renewable energy, and electric vehicle adoption have created a demand for lithium-based materials with enhanced quality control (QC) requirements.

The increasing demand for higher-capacity batteries necessitates improvements in current battery production technology. This improvement involves better control over the raw materials used and their physical properties. For instance, lithium salts are commonly extracted from brine, a concentrated solution of sodium chloride (NaCl) or the extraction of lithium from recycled batteries. During each extraction process, it becomes crucial to remove impurities from the final product to prevent overheating issues in the battery. Therefore, the ability to identify impurities in lithium battery materials is essential for manufacturers and suppliers to ensure optimal battery performance.

In this work, data from the Avio® 550 Max ICP-OES, NexION® 2000 ICP-MS, and NexION 5000 Multi-Quadrupole ICP-MS will be presented. Our aim is to illustrate the correlation between increasing impurity levels in the materials and the need for a more powerful analytical instrument is required to effectively detect all potential impurities. By utilizing these analytical instruments, our objective is to highlight their capacity to accurately identify impurities in lithium battery materials, underscoring the significance of employing suitable instrumentation as impurity levels rise.

(Mon-P34)**Influence of optical configuration on FT-IR imaged breast Tissue Microarrays classification**

Danuta Liberda-Matyja, Tomasz Wrobel, *Solaris National Synchrotron Radiation Centre, Jagiellonian University*

The application of Fourier Transform Infrared Imaging (FT-IR) in combination with machine learning algorithms for tissue type recognition and patient classification was already achieved for numerous pathologies. A typical problem that occurs in this type of study is the low number of samples that are available from healthy patients. It is especially problematic in breast cancer, due to developed diagnosis with numerous steps taken before biopsy collection. Accordingly, we decided to apply machine learning for the recognition of four tissue classes (cancer, necrosis, fibers, benign) in two breast Tissue Micro Arrays and patient classification. In this research, we addressed two issues that need to be overcome to introduce a method to the clinic: measurement time and cost reduction. In the first case, we decided to apply a 3.5x magnification objective giving an 11.4μm pixel size which is the novel aspect for this type of research. To reduce measurement costs we used inexpensive low-e slides. In this study, we compared random forest classification results for data measured in transmission and transflection modes on the pixel and the patient level. Classification results (presented in the Table 1) on the pixel level achieved accuracy values close to 0.8. This similarity in accuracies for both measurement modes, may indicate, that application of the 3.5x objective influences (averages) optical effects. This is also reflected in classification results on the patient level where data measured with 3.5x objective gives high AUC (Area under the receiver operating characteristic curve) values, regardless of measurement mode.

Table 1. Classification results

	Transmission	Transflection
Accuracy on the pixel level	0.7933	0.7906
AUC on the patient level	0.9563	0.9575

This research was supported from the National Science Centre, Poland ("Improvement of histopathological classification based on chemical FT-IR imaging with data augmentation", Grant No. 2019/35/N/ST4/01809). Measurements were done at the CIRI beamline of the NSRC SOLARIS synchrotron facility.

(Mon-P35)**Machine Learning for Classification of Particles from Noisy spICP-TOFMS Data**

Raven Buckman, Hark Karkee, Alexander Gundlach-Graham, *Iowa State University*

Nanoparticles (NPs) and microparticles (μPs) from various sources can be found in consumer products, industrial applications, and environmental samples. Several types of particles, namely titanium (Ti-NPs) and cerium (Ce-NPs), are of particular interest due to their environmental prevalence in soils, water, sewage, cosmetics, and fuels. These particles can be generated by either anthropogenic or natural means; therefore, the ability to precisely and accurately quantify anthropogenic particles in a high particle number concentration (PNC) background is critical for sample analysis.

Previous studies have used both supervised and unsupervised machine learning algorithms to classify NP data from single-particle inductively coupled plasma time-of-flight mass spectrometry (spICP-TOFMS), among other methods. spICP-TOFMS data shows systematic bias in detected elemental compositions of particles as a function of particle size, composition, and analytical sensitivity. To overcome the inherent bias of spICP-TOFMS data for the classification of NP types, we report a multi-stage semi-supervised machine learning (SSML) strategy. We find that the use of SSML enables more robust and accurate classifications compared to supervised or unsupervised learning algorithms.

We developed a multi-stage SSML model for classification of various Ti-rich and Ce-rich particle types, including TiO2 E171, TiO2 rutile, ilmenite, biotite, CeO2, ferrocerium mischmetal, and bastnaesite as representatives for engineered, incidental, and natural particles. In our approach, systematic particle misclassifications are first found and then these "noise classes" are incorporated into the SSML model for the development of a second, more robust classification model. This two-

stage SSML demonstrates low false positive rates, which allow for accurate particle-type classification of mixed samples with variable PNCs and particle-type quantification across more than two orders of magnitude. Overall, our two-stage SSML model for NP classification identifies and overcomes bias in spICP-TOFMS training data to provide a simple and robust approach for the incorporation of machine learning models in spICP-TOFMS particle classification strategies.

(Mon-P36)**Rapid ICP-MS Analysis of Dried Blood Spots via Direct Microextraction from Solid Substrates**

Cameron Stouffer, R. Kenneth Marcus, *Clemson University*

Dried blood spots (DBS) have become a convenient tool in both qualitative and quantitative biological analysis due to their ease of sample collection and their minimally invasive nature. These qualities make these analytical tools suitable for routine medical sample collection as DBS analysis offers the advantage of collecting small sample volumes, making it attractive for infant medical sample collection. However, the concentration and amount collected of the target analyte is typically low, requiring a sensitive and selective method for detection and quantification. Mass spectrometry (MS) has become the most common technique for DBS analysis, but sensitivity is often hindered by the complex nature of the blood matrix. Chromatographic separations (gas chromatography (GC), liquid chromatography (LC), etc.) and additional pre-treatment methods (solid phase extraction (SPE), liquid-liquid extraction (LLE), protein precipitation (PPT), etc.) are often required prior to MS detection leading to extensive analysis times. Analysts typically face challenges of sensitivity, reproducibility, and overall accuracy of DBS quantification by current methods. The extensive sample manipulation on DBS can lead to significant analyte losses due to the small sample size. As an alternative, DBS analysis by the coupling of the Advion Plate Express to the quadrupole-based Advion Solation inductively coupled plasma (ICP)-MS is described. This is a method of direct sampling capable of trace-level detection to provide a method for rapid (< 1 min), highly sensitive, multielement detection of DBS. In this method, a ~2 x 4 mm region of the DBS is exposed to an extraction solvent, with the adsorbed species removed quantitatively in 1 min, and carried to the ICP nebulizer. The Solation ICP-MS has proven to be tolerant of organic matrices with no significant hindrance to the sensitivity of the instrument. The use of aqueous standards of four prevalent heavy metals (Pb, Hg, As, and Cd) with a synthetic blood matrix is presented to allow for the development of a common quantification approach for DBS elemental detection.

(Mon-P38)**Just A Drop: A Simplified Small-Droplet Device And Modified Sampling Substrate for LMJ-SSP-MS**

Daniel Reddy, Lishen Zhang, Thomas Covey, Richard Oleschuk, *Queen's University*

Miniaturization in a broad sense is an expanding, interdisciplinary trend – ranging from computing to medicine to synthesis – that has been colloquially dubbed the "(re)-evolution of analytical chemistry." Given recent interests in laboratory automation and (ultra)miniaturization, the (micro)droplet research space has expanded across research disciplines and sectors. Microdroplets are generally considered to be small-volume liquids ranging in size from pico/femtoliter(s) to nanoliter(s). By performing chemistry in (micro)droplets, laboratories can conserve precious compounds, reduce sample volumes and waste streams, and routinize/speed-up analyses. Because of these features, (micro)droplets are widely used across research fields and serve important roles in both academia and industry. By 2026, the applied (micro)droplet field, that is, the global liquid handling market, is expected to reach nearly $5.1 billion USD in value (Markets&Markets – Market Research Report). In turn, the (micro)droplet field is continually evolving and seeking new methods to generate (micro)droplets, especially in ways that can be integrated into the diverse workflows that are unique to each of the fields that use (micro)droplets. Herein, we present a convenient, low-cost, and potentially re-usable (micro)droplet generation device, which we have termed the "NanoWand," that enables (micro)droplet formation across the nanoliter volume range through modulated surface energy and roughness using commercially-available and readily-assembled materials. Previously, the Oleschuk laboratory group has laser-micromachined surface energy traps (SETs) onto various substrates for liquid capture by employing discontinuously de-wetting surfaces. In this work, we create "open" surface energy traps

(oSETs), wherein the de-wetting surface is maintained but laser-micromachining is used to wholly extrude, or "cut-out," a circular region from hydrophobically-modified borosilicate glass in order to capture a droplet within the hole for subsequent liquid transfer and/or analysis as desired. By adjusting the size of the oSET, the volume of the (micro)droplet can be similarly controlled. This approach provides a user-friendly way to form, sample, and/or transfer (micro)droplets that could be integrated into different applications. Furthermore, this (micro)droplet generation device can be paired with modified low-cost storage substrates like paper or glass to complement the commercially-available liquid microjunction – surface sampling probe (LMJ-SSP) coupled with mass spectrometry (LMJ-SSP-MS) for direct sampling and analysis.

(Mon-P40)**Recent Advances in Continuous Fast Data Acquisition < 100 µs for Single-Particle ICP-TOFMS**
Lyndsey Hendriks, Fredrik Oestlund, Martin Rittner, *TOFWERK*

Single particle inductively coupled plasma time-of-flight mass spectrometry (sp-ICP-TOFMS) is a powerful analytical technique for the detection and quantification of inorganic nanoparticles (NPs) in a wide range of applications. While the technique is rapidly evolving into a well-established tool for NP analysis, constant development and research continue to thrive to achieve the fastest acquisition capabilities.

In the present work, the analytical capabilities of a TOFWERK ICP-TOFMS with improved fast acquisition hardware will be evaluated for high time-resolved single particle analysis. More particularly, single-particle data acquired at acquisitions speeds down to < 100 µs will be presented. As data acquisition rate and data processing are key factors in the accuracy of the characterization of NPs, the effect of different integration times from micro- to milliseconds will be investigated in detail with regard to the distinction of signals from the background, quantification of mass and size, as well as particle number concentrations. Important aspects, advantages, drawbacks, and challenges associated with this new method will be discussed.

Tuesday, October 10, 2023
Oral Presentations

23AES05: Early Career, Southern Pacific F
Chair: Blanca Lapizco-Encinas
Co-Chair: Zach Gagnon

On-Chip Multichannel Cytometry For Phenotypic Monitoring Of Microfluidic Separations

(AES-05.1)**Karina Torres-Castro**, Javad Jarmoshti, Carlos Honrado, Nathan S. Swami *University of Virginia*

Cell separation and sensing (cytometry) are key operations to obtain and quantify different cell subpopulations. For instance, macrophages, a heterogeneous population, show different activation levels during an immune response to injury. These activation levels could exacerbate nerve damage, thus increasing pain in patients. Obtaining these samples could enable biologists to study their immune response and effects on human tissue. To achieve this, we integrated a Deterministic Lateral Displacement (DLD) method with on-chip multichannel impedance cytometry for separating and assessing different macrophage activation levels phenotypes. DLD is a passive technique with moderate cell throughput that exploits cell interactions with post arrays. These post arrays are designed to have a particle size cut-off. Depending on flow conditions, they can also sort by other characteristics, such as deformability or cell positioning along the z-axis. We designed a DLD device to enrich activated macrophages from a custom-made heterogeneous sample (75% max. of activated macrophages) to show the potential of these integrated systems for reducing biased sample loss and

dilution caused by traditional off-chip analyses. We first matched cell/particle flow rates from the DLD separation section into a confined single-cell impedance cytometry stage. We then balanced flow resistance across the separation array, and finally developed our own calibration protocol. This was done by co-flowing polystyrene beads that served as internal standards for in-line assessment of the DLD separation and impedance data normalization. Our results show that the size distributions from on-chip impedance cytometry were comparable with the numbers reported from off-chip cytometry. However, off-chip cytometry showed an apparent biased sample loss due to the inability to effectively collect the fraction of smaller-sized cells from the device outlet, as opposed to our method. Finally, we obtained 72% of cells in the displaced fraction after DLD separation, which is very close to the expected maximum of the 75% of treated cells. Multiparametric on-chip impedance analysis confirmed that the displaced fractions were downshifted in impedance phase levels versus the input sample, indicating enrichment of activated macrophages at the collection outlets.

Exploring The Use Of Low-Frequency Alternating Current Potentials In Insulator-Based Electrokinetic Separations

(AES-05.2)**Nuzhet Nihaar Nasir Ahamed**, Carlos A. Mendiola-Escobedo, Olivia D. Ernst, Victor H. Perez-Gonzalez, Blanca H. Lapizco-Encinas *Microscale Bioseparations Laboratory and Biomedical Engineering Department, Rochester Institute of Technology, School of Engineering and Sciences, Tecnologico de Monterrey, Monterrey, Nuevo Leon 64849, Mexico,Microscale Bioseparations Laboratory and Biomedical Engineering Department, Rochester Institute of Technology, 160 Lomb, School of Engineering and Sciences, Tecnologico de Monterrey, Monterrey, Nuevo Leon 64849, Mexico, Rochester Institute of Technology*

There is an immediate need for rapid and reliable methods for manipulation, separation, concentration, and isolation of target bioparticles; ranging from microparticles to microorganisms. Microscale electrokinetic (EK) methods offer great potential for analysis of bioparticles due to their robustness, rapid response time, low cost, and label-free analysis. Miniaturized EK systems have proven to be effective platform for bioparticle analyses by exploiting the differences between the electric properties of particles and suspending solution. The effect of application of low-frequency alternating current (AC) voltages to separate microparticles with similar characteristics is still unknown. Separating microparticles and cells of similar size, shape, and electrical charge by employing low-frequency AC voltages is an interesting research field as it provides extra parameters like frequency, peak amplitude and DC bias, which can be explored. The present study combines modeling and experimentation to separate a binary mixture of microparticles of the same size (5.1 μm), shape, and substrate material, but with a difference in particle zeta potentials of ~14 mV, by applying low-frequency AC voltages in an insulator-based-EK (iEK) system. Four distinct separations were carried out to systematically study the effect of fine-tuning three characteristics of the applied voltage: frequency, amplitude, and DC bias. The results indicate that fine-tuning each parameter improved the separation from an initial separation resolution of Rs=0.5, to a final resolution of Rs=3.1 of the fully fine-tuned separation. The separation results had good reproducibility for experimental retention time with variations ranging from 6 to 26% between repetitions. The present study demonstrates the potential to extend the limits of iEK systems coupled with carefully fine-tuned AC voltages to perform discriminatory micron-sized particle separations.

Exploring the Insulating Properties of Paper Fibers for Enhanced Electrokinetic Applications in Sample Purification and Liquid Handling

(AES-05.3)**Md Nazibul Islam ,***Texas A&M University*

Over the past two decades paper-based microfluidics has emerged as a cost-effective alternative to traditional PDMS-based microfluidics. The affordability, user-friendliness, biocompatibility, and wide-spread use of paper have made it an attractive option for developing lateral-flow-assay-based devices. In this talk, I aim to explore the insulating properties of paper fibers and their potential to be utilized in developing innovative paper-based electrokinetic applications. We use polymeric laminated non-woven fiberglass paper channels as a source of insulating structures to demonstrate

dielectrophoretic trapping and separation of particles, cells, and nucleic acids. Moreover, we illustrate trapping of DNA from highly conductive mediums and efficient electroporation of E. coli, demonstrating the suitability of paper-based microfluidics for a range of electrokinetic applications. We apply an AC electric field across a paper channel to exhibit insulator-based dielectrophoresis (iDEP) by trapping and separating two different polystyrene particles based on their crossover frequency. In order to a develop point-of-care DEP system, we then demonstrate DC-iDEP by applying a 10V DC field to trap particles, E. coli, and DNA. We have successfully employed this technique to trap DNA from highly conductive fluid media (~15 mS/cm) which is similar to the conductivity of biofluids such as whole blood. In addition, we demonstrate continuous electroporation of E. coli at a lower electric field than what is required for a conventional electroporation device. By utilizing micro computed tomography and finite element analysis, we present a computational model to examine the micro-scale DEP force formation dynamics within a nonwoven structure. We further develop a concept of a "Paper Unit Cell" by employing a micrograph of paper to create a three-dimensional model of a small volume of the paper structure. Using this unit cell model, we demonstrate the electric field amplification effect generated by paper fibers. This new platform enables the development of robust, low-cost, and portable next generation electrokinetic systems for a wide variety of sample purification and liquid handling applications.

A Numerical Approach to Understanding the Effect of Impedance on High-Frequency Dielectrophoresis Applications

(AES-05.4)**Josie Duncan**, *Virginia Tech*

Dielectrophoresis (DEP), a label-free electrokinetic technique, has long been used for characterization, detection, and separation of biological particles such as blood, cancer, and stem cells at low frequencies (<10MHz). High-frequency dielectrophoresis (HF-DEP) (>10MHz) can provide enhanced insight into the intracellular properties of cells corresponding to their biophysical differences. Drag and DEP forces can be coupled to exploit unique characteristics within a heterogeneous sample for detection and separation. When this balance is disturbed or poorly understood, the experimental results can be significantly impacted or misinterpreted. The effects of high frequency on experimental outcome must be studied to preserve the force balance and fully harness the benefits of HF-DEP.

With constant supplied voltage, experimental data consistently displayed decreasing cell trapping at frequencies above 40MHz. To understand this phenomenon, we explore the effect of impedance of HF-DEP on experimental results and device performance using numerical methods. The results yielded the effect of device geometry, material, and supplied signal on the impedance and on performance of the device. Appropriate understanding of the variation between the supplied signal and the signal experienced by the particle of interest will enable more accurate characterization and exploitation of the technology for sensitive separations.

Deep eutectic solvent-based separations for amino acid analysis via CE

(AES-05.5)**Jessica Torres**, Christopher Harrison, Karen Campos, *San Diego State University*

Deep eutectic solvents are a binary mixture of a hydrogen bond donor (HBD) and hydrogen bond acceptor (HBA). Many established DES have HBA and HBD components that are naturally occurring products, such as ethylene glycol and choline chloride. These solvents have unique properties such as exhibiting low volatility, high conductivity, and low toxicity. These solvents are of particular interest to our research efforts due to their potential adaptability towards future space missions and the search of evidence of past life.

CE-LIF has been widely viewed as an ideal method for the identification of biosignatures of life, including amino acids, and can achieve ppb sensitivity while separating analytes via their charge to size ratio. While amino acid detection has been well established, the application of DES in CE has yet to be fully explored. One of the most readily used DES is a 2:1 molar ratio mixture of ethylene glycol

choline chloride DES, however this solvent is not viable for CE separations due to its high conductivity from the excess chloride.

For this reason, we have elected to use an analog to choline chloride: dimethylaminoethanol. Currently, we have employed a 2:1 molar ratio of ethylene glycol dimethylaminoethanol (EGDMAE) DES as a separation buffer for small molecules and fluorescently labelled amino acids. Our preliminary separations using this DES based media shows promise of a new future of non-aqueous separations buffers in the field of separation sciences. We will present our optimizations of the separation parameters for conducting electrophoretic efficiently in DES with a specific focus on the variables that give rise to electroosmotic flow within these solvents. We are also exploring how additives, or even specifically tailored DES can allow for enantiomeric separations in these non-aqueous buffers.

23ATOM01: ICP Time of Flight MS, Central Pacific A/B/C
Chair: Alexander Gundlach-Graham

(ATOM-01.1)**Capabilities Of a Downwards Pointing ICP-TOFMS For Nanoparticle and Cell Characterization**

Detlef Günther, Sandro Fazzolari, Guanghui Niu, Thomas Vonderach, Alexander Gundlach-Graham, *Department Of Chemistry And Applied Biosciences Iowa State University,*

The inductively coupled plasma time-of-flight mass spectrometry (ICP-TOFMS) is widely used for the analysis of single particles, polymers and cells due to its wide linear dynamic range, low limit of detection (LOD), high sensitivity and the simultaneous detection of elements in a single entity. The transport efficiencies reported so far differ in dependence on the sample introduction system used. Significant advantages have been reported for micro droplet generators, where 100 % sample transport efficiency can be reached. Even such a system allows only 400 droplets/s when sizes larger than 50 micron are transported. However, a transport efficiency of 100% has already been reported for cells with average sizes of 3-4 µm but not for larger cells yet [1-2].
To overcome this limitation, a downward-pointing vertical ICP-TOFMS has been developed in our group, which allows to achieve 100% transport efficiency regardless of the sample's mass, size and shape due to the gravitational-assisted sample introduction. Recent studies have already shown that microparticles and cells (PMBC, mouse spleen cell and CHO) can be successfully analyzed using microdroplet generator systems (MDG) into the ICP [3-4]. Furthermore, the vertical oriented ICP allows to introduce samples at a higher throughput of up to 1000 Hz which extends the capabilities of a horizontal plasma by 2.5 [3].
The downward-pointing vertical ICP-TOFMS has been recently used for the analysis of glass microspheres and different algae cells and various sizes of Au NP were quantified and some results will be reported.
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[4] Vonderach T., Günther D., JAAS, 2021, 36, 2617-2630

(ATOM-01.2)**The Origins of Noisy Single-Particle ICP-TOFMS Data and How to Use It**

Alexander Gundlach-Graham, Raven Buckman, Hark Karkee, Sarah Szakas, *Iowa State University*

Single-particle inductively plasma time-of-flight mass spectrometry (spICP-TOFMS) is used to analyze mixtures of nano- and micro-particles from a wide range of sample types. With spICP-TOFMS, researchers aim to classify anthropogenic particle fractions based on multi-element signatures and to record the particle-mass (i.e. size) distributions and number concentrations of diverse particle types. In general, particle types are classified based on the multi-element composition and/or

element ratios measured in individual particles. However, to develop robust classification approaches, a rigorous analysis of sources of variance that may impact classification accuracy is required.

In this presentation, we will consider the impact of three sources of signal variance in spICP-TOFMS measurements: particle size distributions, mass fraction variability, and Poisson detection statistics. We will explore how these sources of variance impact the detectability of elemental signals in individual particles and the accuracy of classification models to discriminate between natural and anthropogenic particle types. To study the impact of noise sources on spICP-TOFMS data, we developed a Monte Carlo simulation that combines the multiple sources of variance typical of an spICP-TOFMS measurement. Comparison of the simulation results with real data shows an excellent match, and thus indicates that Monte Carlo simulations are a promising platform to explore the significance of noise sources on spICP-TOFMS measurements. Details of the Monte Carlo simulation approach and our current understanding of the most prominent causes of signal variability in spICP-TOFMS data will be presented.

(ATOM-01.3)**Expanding the Elemental Coverage by Combining LIBS with ICP-TOF-MS for High-Speed Imaging**

C. Derrick Quarles, Benjamin Manard, Hunter Andrews, Tyler L. Spano, Joseph A. Petrus, Cole R. Hexel, *Elemental Scientific Inc., Oak Ridge National Laboratory*

The use of laser ablation-inductively coupled plasma-time of flight-mass spectrometry (LA-ICP-TOF-MS) has become an intriguing option for the analysis of materials due to the ability to capture the entire mass spectrum with each laser pulse. However, a few hurdles still exist: the speed of analysis must be precisely synced with the laser ablation systemS, the difficulty of measuring light elements with a TOF, and the data treatment of very large data sets when performing large mapping experiments, to name a few. The work presented here will discuss some of these hurdles while providing pathways to address them.

The speed of analysis, including the syncing of the LA systemS (TOF sync) will be displayed using a two-volume laser ablation chamber (TwoVol3) in combination with a dual concentric injector (DCI) to provide ultra-fast washouts with speeds up to 1,000 Hz (in this example the max laser repetition rate was set to 200 Hz). To address the difficulty of detecting light elements, laser-induced breakdown spectroscopy (LIBS) is coupled to the LA systemS to provide coverage to elements such as Li, Be, C, Na, Mg, K, and Ca that may be difficult or impossible with the TOF-MS. LIBS also provides the unique ability to measure H, N, O, and F at the same time when the LA chamber is purged with a inert gas, which is the case when performing LA experiments (e.g. helium is almost always used). Lastly, the entire data processing of the TOF and LIBS data will be conducted in iolite V4, providing a complete, single software platform capable of handling large data sets.

(ATOM-01.4)**Automated Elemental and Isotopic Analysis of Particles with Single-Particle Inductively Coupled Plasma-Time of Flight-Mass Spectrometry**

Veronica Bradley, Benjamin Manard, Brian Sanders, Amber Webb, Lyndsey Hendriks, Hunter Andrews
Oak Ridge National Laboratory, TOFWERK,

Single particle inductively coupled plasma mass spectrometry (sp-ICP-MS) is a unique sampling approach that allows for direct differentiation of individual particles. Isotopic characterization of particles via sp-ICP-MS is of interest to a diverse range of fields, including environmental, nuclear, and biological science. Traditional isotopic characterization with ICP-MS involves a bulk digestion, which homogenizes the sample and obscures any compositional differences between individual particles, and therefore cannot determine the binding efficiency of particles or differentiate between bound ions of interest and dissolved ions. In general, single detector, quadrupole-based ICP-MS instruments have inherently slow detection schemes and cannot measure multiple isotopes for a single nanoparticle. Time of flight (TOF)–based ICP-MS instruments have ultra-fast acquisition times (μs)

and the ability to measure all masses quasi-simultaneously, allowing for the detection of all isotopes present in a single particle. Specialized nebulizers and spray chambers can be used to increase nanoparticle transport efficiency. In an endeavor to increase sample throughput, a long-term stability study was conducted with Au and Ag nanoparticles for an autosampler with sample-mixing capability, which is useful for keeping particles suspended during a long analysis. This single particle (sp)-ICP-TOF-MS method can be utilized for applications in which understanding the isotopic composition of a single particle is important. One such application is in critical material recovery using functionalized magnetic particles, in which the concurrence of a critical material, such as Pt, and the Fe from the particle, provides information on binding capabilities.

(ATOM-01.5)**Accurate Classification And Quantification Of Engineered And Natural Titanium Submicron Particles Using Single-particle ICP-TOFMS**

Hark Karkee, Alexander Gundlach-Graham *Iowa State university,*

The environment contains titanium (Ti) particles from both natural sources and anthropogenic input of titanium dioxide (TiO_2) particles. For example, anthropogenic TiO_2 particles are widely used in food, cosmetics, paint, plastic, and solar energy industries. These engineered TiO_2 particles (Ti-EPs) can enter environmental compartments like the atmosphere, water, or soil via urban run-off, sewage spills, or wastewater treatment plants. Deposition of these Ti-EPs is a significant concern due to their adverse effects, including health hazards to humans.
Here, we investigate the use of single-particle inductively coupled plasma time-of-flight mass spectrometry (spICP-TOFMS) to measure individual Ti-containing particles and classify them as engineered or naturally occurring particles (NPs) based on multi-element signatures. In general, TiO_2 EPs are purer than Ti-NPs and so can be classified based on the lack of minor or trace elements. However, when particles are small, it is more challenging (or impossible) to measure minor and/or trace elements at the single-particle level. For small Ti-NPs, titanium may be measured without minor and trace element signatures, and thus appear to have the same Ti-only signature as TiO_2 EPs. To prevent the misclassification of small Ti-NPs as Ti-EPs, we developed particle-type detection limits that define the minimum Ti signal required to also detect a measurable secondary element (e.g., niobium) in Ti-NPs. This particle-type detection limit establishes a threshold to identify true single-element Ti-EP signals.
We report results from the analysis of mixtures of four Ti-containing particle types: anthropogenic food-grade TiO_2 particles (Ti-EPs) and rutile, ilmenite, and biotite nano-mineral particles (Ti-NPs). Through the characterization of neat particle suspensions, we developed a decision-tree-based classification scheme to classify Ti-EPs and Ti-NPs based on their elemental compositions and mass ratios. Our approach accurately classifies 54% of the Ti-EPs, 34% of rutile NPs, and 74% of each ilmenite and biotite NPs, with Ti-EP false classification rates below 5%. These low false classification rates are essential for quantification of Ti-EPs in real samples. We demonstrate classification of Ti-EPs in presence of Ti-NPs by spiking Ti-EPs into mixtures of Ti-NPs and environmental samples.

23AWD03: Ellis R. Lippincott Award Symposium Honoring Peter Griffiths, Sierra 5
Chair: Peter Griffiths

(AWD-03.1)**Fourier transform spectroscopy and the evolution of infrared imaging: From theory to fast, sensitive measurements**

Rohit Bhargava *University of Illinois at Urbana-Champaign*

Fourier transform infrared (FT-IR) spectroscopy has provided a remarkable level of sensitivity, precision and accuracy for molecular vibrational absorbance measurements. These advantages have allowed for a number of extensions of FT-IR spectroscopy in measuring complex samples. Among the enabling technologies arising from these advantages has been FT-IR spectroscopic microscopy. Here, we provide a brief overview of the key considerations in making spatially-resolved measurements and examples of applications. We present these developments in the context of a complete theoretical understanding for IR microscopy that reveals several interesting results. First, measurements of spatially varying molecular species are convolved with the morphology of the sample that may lead to

complexities in spectral interpretation compared to bulk isotropic samples. Second, the quality of images obtained from absorbance can greatly outperform spectrally-insensitive optical imaging and present new opportunities to exceed long-held beliefs on the potential of microscopy. While FT-IR imaging allows for fundamental studies and examination of light-sample interactions, a translation to practical instrumentation can occur using emerging laser sources. We discuss the evolution of laser-based IR imaging systems, in which physical, optical and acoustic sensing can be used to measure changes in specific properties of the sample upon IR absorption. Finally, we show how these developments lead to fast data recording that can have practical value in fields from polymer to biomedical analyses. We also show how a combination of theoretical understanding and novel optical configurations can allow even more detailed molecular information than previously available. The combination of fast recording of specific spectral features and artificial intelligence can lead to the thorough examination of tissue and disease progression. Here we show how IR imaging measurements of the tumor and it microenvironment can be combined to enhance our ability to prioritize clinical interventions. The author gratefully acknowledges the inspiration and education provided by Prof. Peter Griffiths' that led to his own development as a scientist.

(AWD-03.2)**ATR-FTIR Spectroscopic Imaging for the Analysis of Biopharmaceuticals**

Sergei Kazarian *, Imperial College London*

Sergei G Kazarian
Department of Chemical Engineering, Imperial College London, UK
Email: s.kazarian@imperial.ac.uk

Many new opportunities exist for studying dynamic systems with macro ATR-FTIR spectroscopic imaging. The novel applications of ATR-FTIR imaging included studies of tablet dissolution and drug release, chemical imaging of biomedical samples (live cells and tissues), high-throughput analysis of many samples, developing new forensic approaches, analysis of materials of cultural heritage and imaging of products and processes. In general, our research is focused at the interface between physical chemistry, chemical engineering and materials science where spectroscopic imaging approaches allow us to solve problems of significant industrial and scientific interest by discovering and understanding molecular interactions and using them to engineer new products and processes. Biopharmaceuticals such as therapeutic monoclonal antibodies (mAbs) have shown to be effective drugs for the treatment of a range of diseases, including various types of cancer (1). Aggregation of proteins and in particular biopharmaceuticals results in a loss of time and money to industry. It is essential that any biopharmaceutical is monitored for purity and stability throughout the production processes. Therefore, recently the combination of macro ATR-FTIR spectroscopic imaging with specifically designed devices was used for the monitoring of conformational changes in biopharmaceuticals, including variable angle of incidence accessories that enabled us to investigate the structure and distribution of aggregates close to the surface of the IRE and observe how this distribution changes under stress conditions. The purpose of this research is to understand protein behaviour in static and flowing environments, under a range of stress conditions. Results show interesting behaviours of proteins, particularly IgG monoclonal antibodies, under stress conditions, and the applicability of ATR-FTIR spectroscopy to successfully monitor these proteins in static and flowing set-ups. This research also aims to demonstrate the suitability of FTIR spectroscopic imaging for biopharmaceutical process monitoring.

(1) Tiernan H., Byrne B., Kazarian S. G. ATR-FTIR spectroscopy and spectroscopic imaging for the analysis of biopharmaceuticals.Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy (2020) 241, 118636

(AWD-03.3)**Infrared determination of total particulate mass in mine dust samples: Are cluster models worth the trouble?**

Andrew Weakley, David Parks, Arthur Miller, *Weakley Consulting, National Institute of Occupational Safety and Health,*

Total permissible exposure to coal mine dust has been recommended by the Mine Safety and Health Association to not exceed 1.5 mg/m³ to reduce the risk of developing cardiovascular disease. It is not unreasonable to expect that similar exposure limits may apply to non-coal mines in the future, particularly those abundant in silica-rich geologies.
Determining total sample mass collected on a suitable filter using infrared calibrations, developed from field samples, poses a number of challenges. The simplest way to address most of them is to develop several calibrations from analytical standards or to generate one calibration per active mine if field "standards" are favored. However, such an approach becomes prohibitively expensive as the number of calibrations becomes large, and ultimately fails to consider that field samples from several mines of similar type (e.g., coal, silver) often cluster in terms of their infrared absorption.
Mixture of Expert (MoE) finite mixture models provide an intuitive approach to exploiting any clustering behavior present in the infrared spectra used to quantify total mass. The MoE model tested was trained and validated using paired gravimetric mass measurements from coal, limestone, silica, and silver mines. Scores from a full-wavenumber partial least-squares (PLS) calibration were used as the so-called "expert" (continuous) covariates and "mine type" was used as the "gate" (categorical) covariate. The resulting two-cluster MoE model was compared to the PLS calibration's test sample predictions. An overall reduction in test-set median bias was observed. Grouping samples according to mine type and then calculating median bias revealed that accuracy improved across all mine types, with the exception of silver mine samples.
One final analysis compared the MoE and PLS model to an elastic net (EN) regression, as the latter performs wavenumber selection and calibration simultaneously. The EN method predicted test samples with reduced uncertainty in estimated median bias as quantified by the width of confidence intervals. Overall, this study suggests that finite mixture models improve the quantification of mine dust mass relative to a naïve PLS approach but one should still consider wavenumber selection as a prudent, additional option to minimize uncertainties in performance metrics.

(AWD-03.4)**Quo vadis in process Raman spectroscopy**

Ian Lewis, Maryann Cuellar, Justin Moretto, Karen Esmonde-White, Scott Sutherland, Shajeel Haider, Sean Gilliam, Randy Benedict, Joel Patrow, David Strachan, Herve Lucas, Carsten Uerpmann, *Endress+Hauser Optical Analysis, Endress+Hauser, Endress+Hauser Process Analysis Support, SARL,*

As the field of Process Raman spectroscopy enters its fifth decade, we reflect on the technological, industrial, and scientific milestones that have moved Raman into the forefront as a process analytical technology (PAT). The inception of process Raman spectroscopy in the early 1990's was borne out of need. At the time, inline Raman was a PAT of last-resort when existing FT-IR, FT-Raman, or lab analyses could not work. These early applications relied heavily on bespoke hardware and a high level of spectroscopy expertise which demonstrated a need for high instrument performance that could reliably perform in the field with less spectroscopy expertise. As Raman's value becomes known across many industries to non-specialist, we see a new era in process Raman where non-spectroscopy parameters drive new demands on hardware performance, ease of use, and integration. Raman spectroscopy in a process or manufacturing environment required an integrated approach, with careful attention to the reliability of the analyzer and sampling probe optics and transferability of the data analysis method. Some manufacturing environments have additional environmental or regulatory requirements, which also impacted our technology development and product manufacturing. We broadly discuss these requirements in the context of customer applications and provide examples that illustrate the challenges, successes and benefits of Raman spectroscopy in the process and manufacturing environments.

(AWD-03.5)**Sampling for Field Studies: A Mixed Bag**

James de Haseth, Franklin Barton, *Infrared & Raman Courses, Inc., LLS Instruments, Inc*

Near infrared spectrometry, coupled with chemometrics, is a valuable tool for the evaluation of commodities in food and agriculture industries. Any such study or process requires careful planning. Can the commodity be measured by NIR spectrometry? If so, what resolution and spectrometer bandpass is needed? What is the morphology of the commodity and how many measurements need to be taken to characterize a sample? What is the needed precision to evaluate the commodity? Once those questions have been answered attention must turn to the standards and the independent analysis methodologies. Ideally, the selection of standard samples must be made to reflect the expected precision of the study, and the range of commodity values. In addition, appropriate analyses of the standards must be undertaken to assure reliability. The standard samples must reflect the physical condition of the field samples. All too often, the standard methods do not meet the study criteria. Standard analyses can be expensive and precision is sacrificed. The samples collected for standards may not reflect in situ samples as the standards may have degraded when they are finally analyzed. Even if all the criteria are met, sometimes the client will not accept the evaluation of the commodity by NIR spectrometric analyses. The results may be rejected for economic or even political reasons. Basically, some clients do not want accurate analyses. This leads us to have to consider problems outside the realm of chemistry and spectroscopy.

23BIM04: Celebrating Early Career Scientists in Biomedical and Bioanalytical Spectroscopy, Sierra 2
Chair: Fay Nicolson

(BIM-04.1)**Spatially Offset Raman Spectroscopy Of Biological Tissue – What Can We Get Out Of It?**

Sara Mosca, Megha Mehta, William Skinner, Benjamin Gardner, Francesca Palombo, Nick Stone, Pavel Matousek *Ral, Stfc, Ukri, University Of Exeter, Rutherford Appleton Laboratory*

Spatially Offset Raman Spectroscopy (SORS) is a promising tool for in vivo medical diagnosis[1]. In a clinical context, it is beneficial to identify physical-chemical information (e.g. optical properties, chemical fingerprints) and the depth of a buried object (e.g. a lesion) in biological tissues. SORS allows the non-invasive chemical characterisation of biological tissues at depths of up to two orders of magnitude greater than conventional Raman spectroscopy.
Here we describe how it is possible to estimate the depth of inclusion within turbid media (e.g. biological tissues)[2], the optical properties of the tissue matrix[3] and the mean depth probed by using SORS[4]. Monte Carlo simulations of photon propagation were used to gain an insight into the relationship between the spatial offset and the photon path lengths inside different tissues (i.e. with different optical properties), enabling one to derive the relationship between Raman intensity decay with increasing spatial offset as a function of the reduced scattering coefficient. New approaches will be presented for estimating the depth of a target and the reduced scattering coefficient along with the validation experiments performed using surface-enhanced Raman scattering (SERS) labelled nanoparticles (NPs) acting as an inclusion inside ex vivo porcine tissue. The relative error of the estimated values were 7%[2] and 15%[3] for the depth and the reduced scattering coefficient, respectively.

These results pave the way for future wider applications of non-invasive deep Raman spectroscopy in vivo by enabling, for example, the localisation of cancer lesions or biomarkers for early-stage diagnosis and targeted treatment.

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(BIM-04.2)**Harnessing DNA Hairpin Assemblies for Enhanced Signal Amplification in Point-of-Care Diagnostics**

Samuel Mabbott,*Texas A&M University*

In the rapidly evolving field of point-of-care (POC) diagnostics, it is crucial to develop methods for sensitive and specific biomarker detection that can be adopted in resource-constrained areas. Our group's research focuses on the utilization of DNA hairpin assemblies, exploring their potential to enhance detectable signals in the presence of specific biomarkers.

Our approach exploits the unique properties of DNA hairpin structures. These structures exhibit dynamic behavior that can be harnessed to create intricate, self-assembled formations when interacting with specific targets. This interaction is facilitated by the process of toe-hold induced hairpin opening, a spontaneous mechanism enabling enzyme-free, isothermal amplification of DNA. The strategic design of these DNA sequences, distinguished by short single-stranded regions known as "toe-holds", facilitates hairpin unfolding. In essence, this acts as a biomarker-sensitive switch, triggering an amplification event without the need for enzymatic intervention or fluctuating thermal conditions. This intuitive process amplifies the measurable signal providing a means for visual or spectroscopic detection - a crucial attribute for POC diagnostics.

In my presentation, I will elaborate on our work regarding the synthesis of spontaneous hairpin assemblies for the highly sensitive POC detection of biomarkers associated with myocardial infarction and preeclampsia. Our methodologies leverage the potential of colorimetry and surface-enhanced Raman scattering (SERS) for detection, maximizing the sensitivity and specificity of our assays. This integrative approach allows an exceptional level of sensitivity in biomarker detection, greatly enhancing the effectiveness and efficiency of POC diagnostics.

(BIM-04.3)**Raman spectroscopy for culture-free bacteria detection**

Andrea Locke, Anna Rourke-Funderburg, Timothy Yokley, *Vanderbilt University Biophotonics Center, Vanderbilt University*

With the increasing prevalence of infectious diseases, the need for faster and improved diagnostic technologies at the point of care is on the rise. The gold-standard methodologies of micro-organism culturing and polymerase chain reaction (PCR) are hindered by their complexity and turn-around time for applications at the point of care. Thus, there is a lack of highly sensitive and specific, easy-to-use diagnostic technologies for culture-free detection of disease-mediated pathogens. Raman spectroscopy has gained popularity in addressing this need. Raman spectroscopy is a label-free, non-destructive analytical technique that provides a unique spectral fingerprint representative of the biochemical components of a specimen. For dilute samples, particularly pathogens in biological fluids, conventional Raman can be combined with nanoplasmonic structures to generate a surface-enhanced Raman spectrum (SERS). SERS positions analytes near roughened metallic surfaces to produce spectral enhancements of 10^8 to 10^{14} compared to the conventional Raman scatter. Our research group utilizes this promising analytical tool for the characterization of various bacterial pathogens such as Mycobacterium spp., Lactobacilli spp., Streptococcus spp., and Haemophilus spp., amongst others. Multivariant linear regression analysis is then used to differentiate these species. In conditions of low pathogen counts, SERS can be coupled with the coffee-ring effect to isolate and concentrate pathogens prior to Raman characterization. Coffee-ring-SERS represents the next step toward culture-free pathogen characterization for point-of-care detection.

(BIM-04.4)**Improving Colorectal Cancer early detection with the CanSense-CRC Blood Test**

Cerys Mitchell, Charles Brilliant, Nafiseh Badiei, Nerissa Thomas, Freya Woods, Peter Dunstan, Dean Harris *Cansense Limited, Swansea Universit*

The majority of colorectal cancers (CRC) are diagnosed through primary care after investigation of suspicious symptoms and/or using faecal testing (FIT)[1]. The inclusion of FIT testing to the NICE

pathway has shown improved detection rates from 3% to 16% [2]. However, the clinical features associated with CRC still have poor specificity resulting in large numbers of patients having endoscopy procedures (e.g. colonoscopy) or CT scans to rule out cancer. There are currently thousands of patients awaiting endoscopy tests in the UK with resources unable to keep pace with test demand. There is an urgent need for triage tests to release pressure on diagnostic services by helping to identify those at highest risk of having cancer.

Here, we present clinical results from the prototype CanSense-CRC blood test. CanSense-CRC uses machine learning trained on Raman spectral features collected on serum samples from people with and without CRC (n=300). To validate the test a total of 532 patients aged ≥50 years referred on the USC pathway were recruited from 27 UK primary care practices. Twenty-nine patients (5.0%) were diagnosed with CRC. Raman-CRC identified CRC with sensitivity 95.7%, specificity 69.3% with area under curve (AUC) of 0.80 compared with colonoscopy as the reference test (248 patients) [3]. Stage I and II (early) cancers were detected with 78.6% sensitivity.

Patient/public/ethnic minority and professional engagement work overwhelmingly supports the potential impact of the CanSense-CRC test in primary and secondary care. With 98.5% patient acceptability, and a modelled 65% reduction in those needing to go for colonoscopies CanSense-CRC could become a cost effective and accessible solution for the earlier detection of CRC.

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(BIM-04.5)**New Approaches to Process Analytical Technologies in the Monitoring, Optimization and Scale-Up of Flow Chemical Processes**

Ashley Love , University Of Nottingham

The ability to monitor and optimise reactions in real-time is of upmost importance, particularly in the manufacturing processes of active pharmaceutical agents and essential medicines. Monitoring is usually achieved through the use of process analytical technologies (PAT), which can take the form of off-line, at-line, on-line and in-line approaches. However, by combining in-line optical spectroscopies with flow chemistry, the ability to rapidly monitor and autonomously control reactions becomes possible, allowing efficient, algorithmically driven process control and optimization to take place. This work utilises an array of optical spectroscopic techniques to monitor reactions in real-time and autonomously optimize and probe key reaction parameters (such as yield and selectivity), as well as providing insight into molecular properties, configurations and reaction mechanisms of flow processes. This is achieved by combining vibrational and electronic spectroscopies, including A-TEEM spectroscopy, a new tool for PAT.
In A-TEEM spectroscopy, the excitation emission matrix acts a “molecular fingerprint”, allowing for species which exhibit similar absorption and emission characteristics to be easily identified. This can be performed rapidly and with a high sensitivity, making it an indispensable approach to process monitoring and molecular spectroscopy, complimenting the other optical techniques used.

23IR01: Fluoresence and EEMS: Exploiting A New Approach for PAT and Monitoring, Sierra 3
Chair: Alan Ryder
Co-Chair: Ashley Love

(IR-01.1)**Polarized Excitation Emission Matrix (pEEM) spectroscopy: another dimension for protein analysis.**

Alan Ryder ,*Nanoscale Biophotonics Laboratory, University Of Galway.*

The enhancement of excitation-emission matrix (EEM) fluorescence spectroscopy by the addition of polarization information offers some significant advantages for protein analysis in a variety of environments. Polarized excitation-emission matrix (pEEM) measurements are relatively easily implemented in standard bench-top spectrometers. If deep UV transparent wire grid polarizers are used then this enables the measurement of intrinsic protein emission and thus provides a new suite of label free analysis/measurement methods.
Polarization provides two distinct benefits, first the use of polarizers enables one to selectively enhance the spectra to be more or less sensitive to the presence of particles in samples, by measuring the parallel (EEM∥) or perpendicularly(EEM⊥) polarized spectra. Thus EEM∥ measurements have obvious benefits for the analysis of samples where one needs to identify the presence of particles or aggregation. EEM⊥ measurements are useful in turbid samples as the effect of light scatter can be minimized producing clearer, truer fluorescence emission signals. In addition, one can also use the Rayleigh scattered light signal obtained from the pEEM measurements to provide more detail about presence and size of particles in biogenic samples. Here we show how pEEM has been successfully used for the quantitative analysis of simulated [2] and real clarified bioprocess broth liquids which have a complex sample matrix.
The second benefit is that the pEEM data can provide anisotropy information enabling the extraction of information about protein mobility and local viscosity, which can be used for quality control analysis of proteins [2] in relatively clean sample matrices, such as that found in downstream purification operations.

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(IR-01.2)**SCALABLE CONTINUOUS PHOTOCHEMICAL & ELECTROCHEMICAL REACTIONS: REACTORS AND PAT CHALLENGES: FROM PICOSECONDS TO TONNES.**

Ashley Love, *University Of Nottingham.*

Photochemistry and electrochemistry are potentially very powerful tools for manufacturing not least because energy is delivered to reacting molecules far more selectively than by bulk heating in an atom efficient manner. Indeed, more than a century ago, Ciamician, presented a very powerful vision of where photochemistry could lead us [Science 1912, 36, 385-394]. Its penetration into chemical manufacture remains comparatively modest because of a whole series of issues, mostly centred on the problems of carrying out large-scale photochemical reactions both efficiently and safely. In recent years we have been addressing some of the challenges of making photochemistry and electrochemical synthesis greener, more energy efficient and more widely accessible. This presentation will cover our activity particularly aiming for generic approaches for linking and scaling up multi-step processes in the context of photo-, electro- and thermal- chemistry on the kg/day scale with the emphasis on Process Analytical Technologies (PAT) using excitation emission matrix (EEMs) as a “molecular fingerprint”, allowing for species which exhibit similar absorption and emission characteristics to be easily identified. By combining in-line optical spectroscopies with flow chemistry, the ability to rapidly monitor and autonomously control reactions becomes possible, allowing efficient, algorithmically driven process control and optimization to take place.

(IR-01.3)**Measuring Protein-polymer nanoparticle interactions using polarized Excitation Emission Matrix (pEEM) spectroscopy.**

Matheus de Castro, Alan Ryder , *University of Galway*, *Nanoscale Biophotonics Laboratory, University Of Galway.*

The importance of monoclonal antibodies (mAbs) as therapeutic agents has increased dramatically as they can offer significant benefits over traditional small molecule drugs. The processes required for manufacturing high purity mAbs are complex and might induce protein aggregation, which can be harmful to critical quality attributes (CQAs) and ultimately drug quality, efficacy and safety. Traditionally mAb aggregation quality is measured by size exclusion chromatography (SEC), a complex, relatively expensive and time consuming step. The present work looks at how Polarized Excitation Emission Matrix (pEEM) can be used as a reliable substitute for SEC and overcome some of its constrains. pEEM is a sensitive, label free, non-destructive measurement method as it leverages from protein's inherent fluorescence yielding a 4D2 data system which includes scatter, fluorescence, absorbance and polarization information. In this work, with a shear stress (stirring) Human Serum Albumin (HSA) aggregation model, we investigate the protective effect of a thermoresponsive polymer, Poly(N-isopropylacrylamide), PNIPAm. We compare the efficacy of identifying and then quantifying the degree of aggregation by pEEM with size and aggregation measurements made with Dynamic Light Scattering (DLS) and SEC.
HSA in the absence of PNIPAm aggregates under shear stress, DLS measurements show large aggregates with particle size of roughly 1133 nm, along with higher Rayleigh light scattering intensity (1.600%) and lower fluorescence emission (18%) under VH polarization. PNIPAm containing samples under shear stress exhibited a well-preserved emission pattern and in general less scattered light, indicating some form of protective PNIPAm-HSA interactions. DLS analysis also showed a decrease in particle size as polymer ratio was increase (796.6 nm (1:1); 493.43 nm (1:10); 54.40 nm (1:100) that can be attributed to polymer-protein mixtures. Multi-dimensional fluorescence anisotropy measurements showed similar values for non-stressed HSA and HSA-PNIPAm mixtures, however a striking difference was found in samples absent of polymer, indicating size change (Ex280/Em332: 0.12 (HSA), 0.38 (Stressed HSA), 0.20 (1:1), 0.12 (1:10) and 0.13 (1:100). Future work will correlate pEEM-derived data with SEC and DLS measurements.

(IR-01.4)**Advanced deep UV Raman and fluorescence instruments for industrial process analysis and cleaning verification**

Rohit Bhartia, Ray Reid, Ken Nguyen, Quoc Nguyen, **William Hug**, *Photon Systems, Inc.,*

Introduction
Raman and fluorescence spectroscopy are becoming increasingly common analytical methods for real-time, on-line and in-line, in situ monitoring of product quality in a variety of industrial process analytical technology methods in pharmaceutical, chemical, and biological manufacturing environments. The major shortcomings of Raman spectroscopy conducted in the near UV, visible, and IR are that: 1) highly efficient fluorescence emissions from targeted and surrounding materials within the excitation volume of a sample often obscure the Raman signature of the materials of interest, and 2) Raman signal strength is diminished due to Rayleigh Law and lack of resonance effects. This is especially true of simple organic compounds and biological materials such as amino acids, proteins, peptides, and whole microbial organisms as well as a wide range of pharmaceutical ingredients. In addition, the essential and informative fluorescence features of many organic and biological materials are not excited when at wavelengths longer than 260 nm.
Method
Unless excitation occurs at wavelength less than about 250 nm, there is significant overlap between Raman and native fluorescence spectral regions from a wide array of organic and biological materials including active pharmaceutical ingredients and excipients. This overlap obscures weak Raman emissions and alters the emission spectra of fluorescence emissions due to strong CH and OH Raman bands, both of which reduce the fidelity of spectral classification. This overlap is considerably worse for excitation above 260 nm.
Raman emissions provide information about the chemical bonds within the mixtures present in the excitation volume of detection. Fluorescence emissions provide complementary information about the overall electronic configuration of the targeted material. Together, Raman and fluorescence

information more fully describe the chemical compounds of interest. Simultaneous acquisition of both forms of emissions coupled with chemometric analysis enables detection and characterization of a wide range of organic and biological material not possible when excitation occurs in the near UV, visible, or IR.
Results
We will describe new results using compact, instrumentation employing deep UV excitation to address process analytical issues where fluorescence interference with Raman is a problem and where detection of low concentration target materials are important.

23MASS02: Ion Mobility Separations: Instrumentation, Applications, and Methods, Southern Pacific A/G
Chair: Chris Chouinard

(MASS-02.1)**Clinical Applications of Advanced Ion Mobility Mass Spectrometry**

Ahmed Hamid, Orobola Olajide, Kimberly Kartowikromo, Yuyan Yi, Jingyi Zheng, *Auburn University,*

Rapid and accurate detection and discrimination of bacteria are of increasing importance, with attention arising from public health concerns, clinical diagnostics, environmental monitoring, and food safety surveillance. If left unaddressed, there is a projection of 10 million deaths per year by 2050 due to antimicrobial resistance highlighting the need for rapid unequivocal identification of microorganisms. With rapid diagnostics techniques, early treatment with accurate administration of antibiotics will lead to an increase in recovery rates, shorter hospital stays, and reduced antibiotic resistance. This presentation will demonstrate the use of high-resolution ion mobility mass spectrometry (IM-MS) separations to distinguish bacterial species and strains with 2 minutes analysis time.
Escherichia coli and Bacillus Spp. bacteria were cultured in Luria broth. Then, the analysis was done using liquid chromatography (LC) and paper spray(PS) ion mobility mass spectrometry. Ion mobility and the tandem mass spectra profile of the bacteria lipids were acquired in positive and negative ion polarity modes using LC-IM-MS/MS and PS-IM-MS/MS methods. Multivariate data analysis based on principal component analysis (PCA) followed by linear discriminant analysis (LDA) allowed discrimination of bacterial strains were then performed.
Initially, we utilized an optimized LC-IM-MS/MS platform to discriminate 5 Bacillus Spp. Despite its success, LC-IM-MS/MS requires 10-15 minutes for LC runs. Therefore, we examined the capability of PS-IM-MS and PS-MS/MS in the discrimination of the bacteria species. We optimized several parameters, such as the needed incubation time spray solvent. Surfactins were the predominant ions observed in both negative and positive ion modes. Our results showed that lower prediction rates were observed when only mass spectrometric information was used for discrimination due to the limitation of MS to distinguish isomers. With ion mobility and tandem mass spectrometry information, the prediction rates increased significantly.
Next, we examined the capability of our methods to achieve strain-level differentiation of seven E. coli strains. Using numerical data fusion of negative and positive ion PS-IM-MS/MS data resulted in classification rate of 80.5%. Upon using LC-IM-MS/MS, a prediction rate of 96.1% and 100% utilizing the negative and positive ion information, respectively could be achieved.

(MASS-02.2)**Improved Analysis of Small Molecule Drugs using High Resolution Ion Mobility-Mass Spectrometry Approaches**

Ralph Aderorho, **Christopher Chouinard ,** *Clemson University,*

The opioid crisis has reached an alarming level internationally, as recreational drugs laced with more dangerous substances such as fentanyl and xylazine have pervaded the illicit drug market. This rise is attributed to their ease of synthesis, but black market production has inevitably resulted in introduction of a myriad of structural and stereochemical analogs, which are especially important to characterize because of their wide ranging potency. Historically, tandem and high-resolution mass spectrometry coupled to chromatographic separations have provided straightforward identification and

quantification of simple substances, but in lieu of chemical standards definitive characterization remains challenging. Standalone ion mobility (IM) has also seen utility, especially because of its portability and relatively low cost. The complexity of the drug market today, however, necessitates higher level multidimensional separations, such as by coupling modern, high-resolution IM with existing LC-HRMS/MS workflows. This presentation will demonstrate our most recent work focused on identification and characterization of several isomeric classes of fentanyl and xylazine analogs. With the intent of improving separation, several novel strategies including unconventional metal ion adduction, post-processing strategies, and instrumentation advances will be discussed. Furthermore, structurally studies enabled by a combination of experimental approaches (especially IM-aligned fragmentation) and computational modeling will be shown to provide comprehensive determination of gas-phase structure; this pertains in particular to structural conformers and the presence of ionization site isomers. Finally, we will demonstrate the ability to quantify such isomers in biological samples of forensic/toxicological interest. We expect these methods to have broad applicability to the analysis of small molecule illicit drugs.

(MASS-02.3)**Fingerprinting the Unique Lipidome of Membrane Proteins Using Liquid Chromatography, Ion Mobility Spectrometry and Mass Spectrometry**

Jack Ryan, Yun Zhu, Melanie Odenkirk, Arthur Laganowsky, Erin Baker , *University Of North Carolina At Chapel Hill, Texas A&M University, University of Arizona*

Lipids are a broad group of naturally occurring molecules responsible for energy storage, signaling, and membrane formation. Due to these roles, lipids have been directly implicated in the structure and function of membrane proteins. However, the analysis of membrane protein/lipid complexes is quite complex as >50,000 lipids across 8 distinct lipid categories have been curated in LIPID MAPS. Thus, new analytical methods are greatly needed to characterize the varying structural motifs and chemical characteristics for possible lipids in these complexes. Here, we developed an analytical method for the analysis of saccharolipids as they behave quite differently than other lipid classes due to their oligosaccharide motifs. To evaluate different liquid chromatography, ion mobility spectrometry and mass spectrometry (LC-IMS-MS) conditions, a variety of saccharolipid and lipopolysaccharide (LPS) standards were purchased including Lipid A (Rd mutant), Lipid A (Ra mutant), and Kdo2-Lipid A. Due to the unique behavior of the Lipid A analogues, reversed phase, mixed mode and HILIC based LC separations were assessed for the saccharolipid separations. IMS collision cross sections (CCS) were also obtained and added to our lipid library and collision induced dissociation parameters were evaluated so all-ions fragmentation could be utilized for all detected ions. Extracted protein/lipid complexes were finally evaluated to determine if the any saccharolipids were present.

(MASS-02.4)**Real-time metabolomics of mammalian cell-based biotechnology using mass spectrometry**

Bart Pander, Luke Johnston, Tessa Moses, Sophie Bennet, Lorna Eades, Juraj Bella, Karl Burgess, *University of Edinburgh,*

Mammalian cell cultures are the most common method for producing the highest-grossing pharmaceutical products, therapeutical proteins. Mammalian cells can perform human-identical post-translational modification, which is essential for the efficacy and safety of these proteins. The production process has been optimized considerably in recent decades, but there is ample room for improvement. Real-time process analytical technologies are considered essential for further optimisation. Established technologies such as pH or Dissolved Oxygen probes often measure just one or a select few variables that are often only indirect indicators of the health of the cell culture.

We have developed a mass spectrometry-based real-time metabolomics process analytics system for mammalian cell culture medium. This system allows us to monitor 40 key metabolites at high temporal resolution. Our cultures are furthermore analysed using off-line untargeted techniques such as NMR and LC-IM-MS metabolomics, ICP-MS, and proteomics to improve our understanding of mammalian cell-based bioprocesses. We specifically aim to determine consumption and production of

several key compounds to higher resolution than previously published as well measuring more understudied but important aspects of the cell culture process. With this data we aim to improve metabolic and process modelling, and process control.

We demonstrate the potential of this by comparing the bioreactor growth of a Chinese Hamster Ovary (CHO) cell line producing the cancer therapeutic Herceptin with a genetically similar non-producing CHO-K1 cell line. We aimed to gain insight into how much of the difference in cell physiology is caused by Herceptin production. Our analysis demonstrated striking differences in the metabolism, physiology, and bioprocesses of these cell lines. Recently we have starting to exploit the use of stable isotope labelled C13 substrates, as first step towards metabolic flux analysis. This work has the potential to improve the efficiency and productivity of mammalian cell-based bioprocesses and the engineering of cell lines for this purpose.

(MASS-02.5)**Towards Portable Elemental and Isotopic Analysis with Differential Mobility Coupled to Mass Spectrometry**

Jacob Shelley, Garett MacLean, Ifeoluwa Ayodeji, Sunil Badal, Alexandra Keidel, Theresa Evans-Nguyen, *Rensselaer Polytechnic Institute, University of South Florida,*

Portable mass spectrometry (MS) requires stable, sensitive, robust, and low-power ionization sources. However, elemental analysis with MS necessitates high-energy sources to produce gaseous atomic ions from condensed-phase samples. Alternatively, clustered or chelated metal ions can be produced from lower-power ionization sources operated at atmospheric pressure. However, the open-air nature of these approaches produces significant background and isobaric interferences from ambient species that limit quantitative and isotopic analysis.
In the present study, we explore the use of a differential mobility analyzer (DMA) as compact, post-ionization filter to remove background ions and other interferants in the detection and quantification of elemental species. After DMA ion filtration, analyte ions enter a mass spectrometer for mass analysis and detection. We have tested this setup with two ionization sources and approaches to sample introduction. In one case, elemental species were detected directly with a plasma-based flowing atmospheric-pressure afterglow (FAPA) source. Online complexation reactions with ligands were used to generate volatile metal complexes during ionization. Thus far, 22 of the 29 tested elements have been detected with detection limits in the femtomole range. The FAPA-DMA-MS combination enabled improved detection limits and the ability to differentiate oxidation states of elements in mixtures.
In a second case, nano-electrospray ionization (nESI) combined with DMA-MS was used for the detection and isotopic analysis of uranium-containing solutions. The DMA effectively removed molecular isobaric interferences prior to detection on a low-resolution ion-trap mass analyzer. Isotope-ratio measurements for $^{235}U/^{238}U$ from the uranyl ion were greatly improved in both accuracy and precision. Possibilities for portable elemental and isotopic mass spectrometry with the aid of a DMA will be discussed.

23PAT03: Novel Process Analysis Technologies & Applications, Southern Pacific E
Chair: Shawn Chen

(PAT-03.1)**Real-Time Monitoring of Polymer Manufacturing Process Using Optical Spectroscopy.**
Arindom Saha, *tec5USA, Inc.*

Polymer manufacturing is one of the largest business sectors in the chemical industry. Complexity of polymerization processes has increased significantly as performance demands of polymeric materials keeps growing leading to tighter control of process parameters. Polymer process streams also introduce characteristic challenges for in-situ monitoring due to harsh process conditions, frequent reactor fouling, and unique safety hazards. Nevertheless, process analyzers involving Raman/NIR/UV-Visible spectroscopy enable manufacturers to monitor analyte concentrations in real-time thereby helping operate the processes as safely, reliably, and profitably as possible. Online Spectroscopy is a

relatively fast and reliable technique and on proper installation along with appropriate fiber optics and chemometrics can help manufacturers use spectrometers and statistical models to analyze and monitor various polymerization processes. tec5USA process spectrometers are designed to integrate seamlessly into the existing polymer manufacturing process, with customizable configurations including extended fiber optic cables for remote sampling options to fit with specific in-line monitoring needs. With minimal maintenance requirement and user-friendly software, tec5USA process spectrometers are easy-to-use analyzers that can make a big impact on the manufacturer's requirements.

(PAT-03.2)**Real-time Online Nanoparticle Size Monitoring During Wet Bead Milling With a Microdilution Device**

Nick Koumakis, Carl Schuurmans, Remy Van Tuijn, Marko Verbeek, Michiel Damen, Rut Besseling, Ad Gerich, *InProcess-LSP,*

Abstract.
Wet bead milling is used to produce ultrafine nanoparticles in a variety of industries. Continuous monitoring of particle size during this comminution step is desirable, as it facilitates real-time end-point determination and quality control, which can reducing operating costs and increase insight into particle breakage kinetics. Here, an online Process Analytical Technology (PAT) based on a microfluidic extraction device coupled to a Spatially Resolved Dynamic Light Scattering (SR-DLS) instrument is discussed. By continuously extracting 10-150 µL/min of process fluid and diluting this 10-100× before size measurement in flow, the average nanoparticle size and size distributions (range: 10-2000 nm) can be monitored during (sterile) milling of non-Newtonian suspensions with particle volume fractions up to at least 35%.

INTRODUCTION
Monitoring the progress in particle size reduction during milling of (nano)suspensions is normally done through particle size analysis. Depending on the expected final particle diameter, typically either laser diffraction (practical range ~0.5-500 µm) or Dynamic Light Scattering (DLS, practical range ~10-2000 nm) instrumentation is used. For these methods, a sample is taken from the milling process, which has to be diluted and measured.

Here, we showcase a newly developed Process Analytical Technology (PAT) that can measure average nanoparticle size and particle size distribution of a suspension in real-time during a milling process. Using this system, quality control and milling end-point determinations can be performed continuously, and highly detailed particle size over milling time/energy curves are collected, which can be used for process optimization and dispersion development.

SR-DLS AND ONLINE MICRODILUTION

The developed PAT system utilizes: (i) the Nanoflowsizer, The Nanoflowsizer is capable of measuring particle size distributions in flowing turbid samples. (ii) A microfluidic extraction and dilution device, capable of extracting and diluting ~10-150 µL/min of process fluid from the milling process and providing this to the instrument.

(PAT-03.3)**In Situ Infrared Spectroscopy of a Cementitious Material**

Mark Rickard, Kyoung moo Koh, Stephane Costeux , *DuPont,*

Magnesium oxide (MgO) boards are a relatively new building material that are strong and fire resistant. The primary component of an MgO board is magnesium oxychloride cement, which is formed by the reaction of magnesium oxide, magnesium chloride, and water. Magnesium oxychloride is a crystalline material, and there are several phases and undesired byproducts that can form during the reaction. Both infrared and Raman spectroscopy can be utilized to identify the crystalline phases and byproducts that are formed in magnesium oxychloride cement. In addition, in situ infrared spectroscopy can be used to monitor the formation kinetics of crystalline phases during the reaction.

These techniques can provide key information about optimum ratios of reactants and reaction conditions, which affect the properties of MgO boards.

(PAT-03.4)**Solid-state Raman - the ideal process analytical technology for monitoring & control of industrial fermentation processes**

Brian Marquardt, John Richmond, Thomas Dearing, *MarqMetrix Inc.,*

Raman spectroscopy is gaining more traction in compositional analysis of many industrial processes, due to the speed of measurement and the stability of the device. In the case of industrial fermentation, there is a new wave of companies manufacturing fuels and chemical building blocks from biological or industrial waste & emissions feedstock. Carbon capture technology transforms molecules such as CO & CO2 into products as diverse as jet fuel and bioproducts. Raman spectroscopy is commonly used for compositional analysis of feedstock, transformation and final product quality. This presentation will encompass measurement technology, process sampling, data analysis and real-world application data and results.

(PAT-03.5)**Fiber spectroscopy in-line for PAT by different methods in 0.3-16µm range**

Viacheslav Artyushenko, *Art Photonics Gmbh*

Multispectral fiber probes for 4 spectroscopy methods are developed and produced for in-line PAT in 0.3-16µm range. Robust fiber optic probes enable to run sampling-free remote process monitoring. Depending on the chemical process and media to be analyzed, fiber probes can be based on different fiber types selected for the required spectral range and assembled in various shafts used for Transmission, Reflection, ATR-absorption, Raman & Fluorescence spectroscopies. The newest fiber combi probes allow to use two spectroscopic methods in the same probe shaft - such as Mid-FTIR+Fluorescence, Raman+DRS (Diffuse Reflection Scattaring), etc., - providing more accurate spectral analysis results due to the synergy of data fusion from 2 different methods.
Recent advances in precision opto-mechanics and probe design enable to create fiber optic probes with high useful signal to noise and low stray light. Probes reliability was tested in a variety of applications under harsh environments, including wide temperature range from -150°C to +250°C, high pressure up to 200 Bar, in aggressive or toxic liquids and gases, in microwave electromagnetic fields, etc. On the other hand, fiber optic probes for medical applications have been produced with very compact design – up to a mono-fiber needle-probes for Raman spectroscopy already used to define tumor margin shape in onco-clinical diagnostics.
In the case of complex samples, single spectroscopic technique may be not sufficient for reliable process-control. Such challenging analytical tasks may require an optical diagnostic where different spectroscopic methods are combined to achieve better accuracy, while the complimentary spectral information is collected from the same spot of media. The best choice of spectral method selections for a distinct process can be investigated in the lab using all kinds of fiber spectroscopy techniques. Several examples of fiber spectroscopy applications will be presented using different methods and their combinations. Based on the results, portable and low cost spectral fiber sensors can be developed to match demands on distinct process-control using the only few most informative spectral bands.

23PMA04: Nanomedicine Applications, Southern Pacific D
Chair: Zahra Rattray

(PMA-04.1)**Analytical Pipelines for Profiling Nanomedicine Biological Interactions**

Karim Daramy, MS Panida Punnabhum, Callum Davidson, Ms Rand Abdulrahman, **Zahra Rattray,** *University Of Strathclyde,*

Nanoparticle-based therapies are increasingly being developed for treating a diversity of clinical conditions. However, the bench to clinic translation of nanomedicines remains low (Mitchell et al., 2021). A reason for this low translation rate is limited knowledge of interactions occurring at the nano-bio interface. Nanoparticles are highly reactive, and in biological systems spontaneously interact

with proteins leading to surface-adsorption onto the nanoparticle surface- this phenomenon is referred to as the 'protein corona'.
The protein corona alters the biological safety and efficacy of nanomedicines (Rampado et al, 2020). Therefore, it is crucial to understand how physiological parameters influence nanoparticle-protein interactions. Our team investigates changes occurring in nanoparticle parameters following treatment with complex biological media.
Here we discuss the importance of media composition and environmental incubation parameters (e.g., flow) in studying the nanoparticle protein corona. We use a range of analytical techniques such as electrophoretic light scattering (ELS), dynamic light scattering (DLS), field-flow fractionation (FFF) and particle tracking analysis (PTA) to measure changes in particle parameters.
Overall, the incubation of model polystyrene nanoparticles in the presence of protein results in an increase in nanoparticle size. A significant increase in particle size was also observed following incubation in serum under flow (0.85 cm/s, 8.5 cm/s), accompanied by higher surface-adsorbed protein content and changes in nanoparticle zeta potential. These findings are supported by previous work (Jayaram, Pustulka, Mannino, Lam, & Payne, 2018) showing that circulatory shear flow conditions alter protein corona composition.
Our results show that the routinely-used protocol for nanoparticle protein corona analysis, leads to nanoparticle loss and perturbations in size, which is not reflected in in-situ measurements. We show that physiologically-relevant flow conditions yield different nanoparticle parameters and protein composition in comparison to static incubation conditions. We also demonstrate the early assessment of lipid nanoparticle prototype interactions with biological media. Overall, our findings demonstrate the importance of using biologically-relevant protocols for early evaluation of the nanoparticle protein corona.

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(PMA-04.2)**Field Flow Fractionation Applications For The Analysis Of Nanomaterials For Health**

Panida Punnabhum, Karim Daramy, Callum Davidson, Ms Rand Abdulrahman, Zahra Rattray,
University of Strathclyde,

Nanoparticle-based therapeutics represent a rapidly growing area of the pharmaceutical industry portfolio, with >55 nanomedicine-based products under clinical evaluation as of 2020, and a new wave of next-generation nanomedicines entering the clinical arena. While there are no universally accepted conventions for measuring nanomedicines, it is widely known that their safety and efficacy are directly correlated to their critical quality attributes, including particle size, drug loading, polydispersity, shape, as well as stability. Field-Flow Fractionation (FFF) as a high-resolution separation method has rapidly grown in interest over recent years, where it has been implemented for the separation and hyphenation with in-line detectors for the analysis of nanoparticle physicochemical parameters.

Here, using a series of case studies we present the optimization of different FFF modes (frit inlet, asymmetric, and electrical field-flow fractionation), investigating various nanoscale materials including polystyrene nanoparticles, immunoglobulin G (IgG), and lipid nanoparticles with variations in conditions based on the parameters. We investigated parameters influencing the effectiveness of FFF, including membrane composition, molecular-membrane weight cut-off (MWCO), channel composition, carrier fluid and flow rate. We demonstrate that sample loss due to membrane interactions is a key challenge impacting the FFF-based separation of analytes. We present multiparametric experimental data showing the high-resolution analysis of nanoparticle and antibody formulation stability and compare them with orthogonal data, alongside the potential it holds for early stage analysis of bionanotherapeutics.

References:

Mitchell, M. J., et al (2021). Nat Rev Drug Discov, 20(2), 101-124.
Caputo, F., et al (2021). J Chromatogr A, 1635, 461767.
Quattrini, F., et al. (2021). Drug Deliv Transl Re, 11(2), 396-396.

(PMA-04.3)**Orthogonal Pipelines for Lipid Nanoparticle Evaluation**

Callum Davidson, Rand Abdulrahman, Panida Punnabhum, Yvonne Perrie, Zahra Rattray, *University of Strathclyde*

Ribonucleic acid (RNA) drugs pose promising candidates for gene therapy in treatment resistant conditions and rare diseases. The FDA approval of siRNA Onpattro® in 2018 mRNA-LNP Spikevax® and Comirnaty® COVID-19 vaccinations in 2021[1] ignited research interests as these were the first siRNA and mRNA candidates to utilize lipid nanoparticles (LNPs) as a drug delivery platform. As the RNA-LNP research field is rapidly growing, robust, high-resolution separation techniques coupled to in-line detectors are required to analyze particle key quality attributes and accelerate the successful clinical translation of RNA-LNP therapies. Asymmetric-Flow Field Flow Fraction (AF4) and Size Exclusion Chromatography (SEC) are robust approaches for the characterization of oligo-LNPs [2, 3]. AF4 utilizes perpendicular field induction and particle diffusion-based separation, whereas SEC uses LNP-stationary phase interactions for separation.

The goal of this study was to develop separation pipelines for the high-resolution analysis of LNPs. Briefly, we prepared (6Z,9Z,28Z,31Z)-heptatriaconta6,9,28,31-tetraen-19-yl-4-(dimethylamino)-butanoate:cholesterol: 1,2-distearoyl-sn-glycero-3-phosphocholine: 1,2-dimyristoyl-rac-glycero-3-methoxypolyethylene glycol-2000 (MC3:CHOL:DSPC:DMG-PEG2000) LNPs and 8-[(2-hydroxyethyl)[6-oxo-6-(undecyloxy)hexyl]amino]-octanoic acid, 1-octylnonyl ester:cholesterol: 1,2-distearoyl-sn-glycero-3-phosphocholine: 1,2-dimyristoyl-rac-glycero-3-methoxypolyethylene glycol-2000 (SM-102:CHOL:DSPC:DMG-PEG2000) using microfluidics at 50:38.5:10:1.5 mol% ratio.

Here, we performed AF4, combined with in-line dynamic light scattering, multi-angle light scattering, and UV detection. Using these detectors, we measured key particle quality attributes including particle size, polydispersity index (PDI), and shape factor. The properties were evaluated alongside oligo-LNP samples that had not been subjected to separation.
Manufacture of LNPs using microfluidics-based analysis led to PolyA MC3-LNPs in the 56.5 nm ± 1.2 nm size range with a corresponding PDI of 0.12 ± 0.02, and PolyA SM-102-LNPs of 48.5 nm ± 1.1 nm with a PDI of 0.10 ± 0.01. Our findings show the presence of sub-populations within LNP samples, which cannot be detected using routine particle metrology techniques such as nanoparticle tracking analysis and dynamic light scattering. Our results highlight the need for developing more high-resolution approaches for the analysis of LNPs and linking these to input materials and process parameter design.

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2. Mildner, R., et al., Eur J Pharm Biopharm, 2021. 163: p. 252-265.
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(PMA-04.4)**Investigating the Impact of Shear Flow on Polymeric Nanoparticle-Protein Interactions**

Karim Daramy, Panida Punnabhum, Yvonne Perrie, Zahra Rattray, *University of Strathclyde*

Nanomedicines, are increasingly being developed to address unmet clinical need. Upon administration to biological systems, nanoparticles spontaneously interact with and adsorb proteins onto the particle surface to form the ‘protein corona’. The nanoparticle protein corona leads to changes in the physical and chemical parameters of nanoparticles, which alters its biological safety and efficacy profiles. Therefore, it becomes crucial to characterise the changes in particle parameters following protein corona formation. Here we report the changes in unmodified, amine-, and carboxylate-modified polystyrene latex nanoparticle parameters following treatment with fetal bovine serum under various physiologically-relevant conditions. We employed a range of analytical techniques to measure changes

in nanoparticle parameters including particle tracking analysis (PTA), and asymmetric field flow fractionation (AF4) coupled with multiangle light scattering (MALS), and SDS-PAGE. Overall, an increase in mean particle size was seen following incubation within protein-containing medium. A significant increase in particle size was observed for nanoparticles subjected to physiologically-relevant shear flow conditions with a 31% increase in mean diameter observed for unmodified particles, and a 24% increase and 118% increase in diameter for carboxylate-, and amine-modified nanoparticles respectively when incubated at (0.85 cm/s). Unmodified nanoparticles showed a 49% increase in mean diameter when incubated at (8.5 cm/s). The increase in particle size under shear flow was accompanied by changes in the protein corona composition when compared to static incubations. These findings correlate with previous studies (Jayaram et al., 2018) which show similar trends in particle size and corona composition following incubation under flow conditions. Our results show that the centrifugation-wash method routinely used to study the nanoparticle protein corona is disruptive and leads to changes in measured nanoparticle parameters (Böhmert et al., 2020), including an increase in mean measured size and a loss in particle concentration which is not reflected with in-situ measurements (FFF/PTA). We recommend the use of more gentle separation techniques such as AF4. Our results demonstrate that careful consideration is required for sample handling protocols when studying nanoparticle-protein interactions under physiologically-relevant conditions.

Böhmert, L. et al. (2020). Nanoscale Advances, 2 (2), pp. 563-582.
Jayaram, D.T. et al. (2018). Biophys J, 115 (2), pp. 209-216.

(PMA-04.5)**Raman Spectroscopy Characterization Methods for CVD-grown Graphene in Biosensors Applications**

Elizabeth Legge, Andrew Pollard, Andrew Wain, *National Physical Laboratory*

Due to graphene's desirable properties, such as the ease of chemical functionalization, high electrical conductivity and high surface area, graphene biosensors are a rapidly growing area of research. To achieve high selectivity, graphene must be functionalized with appropriate molecules, such as amine groups which are required to bind to antibodies then used for the detection of specific biomarkers. Raman spectroscopy is frequently used to characterize graphene biosensors, however there is no standard method for determining the success and durability of the different stages during the fabrication process. The ID/IG ratio from the Raman spectra of graphene can be used as an indication of the level of disorder, and therefore can approximate the level of sp3 defects due to molecules bound to the graphene. Consequentially, it is typically used to confirm the success of a functionalization process. When functionalizing graphene, an increase in the ID/IG ratio typically indicates higher disorder, however external forces such as strain, chemical processes and scanning tunneling microscopy can later decrease the ID/IG ratio, which suggest it is also important to test the durability of the functionalization.
While covalent functionalization is usually the method of choice, it is not necessarily preferable over non-covalent alternatives. We investigate Raman spectroscopy methods for determining the coverage of graphene on a substrate, the coverage of functional groups on the graphene, and the durability of these functional groups. By utilizing a combination of imaging techniques, including contact-mode and tapping-mode AFM, Raman spectroscopy and x-ray photoelectron spectroscopy (XPS), we demonstrate some imaging challenges and the importance of using different analytical techniques to investigate the physicochemical properties of biosensors.
The work presented here on functionalized, chemical vapor deposition (CVD) graphene shows methods for testing the durability of covalent and non-covalent functionalization. Removal of functional groups from CVD graphene with contact-mode atomic force microscopy (AFM) explains unexpected changes in the Raman spectra and suggests the durability of non-covalent functionalization can be similar or better than covalent functionalization.

23RAM10: Low Frequency Raman, Cascade 1

Chair: Robert Chimenti

(RAM-10.1)**THE BOSN PEAK AND HETEROGENEITIES IN LIQUIDS AND DISOREDER SOLIDS**

Alexei Sokolov, *University of Tennessee*

This talk overviews experimental results on low-frequency Raman, inelastic neutron and X-ray scattering studies of glasses, liquids and polymers with the focus on the boson peak. We demonstrate a connection of the frequency and the amplitude (relative to the level expected in the Debye model) of the boson peak to characteristic length scale and amplitude of structural heterogeneity in disordered systems. Presented analysis reveals clear correlations of the boson peak amplitude to the strength of damping of acoustic waves in the THz frequency range. At the end we discuss changes in the boson peak spectra in polymer nanocomposites where additional heterogeneity length scale appears due to formation of the interfacial polymer layer.

(RAM-10.2)**Kinetic Study of Rheology-Modified Interpenetrating Polymer Network (IPN) Resins Using Ultra-Low Frequency Raman**

Alexandra Lehman-Chong, Robert Chimenti, Jianwei Tu, Samuel Lofland, Joseph F. Stanzione, III, *Rowan University*

Curing kinetics of photopolymerizable resins is essential when determining the printing parameters for resin-based additive manufacturing. Most traditional methods of analyzing chemical and structural dynamics, such as photo-differential scanning calorimetry or infrared spectroscopy, can be challenging to perform in situ, but Raman spectroscopy is ideally suited for such studies. Traditionally, Raman-based kinetic studies have focused on the fingerprint region where chemical changes result in changes in peak heights or steric changes result in peak shifts or broadening. In addition to the chemical vibrational bands, amorphous materials display low-frequency Raman features related to the phonon density of states, which result in a broad disorder band below 100 cm -1. This disorder band is directly related to the conformational entropy of the system and has a broad asymmetric shape with a well-defined shoulder dominated by the van Hove peak. In previous studies, we have demonstrated the ability to measure kinetic processes such as glass transition and polymerization kinetics by normalizing the disorder band to the shoulder and monitoring the integrated intensity of the peak near the apparent maxima.

In this work, we used traditional and structural Raman to investigate the photocure kinetics of epoxy-methacrylate interpenetrating polymer networks (IPNs) with and without unreactive rheological modifiers. We also analyzed a fully methacrylate resin formulation as a prototypical system. Based on the chemical-to-structural conversion proportionality constant, we demonstrated that a dimethacrylate IPN resin was more “structurally cured” than an IPN resin containing a dual-functional epoxy-methacrylate monomer after photocuring. Additionally, the modified resins systems were found to be less “structurally cured” than the unmodified resins. These results are consistent with expectations since dimethacrylate IPN resins allow for crosslinking upon photocuring, and unreactive fillers increase the overall disorder of the system. Next, we investigated the cure kinetics of each resin system at various illumination intensities to determine the dose dependency of each resin formulation and produce master conversion profiles for both the chemical and structural cure kinetics. Based on the master curves, we were then able to determine the ultimate conversion, rate constant, and reaction order for each resin system.

(RAM-10.3)**Catching nucleation in action by Raman and terahertz Raman spectroscopies**

Mark Christie, Jan Sefcik, Karen Faulds, *University Of Strathclyde*

Nucleation and subsequent crystallisation are extremely important processes within numerous commercial fields, notably pharma and metallurgical. It is widely accepted that nucleation is a stochastic process whereby molecules randomly collide, resulting in assemblies which generate a stable nucleus upon which more molecules can attach.

Monitoring this process provides new insights into the mechanisms behind nucleation and crystallisation and opens new possibilities for controlling the final crystal form. Historically, the study of nucleation relied on visual identification of an already established macro-phase by acquiring large numbers of images to monitor the progress of nucleation, and heat of crystallisation by differential scanning calorimetry. The one aspect which still provides some uncertainty is structural information of the nucleating phase. Currently, nucleation mechanisms are proposed and supported by a multitude of computational and mathematical approaches yet there has always been difficulty in measuring this practically.

Raman spectroscopy is a sensitive technique which can be utilised to identify polymorphic forms and understand the behaviour and changes undergone by molecules in different environments. Beyond the classical fingerprint region (200-1800 cm^{-1}), the terahertz region (30-200 cm^{-1}) offers insight into the intermolecular interactions which may be key to identifying nucleating clusters.

This work focusses on the application of noble metal nanoparticles with Raman spectroscopy, both in the fingerprint and terahertz regions, as a method of detecting nucleation in solution. Here, owing to its simplicity and known polymorphs, the amino acid glycine was used as a model molecule. It was determined that synthesising nanoparticles with glycine as the capping ligand was the most effective approach for monitoring glycine nucleation within supersaturated solutions, at near-neutral pH. We will discuss a proposed pathway by which crystallisation of glycine occurs heterogeneously in solution, in the presence of noble metal nanoparticles. Overall, this work demonstrates potential new applications of Raman spectroscopy, particularly in the terahertz region.

(RAM-10.4)**Size effects in nanoparticles of WO3, Y2O3, and TiO2: quantum confinement**

Sergey Mamedov, *HORIBA Scientific*

Nanoparticles of metal oxide are attractive for many applications but have recently become essential to highly efficient catalysis materials. The material's efficiency depends on size, shape, and surface chemistry, which critically determine their properties and interaction.
Raman spectroscopy is a powerful method to investigate nanoparticles' vibrational properties, as the Raman band's peak and width are very sensitive to the local structure. Besides, phonons' behavior at the nanoparticle boundary strongly depends on the particle size and is a critical factor in creating a highly efficient material. The samples of Y2O3, WO3, and TiO2 with a mean size ranging from 5 to 40 nm were investigated by Raman spectroscopy in the broad spectral and temperature range.
The Full Width of Half Maximum (FWHM) of 377.6 cm-1 band of Y2O3 shows symmetric broadening with a decrease in the size of the nanoparticles without changes in the band's position. The changes in FWHM are due to the crystalline lattice distortion. Based on the FWHM of the peak, the size of the nanoparticles can be calculated. Raman spectra of TiO2 nanopowder show an asymmetric broadening and shift of Eg peak at 142.9 cm-1 with decreasing the nanoparticle's size. The position and FWHM of Raman bands of WO3 show a mixture of effects. The nanopowder with a size of 8 nm undergoes significant changes under heat treatment or laser irradiation due to phase transformation. The phonon confinement model describes the experimental data for TiO2 and WO3 nanopowders. The phonons' correlation length calculated from nanoparticle spectra of TiO2 shows a good agreement between grain sizes obtained from Raman spectra and XRD. Raman spectra are sensitive to nanoparticles' structural motive and reflect the differences in nanoparticles' surface structure.

23SPR03: Structuring Plasmonic Particles for Applications, Cascade 4
Chair: Matthew Sheldon

(SPR-03.1)**Broad Spectral Range Linear and Circular Dichroism Responses of Gold and Aluminum Nanostructures**

Jennifer Shumaker-Parry, *University of Utah*

Plasmonic nanostructures demonstrate linear and circular dichroism (CD) behavior that depend on both structural characteristics and the properties of incident light, leading to tunable complex light-matter interactions. Nanosphere template lithography (NTL) provides an approach to tailor the structural properties and explore the structural dependencies of the optical responses. Using copper mask NTL, both gold (Au) and aluminum (Al) structures with different symmetric and asymmetric features were produced. The versatile NTL fabrication controls structural properties, leading to optical responses tunable across a broad spectral range from the ultraviolet (UV) to the infrared (IR). For simple Au nanostructures, CD responses representing extrinsic chirality were observed at tilted, out-of-plane angles. For Al nanostructures, more complex CD behavior was observed and attributed to the native oxide layer that impacts the path of plasmon electrons in the structures, especially in dimer-based configurations. Comprehensive optical analyses combined with modeling support studies of the origin of the optical responses. The studies form a basis for developing the plasmonic nanostructures for applications in spectroscopies involving circularly-polarized light including vibrational circular dichroism (VCD) and CD-based sensing.

(SPR-03.2)**Tunable Gold and Aluminum Nanocrescents as a Platform for Circular Dichroism Spectroscopy**

Anh Nguyen, Jennifer Shumaker-Parry, *The University Of Utah*

Gold (Au) has been a common material for studies of plasmonic nanostructures. Although aluminum (Al) has several advantages over gold, the ubiquitous alumina film is a challenge for fabrication of nanostructures, limiting the studies of Al nanostructures. With the copper mask nanosphere template lithography fabrication method, our group has successfully fabricated both Au and Al nanocrescents (NCs) despite the challenges that arise from the native oxide layer of Al. Both AuNCs and AlNCs exhibit multimodal, polarization-dependent plasmons that can be tuned in different spectral regions. Furthermore, we investigated the chiroptical behavior of AuNCs and AlNCs. The structures demonstrate extrinsic chirality with circular dichroism (CD) responses when titled to an out-of-plane incident angle (θ) with respect to the incident circularly polarized light. At $\theta=\pm30°$, the true CD responses of the AuNCs and AlNCs are equal in ellipticity but opposite in handedness. In addition, the AuNCs and AlNCs exhibit the opposite chiral response when rotated 180° relative to the sample orientation at $\theta=\pm30°$. No chiroptical response is observed for true CD of both AuNCs and AlNCs at normal incidence, which confirms the extrinsic chirality of the structures. Conversely, there is negligible handedness response for the birefringence of AuNCs and AlNCs at either normal incidence or out-of-plane incident angle. These chiroptical responses indicate that the nanocrescents produce isotropic orientation averaging where there is little to no change in the original substrate surface forward/backward CD response as well as exhibit true CD behaviors. The studies have provided insight into understanding the chiroptical and plasmonic behavior, providing a foundation for further investigation into the use of Au and Al nanostructures in CD-based sensing and detection.

(SPR-03.3)**A Novel Method for the Synthesis of Core-Satellite Plasmonic Nanostructures for Single Particle SERS and CARS**

Sanjun Fan, Brian Scarpitti, Zachary Schultz, *department of chemistry and biochemistry, The Ohio State University,*

Engineering nanoparticles to support localized surface plasmon resonances (LSPRs) has attracted much attention due to their ability to enhance light−matter interactions and manipulate light at the subwavelength level. The plasmonic properties (optical, electronic and magnetic) of noble nanoparticles are highly dependent on the size, shape, and the composition of nanoparticles and can be tuned from the ultraviolet (UV) to the visible and infrared regions of the electromagnetic spectrum. Strategies for the precise control of noble metal nanoparticle assembly and morphology are used in a variety of applications like plasmonic sensing, surface enhanced Raman scattering (SERS)/coherent anti-Stokes Raman scattering (CARS), fluorescence, electrochemiluminescence, photovoltaic, cancer therapy, catalysis, etc. In particular, core-satellite nanoparticles have gained much interest for the strong SERS enhancement due to the hot-spot generation arising from the high electromagnetic

coupling between the core and satellite structures. These particles have electric field intensity 600x higher than that at the surfaces of the core alone.
Typically, core-satellite nanoparticles are prepared connecting a larger core to separately synthesized smaller satellite nanoparticles, where the core or satellite particles can be gold or silver nanoparticles. The nano-spacer linkers for the connection can be divided into several categories: 1) DNA-based assembly; 2) polymer-based assembly; 3) protein-based assembly; 4) covalent or electrostatic interactions of the functional groups.
Herein, we have developed a new method for the preparation of core-satellite nanostructures without using the above linking molecules, covalent bonds, or electrostatic interactions. This facile and convenient approach can synthesize core-satellite nanostructures using Au nanostar as core and Au, Ag, Pt, or Pd nanoparticles acting as satellites that are grown on the tips to different sizes. Interestingly, the Au core/Au satellite nanoparticles can form several gap-enhanced Raman tags (GERTs) at the tips. The GERTs have strong electromagnetic hot spots from interior sub-nanometer gaps between external petal-like shell structures, larger immobilization surface area, and a large net Raman cross section that is useful as an ultrabright Raman probe for single nanoparticle SERS. These core-satellite nanostructures with intraparticle gaps allow for single-particle SERS and single-particle CARS, making them attractive for single particle applications in the field of SERS/CARS biosensor field.

(SPR-03.4)**Polydopamine as a Versatile Tool to Develop Surface Enhanced Raman Scattering Substrates**

Ahmed Mahmoud, Alexandra Teixeira, Maria Sousa-Silva, Antonio Fernández, Sara Abalde-Cela, Lorena Diéguez, *NL - International Iberian Nanotechnology Laboratory (INL), RUBYnanomed LDA, Centro de Estudios Superiores Aloya,*

In the past few decades, portable and handheld Raman spectrometers have been emerging as powerful analytical tools that led to the expansion of the applications of Raman and surface-enhanced Raman scattering (SERS) beyond lab settings. However, most of these devices are designed mainly for qualitative and semi-quantitative purposes, which may limit their analytical performance, especially in SERS platforms. For example, some of these devices do not allow the user to fully control or modify many critical experimental parameters, such as the laser focus, laser power, and/or acquisition time. One way to circumvent these limitations is to develop SERS substrates that are systematically optimized to be accompanied by these devices.
In this talk, we will show how polydopamine can be used as a versatile multifunctional polymer to develop and optimize inexpensive SERS substrates for these portable devices. Polydopamine, a mussel-inspired biopolymer, can adhere to almost any surface through self-polymerization in a basic medium. Polydopamine can also act as a reducing agent where plasmonic nanoparticles can be reduced and deposited. We will discuss how the SERS performance can be systematically optimized through different in-situ seedless and seeding growth protocols of plasmonic nanostructures. Moreover, we will show and discuss how these methods can be transferred and applied to fabricate SERS substrates in glass vials, paper-like materials, and microfluidic devices for some biomedical applications. Using polydopamine to develop SERS substrates can provide a promising inexpensive avenue for developing sensitive and reproducible sensing platforms.

Plenary Sessions: FACSS Charles Mann Award for Applied Raman Spectroscopy; Juergen Popp, Sierra 5

(PLEN-L2.1)**Applied Raman spectroscopy for clinical diagnosis and therapy to fulfill currently unmet medical needs**

Juergen Popp, *Leibniz Institute of Photonic Technology*

Within the last years we witnessed a rapid grow in utilizing Raman spectroscopic approaches together with the phantastic possibilities of artificial intelligence (AI) for applied clinical diagnosis and therapy. Numerous proof-of-concepts studies have been published showing the great possibility of Raman spectroscopy for medicine. The next big step will be translating these Raman approaches into routine

clinical applications. This translation will be also the biggest challenge over the next years. Within this contribution we will highlight our recent efforts in translating a variety of linear and non-linear Raman spectroscopic approaches in combination with innovative AI-based spectral analysis routines towards routine clinical applications. We address two important fields of medical application of unmet medical needs: (I) the instantaneous intraoperative histopathological diagnosis of tumors and (II) the reliable on-site diagnosis and treatment of infectious diseases. For both clinical issues, time is a critical parameter, i.e., the faster an infectious agent (together with its immune response, in case of bacterial infections its resistance pattern, or response to treatment) is detected or the faster a tumor is completely removed by Raman spectroscopic surgical guidance and intraoperative histopathological tissue staging and grading the better the chances of recovery for the patient. The introduced approaches comprise the entire process chain i.e., from sampling to the final diagnostic result, and have a high potential to significantly reduce the critical parameter 'time' to initiate individualized therapy plan tailored to the patient as quickly as possible. In order to ensure that the progress made in this research reaches patients more quickly, translational infrastructures to quickly overcome the valley of death from the idea to product are also introduced.

Acknowledgements
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Plenary Sessions: RSC Joseph Black Award; Mathew Horrocks, Sierra 5

(PLEN-L2.2)**Visualizing neuroscience at the single-molecule level**
Mathew Horrocks, *The University of Edinburgh*

Neurodegenerative disorders, such as Parkinson's and Alzheimer's disease, are characterised by the misfolding and aggregation of soluble monomeric protein into insoluble amyloid fibrils. Despite these being the pathological hallmark of the diseases, it is the earlier oligomers composed of a smaller number of misfolded monomers that are thought to be the most cytotoxic species. These are challenging to study due to their low abundance, dynamic nature, and high heterogeneity. For this reason, we have developed and utilised single-molecule and super-resolution microscopy approaches to visualize individual protein aggregates, characterising their shapes and sizes in a range of complex biological samples. We've also applied these approaches to understand how the healthy brain functions, and have invented a new super-resolution microscopy technique that works in live cells.

23ART03: Historic Perspective and Recent Advances in Art Analysis using Vibration Spectroscopy, Southern Pacific F
Chair: Marc Vermeulen

(ART03.1)**Tales of Art and Raman Spectroscopy**

Anastasia Rousaki, *Ghent University, Raman Spectroscopy Research Group, Department of Chemistry*

Raman spectroscopy is considered to be an ideal molecular technique for the analysis of the materials of cultural heritage objects. Except from the original 'palette' of the artist or the different components of the work of art, the technique can successfully identify restoration materials, degradation products and thus access the preservation state of the artefact.

Although the technique is used in cultural heritage analysis for almost 40 years, still it is considered to be promising with new approaches emerging to upgrade the experience. An undeniable asset of Raman spectroscopy is that the technique can be applied directly and on the field without jeopardising the artefact. The latest instrumental advantages and the coupling of different laser excitations on mobile systems enhanced the analysis of the organic and inorganic components. Except of the use of the

‘traditional’ 785 nm laser on cultural heritage, the coupling of a 1064 nm laser to a dispersive Raman system, can allow to travel to the artefact and be sufficiently adequate to identify (in)organic materials and supress the fluorescence. The incorporation of dedicated algorithms on compact Raman devices for the fast detection of materials makes the application of the technique more appealing to non-Raman users.

After nearly 40 years of applications of Raman spectroscopy on works of art, and almost 20 years of mobile Raman research, the technique has managed to decode the past and pave the future. The connection of different Raman devices (different characteristics, lasers, advances etc.) to the analysis of artefacts ranging from prehistory to contemporary art will be discussed. Raman spectroscopy crafts interactive stories of beautiful objects and their analysis. Tales of origin, materiality and time.

Acknowledgements
A. R. acknowledges the Ghent University Special Research Fund (BOF) for her postdoctoral fellowship.

(ART03.2)**A Well of Information: Mutli-Technical Studies of Della Robbia's Christ and the Samaritan Woman**

Alicia McGeachy, Elena Mars, Colleen Snyder, Gwen DePolo, Adrienne Gendron, Beth Edelstein, Marc Vermeulen, Marc Walton, *The Metropolitan Musuem of Art, The University of Arizona, The Cleveland Museum of Art, Northwestern University, Harvard Art Museums, The National Archives, M+ Museum*

Christ and the Samaritan Woman at the Well, in the collection of the Cleveland Museum of Art, is a large-scale painted terracotta relief attributed to the workshop of Giovanni della Robbia. The relief, created in the 16th century for the Sacro Monte di San Vivaldo, was an important outdoor fixture of this Franciscan pilgrimage site and was repeatedly conserved and restored over its lifetime. Analysis of the materials and techniques of the relief, including a study of the ceramic types, ground and paint layers, and restoration materials, will illuminate the work’s history and inform the course of planned conservation treatment. As a relatively rare and important example of painted, rather than glazed, terracotta, it expands on the current scholarship on the della Robbia workshops. Moreover, as an audience favorite and an important part of the early Renaissance holdings in the collection of the Cleveland Museum of Art, the outcomes from this study may have potentially significant public and scholastic impact as well. To this end, we employ a combinatorial approach employing X-ray fluorescence spectroscopy, Fourier transform infrared spectroscopy, reflectance imaging microscopy, Raman microscopy, and scanning electron microscopy with energy dispersive X-ray spectroscopy to develop a comprehensive understanding of the stratigraphy and overall material use across the large-scale relief. This project is a collaborative effort between the Center for the Scientific Studies in the Arts and the Cleveland Museum of Art.

(ART03.2)**Innovations for rapid x-ray fluorescence and infrared chemical imaging of cultural heritage objects**

Thomas Tague, Michael Beauchaine, *Bruker Scientific, Llc, Bruker Nano*

Infrared (IR), Raman, and x-ray fluorescence (XRF) object analyses have become important components of a complete characterization of art objects for conservation and authentication. IR and Raman are highly specific for the molecular analysis of binders, pigments, and other objects containing features organic in nature. XRF spectroscopy is commonly used for the analysis of art objects as it allows for the analysis of the elemental makeup of objects. When used together they can give a good indication of the chemical composition. X-ray fluorescence is particularly important for the analysis of pigments, as most paint before 1900 contained organometallic pigments. When analyzing paintings that may have been painted over an underlying painting, XRF is particularly useful as the depth of penetration is such that both layers will be analyzed. New large scale XRF mapping of large paintings and other objects is now possible with improvements made in signal processing

electronics and detector technology, as well as the implementation of novel polycapillary optics. Additionally, new state-of-the-art data processing tools to extract and evaluate the information have been employed to greatly facilitate large scale image processing.
IR and Raman are very much surface techniques where molecular absorptivity frequently precludes analysis below the surface. For IR analysis, ATR has been traditionally used for paint cross section analysis. Unfortunately, ATR requires sample contact. Direct reflection analysis is possible but typically has a low signal. New IR sources have been utilized to significantly improve the signal strength such that many art objects can be analyzed using direct reflectance. In fact, new reflectance-based IR imaging can now be conducted for the analysis of art objects to include cross sections and other objects.

(ART03.4)**Development Of Advanced Micro-SORS For Heritage Science**

Alberto Lux, Alessandra Botteon, Marco Realini, Pavel Matousek, Pietro Strobbia, Claudia Conti, *CNR-ISPC, Rutherford Appleton Laboratory, University Of Cincinnati*

The development of two micro-SORS prototypes and their optimization for Heritage Science applications are the result of the research carried out over the last few years at CNR-ISPC Raman Laboratory in collaboration with the Rutherford Appleton Laboratory and the University of Cincinnati [1, 2]. First, a commercial benchtop micro-Raman instrument has been modified for increasing depth sensitivity and 3D mapping capabilities; micro-SORS can be deployed using different modalities (defocusing, internal beam-steered and point-like) to fit specific application effectively.
Painted layer sequences and diffusion of conservation or decay products were investigated using defocusing for obtaining an average distribution of compounds and point-like for increasing the contrast between external and inner portions of the materials; extremely thin layers (10-15 μm) required small spatial offsets provided by internal beam-steered modality. Different imaging/mapping modalities (conventional, StreamLine and StreamHR) were coupled with micro-SORS variants paving the way for studies of high-resolution molecular distribution of compounds hidden by external opaque layers in art objects, as in the case of hidden letters in sealed or closed documents.
Secondly, an in-house portable micro-SORS prototype was developed to enable conventional and micro-SORS measurements in situ. This device represents a technological evolution of existing commercial portable Raman since the detection of Raman signals of the surface and subsurface is achieved by using a micrometric linear fiber bundle to conserve the offset information on the detector, permitting simultaneous acquisition of Raman photons emerging from the surface and subsurface in separate spectra in analogy to approaches also used in macro-SORS spectroscopy [3]. The system is particularly well suited to non-invasive analyses in museum collections, archaeological or conservation sites, where vibrations could be an issue, since no additional mechanical movements are required for setting spatial offset. Designs and applications to Heritage Science will be discussed.

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(ART03.5)**Using Vibrational Spectroscopic Imaging Techniques to Visualize Subsurfaces in Cultural Heritage Studie**s

Marc Vermeulen, *The National Archives*

Archival collections are invaluable resources for understanding the material and culture of past societies. These well dated artefact collections often contain hidden layers that are difficult to access without damaging the object. In recent years, non-invasive analytical techniques such as μ-XRF spectroscopy imaging have allowed access to concealed layers, but they do not provide molecular

information. Raman and Fourier Transform Infrared (FTIR) are commonly used to supplement the shortcomings of XRF.
In this study, we used micro-spatially offset Raman spectroscopy (μ-SORS) imaging and μ-ATR FTIR imaging to investigate the composition and structure of concealed layers in various materials from archival collections, including closed letters, and documents glues into larger volumes.
This study found that both techniques were effective in identifying and mapping the distribution of organic and inorganic compounds within the concealed layers of the artefacts. This revealed hidden historical contents, providing invaluable insights into the customs and material culture of past societies.
The findings of this study have significant implications for the study and preservation of cultural heritage objects found in archives and libraries around the world. By utilizing these non-destructive analytical techniques, researchers can gain access to concealed information of historical artefacts without causing damage. This enables a greater understanding of the composition and structure of these objects, which can also inform conservation and preservation efforts. Additionally, these technique can guide the decision-making process for more invasive approaches and provides invaluable information for historical and art historical research.
The study demonstrates the potential of μ-SORS and μ-ATR FTIR for the non-destructive and non-invasive study of concealed layers in archival collections. The information gained from these techniques can be used to inform conservation and preservation strategies, historical and art historical research, and ensure the long-term protection and accessibility of these valuable cultural heritage objects.

23ATOM04: Single Particle and Single Cell ICPMS, Central Pacific A/B/C
Chair: Antonio Montoro
Co-Chair: Derrick Quarles

(ATOM-04.1)**Overcoming interferences in single event-ICP-MS**

Martin Resano, Maite Aramendía, Flávio Nakadi, Antonio Bazo, Raúl Garde, Javier Resano, Juan Carlos García-Mesa, Elisa Vereda Alonso, *Universidad De Zaragoza, Universidad de Málaga, University of Oviedo*

The occurrence of matrix effects hinders the application of single particle-ICP-MS to real, complex samples for which its use could be more beneficial. Novel approaches for overcoming such matrix effects are presented in this work. One such approach is based on the application of the standard addition method in such a way that the calibration of the particle size is performed by two different methods: (i) by spiking a suspension of NPs standards of known size containing the analyte, or (ii) by spiking the sample with ionic standards. Another possibility makes use of the mass spectrometer (quadrupole) operating in bandpass mode, enhancing the sensitivity for the monitoring of NPs while also allowing for the detection of NPs of a different type in the same measurement run, such that they can serve as an internal standard. Examples of both approaches applied to the characterization of NPs in complex matrixes will be provided.

(ATOM-04.2)**Novel tools for the characterisation of micro- and nanostructures in biology and the environment**

David Clases, Thomas Lockwood, Lukas Schlatt, Marko Simic, Christian Neuper, Christian Hill, Raquel Gonzalez De Vega, *University Of Graz, University of Technology Sydney, Nu Instruments, Brave Analytics,*

Nano- and microstructures play a fundamental role in biology and geology, but are often neglected. In the past, one reason for this was a lack of suitable methods to provide complementary perspectives on integrated and discrete structures and to establish models on parameters such as sizes, masses, composition and number concentrations. Inductively coupled plasma – mass spectrometry (ICP-MS) and its associated techniques initiated a paradigm shift for the investigation of micro- and nanostructures. On the one hand, laser ablation (LA)-ICP-MS enables the inquiry of integrated micro-

sized structures and on the other hand, single particle acquisition techniques enable the detection and characterisation of discrete nanomaterials.
This work presents new approaches, models and tools which advance the characterisation of both nano- and microstructures. This includes novel software solutions which facilitate the processing of increasingly large data sets while providing useful tools to investigate distributions of number concentrations, sizes, masses and composition as well as to perform non-target nanoparticle analyses. One focus is directed to opportunities to improve the duty cycle in ICP-MS translating into improved ion transmissions. However, strategies to bypass interferences and to enhance signal to noise ratios are considered as well. A second focus is directed to ICP-ToF-MS which enables entirely new approaches for single particle analysis. Especially in conjunction with new hyphenated techniques, it becomes possible to combine optical and mass spectrometric characterisations and to promote more holistic investigations. We will showcase these new techniques and tools in the context of recent studies focussing on the analysis of discrete inorganic and organic (microplastics) particles in the environment as well as emerging nanomaterials relevant in a biomedical framework.

(ATOM-04.3)**Real-Life Applications of Single-Cell ICP-Mass Spectrometry in the Biomedical Sciences**

Eduardo Bolea-Fernandez, Tong Liu, Rinus Dejonghe, Mina Nikolić, Olivier De Wever, Kevin Braeckmans, Frank Vanhaecke, *University of Zaragoza, aGhent University, Department of Chemistry, Atomic & Mass Spectrometry (A&MS) research unit,*

Cell biology and nanomedicine strongly benefit from having access to information on the metal contents with single cell resolution, rather than at population scale. Metals are intrinsically present in cells to fulfil a wide variety of biochemical functions or are deliberately imported into cells, e.g., in the context of novel cell therapies, relying on the use of nanotechnology. However, very few analytical techniques are capable of quantifying metals at single-cell resolution. Novel technological breakthroughs in ICP-mass spectrometry (ICP-MS) have provided this technique with the potential for high-throughput single-cell analysis.[1]
Single-cell ICP-MS (SC-ICP-MS) provides information on the absolute amount(s) of the target element(s) in individual cells (e.g., average and median concentration, concentration distribution). Among others, this technique shows promising features for the development of better targeted cell-based therapies, but this approach is still in a very early phase.
This presentation will discuss the (bio)analytical challenges still hampering a wider use of the technique and will provide an overview of the application range through different case studies. First, results for the quantification of several endogenous elements and of Pt, present as a result of exposure of various cell types to cisplatin as a Pt-containing chemotherapeutic drug, will be presented, and the differences in Pt uptake between cell types will be linked to chemosensitivity and chemoresistance.[2] In addition, the role of metals in health and disease will be evaluated by quantifying endogenous metals inside blood cells. Special attention will be paid to QA/QC of the (bio)analytical protocol, while results of a biological consistency test and of a serial dilution experiment will be used to further demonstrate the validity and relevance of the method. Finally, a novel methodology for quantifying the number of nanoparticles (NPs) attached to the membrane of an individual cell is being optimized. This approach shows potential for finetuning cell-based therapies, such as NP-mediated photoporation.

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(ATOM-04.4)**Considerations for the Measurement of Individual Atmospheric Mineral Dust Particles Entrapped in Glacial Ice Cores by Single Particle Inductively Coupled Plasma Time of Flight Mass Spectrometry**

Madeleine Lomax-Vogt, Stanislav Kutuzov, Susan Welch, Lucas Carter, Paolo Gabrielli, Jonas Wielinski, Greg Lowry, Ryan Sullivan, John Olesik, *The Ohio State University, Italian Glaciological Committee, Carnegie Mellon University,*

Insoluble mineral atmospheric microparticles (μPs) and nanoparticles (NPs) make up a small fraction of the total mass of particulate matter in the atmosphere but play a large role in the systems that regulate the Earth's climate and environment. The size and mineralogy of each particle affects its bioavailability and reactivity in the atmosphere. Ice cores from remote regions act as an archive of the past atmosphere unaffected by humans. Particles deposited onto and then entrapped in the ice can be preserved up to hundreds of thousands of years.

Until now, our understanding of the size, elemental chemical composition, and number concentration of these particles has been limited by analytical methodology. Coulter Counter provides the size distribution of particles with a diameter >0.6 μm but cannot measure the elemental chemical composition of particles. Bulk measurements of ice core samples are made by dissolving the particles and measuring the average concentration of each element (typically by inductively coupled plasma sector field mass spectrometry (ICP-SFMS)). However, ICP-SFMS cannot determine estimated diameter or elemental chemical composition of individual particles. The average concentration elements may be dominated by the signal due to a few larger particles. Measuring uncommon elements (e.g. Ir, Pt) is challenging and requires ultra-clean techniques to avoid contamination.

single particle Inductively Coupled Plasma Time of Flight Mass Spectrometry (spICP-TOFMS) was used to measure hundreds of thousands of individual atmospheric NPs and μPs entrapped in ice from the Taylor Glacier, East Antarctica spanning the last glacial-interglacial cycle (44,000 to 9,000 years before present); a diameter is estimated for each particle using the total detected mass (fg) of elements (up to 70 at once) and a density. Particle size distributions and number concentrations are calculated for each sample. Possible mineral composition(s) of each particle can be inferred by comparing known mineral chemical compositions to the ratio of detected elements in each particle.

Initial results from each Taylor Glacier sample, including size distributions, number concentrations, and common and uncommon particle compositions will be compared over the glacial-interglacial cycle. spICP-TOFMS measurement challenges and limitations, such as element dependent backgrounds and detector non-linearity, will also be discussed.

(ATOM-04.5)**Comparison of Direct and Indirect Measures of Transport Efficiency in Single Particle ICP-MS**

Antonio Montoro Bustos, Karen Murphy, Lee Yu, Monique Johnson, Michael Winchester, *NIST,*

Accurate calibration of the fraction of introduced sample that is transported to the plasma, termed "transport efficiency" (TE), is required for particle sizing and number concentration determination by single particle inductively coupled plasma mass spectrometry (spICP-MS). In this study, we systematically compare three methods for the measurement of TE: the particle frequency (TEF), particle size (TES), and dynamic mass flow (DMF) methods. The TEF and TES methods provide a direct measure of TE but require a single nanoparticle reference material accurately value-assigned for particle size and number concentration of which few are available. The DMF method provides an indirect measure of TE and only requires measurement of the mass difference between the amount of sample solution introduced to the instrument and the amount of effluent exiting the spray chamber but assumes that the mass difference represents the fraction of sample transported to the plasma. The three measures of TE are compared for three different spray chamber types, Scott-type double pass, conical impact bead, and baffled cyclonic spray chambers operated at cooled (2 °C to 10 °C) and ambient temperature (19 °C to 21 °C) conditions and using different nebulizers and ICP-MS platforms. When operating the spray chamber at ambient temperature, the DMF method yielded systematically higher measures of TE than the TEF and TES methods regardless of nebulizer type, spray chamber type, or ICP-MS platform. While better agreement between the three measures of TE was achieved when operating the spray chambers at 2 °C, DMF repeatability was poor. We found that while the indirect measure of TE (DMF method) could yield unbiased and precise results for particle number concentration and particle size of gold nanoparticle suspensions in some cases (cyclonic spray chamber at 2 °C and Meinhard nebulizer), only the direct measures of TE (TEF and TES) yielded unbiased results across all use conditions.

23AWD04: FACSS Charles Mann Award Symposium Honoring Juergen Popp, Sierra 5
Chair: Juergen Popp

(AWD-04.1)**FLIM in Surgical Oncology**

Laura Marcu, *University of California Davis*

This presentation reviews the development of clinically-compatible fluorescence lifetime imaging (FLIM) technology and applications in surgical oncology. Emphasis is placed on the integration of FLIM in surgical workflow and the potential of this approach to improve surgical decision-making during trans-oral robotic surgery (TORS) and neurosurgical procedures. Clinical outcomes and results will be discussed. We demonstrate the straightforward coupling of FLIM apparatus with the da Vinci surgical platform and the neuronavigation system. Also, we show innovative methods for real-time dynamic augmentation of imaging parameters on the surgical field of view as seen on the da Vinci console and surgical microscope. Current results demonstrate the utility of FLIM-derived parameters detecting tissue biochemical and metabolic characteristics to distinguish oral and oropharyngeal cancer in real-time from surrounding normal tissue in patients in-situ during TORS as well as to sense infiltrative brain cancer at the resection margins. Our findings suggest that label-free FLIM-based tissue assessment, characterized by simple, fast and flexible data acquisition and display, could find applications in a variety of surgical procedures.

(AWD-04.2)**Ultraviolet Raman spectroscopy as a novel approach for testing the stability of mRNA vaccine in situ**

Igor Lednev, Lamyaa Almehmadi, Sergei Reverdatto, Vladimir Ermolenkov, Alexander Shekhtman, *University at Albany, SUNY, University of Albany*

The recent success of mRNA-based COVID-19 vaccines has highlighted the potential of RNA-based therapeutics. However, using RNA-based therapeutics is limited by special storage conditions needed to keep RNA stable. For example, an improper temperature control results in the loss of approximately half of the vaccines distributed worldwide. The current methods used for assessing the quality of vaccines are labor-intensive, require specialized training and laboratory environments, and are resource-intensive and time-consuming. Notably, these methods cannot be performed in situ and require the destruction of samples before analysis.
Here, we propose using deep–UV resonance Raman (DUVRR) spectroscopy as an in situ, direct, label-free, and rapid method for probing mRNA degradation in an mRNA vaccine model system. DUVRR spectroscopy allows the selective probing of nitrogenous bases in RNA by tuning the excitation laser wavelength within the absorption band of the bases. As a result, the resonance effect occurs, allowing probing of the nitrogenous bases with higher sensitivity and selectivity. Compared to nonresonance conditions, the resonance enhancement of the Raman signal allowed for a significant reduction in spectral interference from the matrix and a focus on the analysis of the nitrogenous bases. Extensive literature is available on the application of DUVRR spectroscopy for the characterization of nitrogenous bases. Accordingly, DUVRR spectroscopy could characterize RNA at various manufacturing stages, including product quality validation and establishment of storage condition parameters, where RNA integrity must be verified. Considering that portable DUVRR spectrometers are commercially available, the developed methodology opens a worldwide opportunity for testing the stability of mRNA vaccines in situ before the usage.

(AWD-04.3)**The Wonderful Land of Miniaturization in Near-Infrared Spectroscopy**

Christian Huck, *University Of Innsbruck, Institute Of Analytical Chemistry*

Vibrational spectroscopy (mid-infrared, MIR; near-infrared, NIR; and Raman) has become increasingly important tool of analytical chemistry. Past decade was a particularly vivid period of miniaturization of spectrometers with aim reaching the concept of a ‘lab on a chip’. In this context, particular attention should be directed at near-infrared (NIR) spectroscopy. It offers especially potent

suite of qualities forming rapid, non-destructive, and cost-effective analytical tool. It has widespread over wide field of applications, with the most prominent ones including agriculture, food analysis, forensics, security, and industry, where it often serves as the primary quality control tool [1]. However, the portability and miniaturization of the spectrometers forms particularly strong bond with the conventional advantages of NIR spectroscopy that have opened a new era in its analytical applications [2]. It enabled bringing all the practical qualities of NIR analysis directly to the measurement site making it possible to perform analysis in the field and in real-time. Overall, this new technology has expanded the potential applications of NIR spectroscopy analysis far beyond traditional laboratory-based systems.
The dynamic development of the synergy between miniaturized, on-site capable NIR spectrometers and new tools for spectral analysis has increased the potential and reliability of NIR spectroscopy in various applications. Current trends favor development of devices, which can be used by non-experts for rapid and routine analyses. This has expanded the use of NIR spectroscopy beyond research laboratories and into industries where quick and accurate analysis is critical, such as food and agriculture. This progress revolutionizes NIR spectroscopy, making it a more versatile and accessible analytical technique with a wide range of potential applications including everyday life use.

[1] Beć, K.B.; Grabska, J.; Huck, C.W. Miniaturized near-infrared spectroscopy in current analytical chemistry: from natural products to forensics. In: Molecular and laser spectroscopy - Advances and applications. Vol. 3, Gupta, V.P. Ed.; Elsevier, 2022, pp. 141-188. DOI: 10.1016/B978-0-323-91249-5.00009-0
[2] Beć, K.B.; Grabska, J.; Huck, C.W.; Principles and applications of miniaturized near-infrared (NIR) spectrometers, Chem. Eur. J. 2021, 27, 1514-1532. DOI: 10.1002/chem.202002838

(AWD-04.4)**Spectroscopic Approaches to Biomedical Analysis**

Duncan Graham, Waleed Hassanain, William Tipping, Sian Sloan-Dennison, Karen Faulds *Spring SciX, University of Strathclyde,*

Raman spectroscopy is an attractive technique for the analysis of biomolecules due to the rich information provided. However, due to the lack of sensitivity, Raman spectroscopy has struggled to become adopted in many widespread biological applications. To enhance the sensitivity of Raman spectroscopy, surface enhanced Raman spectroscopy (SERS) can be used and this presentation will focus on the latest developments of using SERS from Strathclyde for point of care applications. In the first example a SERS lateral flow assay has been devised to detect the presence of the infection clostridium difficile and shows the ability to perform this detection in a faster time and more sensitively than currently available approaches. The second example will focus on the detection of drug induced liver injury and shows the ability of SERS to be used to detect the specific biomarker, keratin 18 to indicate the presence of liver injury. These two examples demonstrate the utility of SERS for point of care applications and are the basis for further translational efforts into moving SERS from the laboratory to the clinic.

(AWD-04.5)**Strategies for the Successful Implementation of Molecular Spectroscopy Across the Biopharmaceutical Value Chain**

Andrew Whitley, Linda Kidder, Sean Travers, Jeffrey Julien, *HORIBA,*

The implementation of spectroscopy in the biopharmaceutical industry is much more that a well designed and successful experiment in the laboratory. Success requires collaboration between spectroscopists, biopharmaceutical scientists, manufacturing engineers and regulatory experts. Transferring success in a cuvette to long term implementation and acceptance in the biopharmaceutical value chain is not a trivial task and a failure can happen for many reasons. Some of the biggest failure points are sample handling, automation, software and regulatory acceptance. We will discuss these requirements using examples of Raman and fluorescence spectroscopy that highlight both the opportunity and challenges to enable successful solutions in development, formulation, manufacturing, quality and product rapid release.

23CHEM04: Chemometrics in Food and Agriculture, Southern Pacific E
Chair: Mengliang Zhang

(CHEM-04.1)**Authentication of Edible Oils Using an Infrared Spectral Library and Digital Sample Sets for Calibrated and Uncalibrated Adulterants**

Barry Lavine, DIsio Sota-Uba, Collin White, Karl S. Booksh, *Oklahoma State University, University of Deleware*

A machine learning method to determine whether two varieties of edible oils can be differentiated by Fourier transform infrared (FTIR) spectroscopy is demonstrated using digitally generated data of adulterated edible oils from an FTIR spectral library. The first step is the evaluation of digitally blended data sets. IR spectra of adulterated edible oils are computed from digitally blending experimental data of the IR spectra of an edible oil and the adulterant (a less expensive edible oil) using the appropriate mixing coefficients to achieve the desired level of adulteration. To determine whether two edible oils can be differentiated by FTIR spectroscopy, pure IR spectra of the two edible oils are compared to IR spectra of two edible oils digitally mixed using machine learning to solve a ternary classification problem. If the IR spectra of the two edible oils and their binary mixtures are differentiable, then differences between the IR spectra of these two edible oils are of sufficient magnitude to ensure that a reliable classification by FTIR spectroscopy can be obtained. Using this approach, the feasibility of authenticating edible oils such as extra virgin olive oil directly from library spectra is demonstrated. For this study, both digital and experimental data are combined to generate training and prediction sets for method validation and to assess detection limits in FTIR spectroscopy for both calibrated and uncalibrated adulterants.

(CHEM-04.2)**Infrared Solutions for Rapid Sensing of Food Contaminants**

Luis Rodriguez-saona, *The Ohio State University*

Unintentionally chemical contaminants in food, such as environmental, process contaminants and natural toxins, can pose public health concerns if their concentrations are not kept at their appropriately low levels, as the health risks from dietary exposure cannot be fully avoided. Their potential harm to human health is that they may cause chronic diseases from the long-term low-level dietary exposures. Effective surveillance and response systems are required to prevent chemical hazards from entering the food supply and posing harm to the public. Current methods for testing chemical contaminants rely on chromatography combined with mass spectroscopy (GC-MS and LC-MS/MS) offering analytical sensitivity, selectivity, specificity, and reliability, but they are time-consuming, expensive, labor-intensive, require complex procedures of sample pretreatment and well-trained technicians to operate the instrumentation, and do not allow field deployment. Combining infrared spectroscopy with chemometric analysis would permit to uncover the unique spectral signature profiles (“fingerprints”) of contaminants present in foods based on subtle biochemical changes in the matrices. We will present the use of supervised pattern recognition techniques such as Soft Independent Modeling Class Analogy (SIMCA) and Orthogonal Partial Least Squares Discriminant Analysis (OPLS-DA) to screen for the presence of pesticides in cocoa beans and aflatoxins in peanuts. Infrared technology in tandem with machine learning can provide efficient, throughput and reliable methods for screening for contaminants, allowing for "real-time" decisions that safeguard the safety of high-risk products.

(CHEM-04.3)**Use Of Chemometrics And Factorial Multivariate Analysis Of Variance For Identification Of The Impact of Genetics, Environment, Management, And Processing On Plant Food Composition**

James Harnly, *US Department of Agriculture*

The variance of food composition depends on numerous factors, the organism genus and species (Genetics), Growing location and climate (Environment), farming methods (organic, conventional, fertilization (Management), and all aspects of commercial handling (Processing). Each of these

factors can cause variation in both macro and micro components. Over the years, MAFCL has applied numerous analytical methods (IR, NIR, LC, LC-MS, FI-MS, and NMR) to a wide range of plant materials (e.g., broccoli, grapefruit, mushrooms, and cranberries) and botanical supplements (e.g., Ginseng, Echinacea, Maca, Black Cohosh). Score plots from Principal Component Analysis is the most useful means of non-supervised inspection of a data set. The derived information is dependent of the metadata, data concerning G, E, M, and P. The following step includes identification of those components that are different and supervised determination of statistical significance of the differences. Finally, evaluation of each of the factors using all the components or complete chromatograms and spectra is achieved using factorial multivariate analysis of variance (ANOVA). Analysis of food composition is only a snapshot in time and understanding the degree and sources of variance are essential to evaluating the human diet.

(CHEM-04.4)**Detection of Insect Infestation of Milled Grain-Focus on Tribolium castaneum**

Rabi Musah, Amy Osborne, Samira Beyramysoltan, *University At Albany-SUNY, GSK*

Insect infestation of agricultural stored-products is a significant challenge to food security across the globe. One common pest is Tribolium castaneum (red flour beetle). In a new approach to addressing the threat of these beetles, Direct Analysis in Real Time-High Resolution Mass Spectrometry was used to process infested and un-infested flour samples. These samples were then distinguished through statistical analysis techniques, including EDR-MCR, in order to highlight the important m/z values contributing to the differences in the flour profiles. A subset of these values responsible for the identification of infested flour (nominal m/z 135, 136, 137, 163, 211, 279, 280, 283, 295, 297, and 338) were further investigated and compounds responsible for these masses included 2-(2-ethoxyethoxy)ethanol, 2-ethyl-1,4-benzoquinone, palmitic acid, linolenic acid and oleic acid. These results have the potential to lead to a rapid technique by which flour and other grains can be tested for insect infestation.*

*Osborne, A.M., Beyramysoltan, S., Musah, R.A., Distinguishing Infested Flour from Uninfested Flour through Chemometric Processing of DART-HRMS Data─Revealing the Presence of Tribolium castaneum, the Red Flour Beetle, Journal of Agricultural and Food Chemistry, DOI: 10.1021/acs.jafc.3c00685

(CHEM-04.5)**Application of Self-Optimizing Support Vector Classifier-Radial Basis Function for Multivariate Classification of Maca Metabolomic Mass Spectral Profiles from China and Peru**

Qudus Thanni, Peter Harrington, *Ohio University*

Support vector classifier (SVC) is frequently used with kernels when linear models are inadequate to define class boundaries. One of the most commonly used kernels in conjunction with SVC is the radial basis function (RBF). The RBF function and SVC were optimized by adjusting the width parameter (s) and the cost parameter (λ), respectively. Internal bootstrapped Latin partition (BLP) was incorporated into classifiers to enhance parameter optimization and model generalization. This technique reduces bias by randomly selecting prediction subsets, and the parameters with the lowest average prediction errors across each bootstrap iteration are then chosen. Maca mass spectra in the negative and positive ion modes were classified by country of origin: China and Peru. The Peruvian samples were six times the size of the Chinese samples, requiring synthetic data construction to address the dataset imbalance. The synthetic and half of the real data were used for model-building while the remaining half of the real data was used for validating both models via an external BLP. The performance metrics of the self-optimized radial basis function - support vector classifier (SO-RBF-SVC) were compared to those of other classifiers, such as super SVC (sSVC) and super partial least squares-discriminant analysis (sPLS-DA). The result of the pairwise model comparison showed an improvement in prediction accuracies obtained for SO-RBF-SVC and sPLS-DA when using synthetic data compared to real data. Generalized sensitivity analysis (GSA) was used to evaluate the nonlinearity of the SO-RBF-SVC model. All data analysis was conducted through the MATLAB® programming language environment, employing in-house developed functions and computer systems.

23CTP/EARLY02: AMA: Women in Analytical Sciences, Sierra 2
Chair: Andrea Locke

(CTP-02.1)**An Imaging Chemist in a Cancer Biology World: (Scientific) Imagination, Life is Your Creation**

Fay Nicolson, *Dana-farber Cancer Insitute / Harvard Medical School*

Fay Nicolson obtained her PhD from the University of Strathclyde, UK in 2018. She then joined the Department of Radiology at Memorial Sloan Kettering Cancer Center (New York, USA) as a Postdoctoral Research Scholar and relocated to Boston where she is currently a Postdoctoral Research Fellow in the Cancer Biology at Dana-Farber Cancer Institute and Harvard Medical School. In January 2024, she will open her own independent lab as a Tenure-Track Assistant Professor in the Department of Imaging at DFCI and the Department of Radiology, Harvard Medical School. Her research interests lie in the development and translation of molecular imaging technologies and radiotheranostic agents for the combined detection, evaluation, and treatment of cancer. Her work in this area has been recognized through awards and fellowships including the Metrohm USA Young Chemist 2020 (Runner-up), DFCI Trustee Science Committee Postdoctoral Fellowship and a K99/R00 Pathway to Independence Award from the National Cancer Institute/National Institutes of Health. Fay Is an active member of the World Molecular Imaging Society's "Women in Molecular Imaging Network", and the Society for Applied Spectroscopy's (SAS) "Early Career Interest Group" where she serves as founding chair. She is grateful to the mentors who have encouraged and supported her thus far in her career and is committed to promoting and advancing the next generation of scientists. In this talk, Fay will discuss her career journey so far including the transition from a PhD in spectroscopy in Scotland, to a postdoc in the United States in Cancer Biology, and beyond.

(CTP-02.2)**A Scientific Journey From Scotland To Silicon Valley**

Kristy Mckeating, *Google*

Academia or industry? It's the perpetual question that can plague scientists at many points in their career. For some the decision is easy, for others like myself, it can be a bit more daunting. What path to pursue can often be blurred by factors outside of professional aspirations such as global location, friends, family, mentors…imposter syndrome. Everyone's journey through the decision is different, but during this talk I will share my own experiences after completing a PhD as I pursued two post-doc positions before taking a job in industry at Fitbit. I will talk about the move from academic research to the tech industry, with an emphasis on how I feel being a woman in the sciences has influenced both roles. Hopefully during this talk I can provide some useful professional and personal insights from my career journey so far and can lend some help to anyone currently in the grips of the academia versus industry debate.

(CTP-02.3)**From Small Town to Big City: One Girl's Journey**

Cristina Zavaleta, *University Of Southern California*

Growing up in an isolated border town on the most Southern tip of Texas isn't the most likely start to an academic career, but that's where my journey began. I had no clue what a Ph.D. was, nor had I ever had the opportunity to meet a scientist, but somehow, I was destined for academia. During my talk, I will share some of my experiences along the way that influenced my decision to pursue a career that focuses on research, teaching, and service within the academic infrastructure. I will also discuss what I've learned in the last 5 years as an assistant professor and try to pass along some helpful "nuggets of wisdom" to those who are interested in forging an academic path.

23FORENS01: Nuclear Forensics, Southern Pacific A/G
Chair: Robert lascola

(FORENS-01.1)**Sensor fusion, experimental design, and chemometrics for monitoring uranium(VI) in the presence of lanthanides and corrosion products**

Luke Sadergaski, Hunter Andrews, Brandon Wilson, *Oak Ridge National Laboratory,*

Chemometric analysis of fused spectra from multiple optical sensors, including laser-induced fluorescence spectroscopy, Raman scattering, and UV–visible–near-infrared spectrophotometry, indicate the potential for real-time quantification of uranium(VI) (0–100 μg·mL−1), europium (0–150 μg·mL−1), samarium (0–250 μg·mL−1), praseodymium (0–350 μg·mL−1), neodymium (0–1,000 μg·mL−1) and nitric acid (2–4 M) with varying corrosion product levels (iron, nickel, and chromium). Partial least squares regression models were built from a training set selected by D-optimal design to minimize the required samples in the seven-factor design space and cover the anticipated conditions determined by simulated burnup and ingrowth. The regression analysis was optimized by evaluating numerous preprocessing combinations and a genetic algorithm for feature selection. The top models resulted in root mean square error of prediction values of 5% or less. This approach can be leveraged for monitoring chemical processing streams in the nuclear field and potentially characterizing the burnup of irradiated nuclear fuel.

(FORENS-01.2)**On-Line Monitoring of Nitric Acid Concentration in Advanced Nuclear Fuel Reprocessing**

Catriona McFarlan, Alison Nordon, Mark Sarsfield, Robin Taylor, *University of Strathclyde, National Nuclear Laboratory, Sellafield, UK*

The plutonium uranium reduction extraction (PUREX) process is a common industrial method for the recycling of uranium and plutonium from spent nuclear fuel. It is based on the extraction of actinides from an aqueous phase of nitric acid to an organic phase of tributyl phosphate in a hydrocarbon-based diluent. The concentration of nitric acid affects the extraction efficiency, and is generally measured by titration. However, titration is time-consuming and generates significant volumes of additional waste. Optical spectroscopic techniques can be utilised as an alternative to perform fast, automated measurements on-line, without generating waste.

The effectiveness of Raman and mid-infrared (MIR) spectroscopy was first evaluated for the quantification of nitric acid in PUREX-relevant mixtures. Samples of 0 – 12 M nitric acid in the aqueous phase and 0 – 1.10 M nitric acid in the organic phase were analysed and partial least squares (PLS) regression models were built to predict nitric acid concentration. More accurate predictions of nitric acid concentration were obtained for MIR spectroscopy than for Raman spectroscopy, with root mean square error of prediction (RMSEP) values of 0.099 M versus 0.148 M obtained for the aqueous phase and root mean square error of cross validation (RMSECV) values of 0.006 M versus 0.013 M obtained for the organic phase.

There are challenges associated with acquiring calibration data on-line in a nuclear process environment and so the potential to apply models built in a non-active environment on-line during the PUREX process was investigated. The PLS models built to predict nitric acid concentration in the non-active organic phase samples were applied to spectra of organic phase nitric acid samples containing uranyl nitrate, acquired on different instruments. Systematic prediction error correction (SPEC) was used to correct the predictions for the presence of uranyl nitrate. An improvement in RMSEP of an order of magnitude could be obtained after the application of SPEC for both Raman and MIR spectroscopy. This provides a potential solution to the challenge of acquiring calibration data in a nuclear process environment, to facilitate on-line monitoring of nitric acid concentration and ensure the efficiency of the PUREX process.

(FORENS-01.3)**Quantifying Dense Multicomponent Slurries with In-line ATR-FTIR and Raman Spectroscopy: A Hanford Case Study**

Rupanjali Prasad, Steven Crouse, Ronald Rousseau, **Martha Grover,** *Georgia Tech,*

The multiphase nature of slurries can make them difficult to process and monitor in real time. For example, the nuclear waste slurries present at the Hanford site in Washington State are multicomponent, multiphase, and inhomogeneous. Current analytical techniques for analyzing radioactive waste at Hanford relies on laboratory results from an on-site analytical laboratory, which can delay processing speed and create exposure risks for workers. However, in-line probes may provide an alternative route to collecting necessary composition information. In this work, Raman spectroscopy and attenuated total reflectance - Fourier transform infrared (ATR-FTIR) spectroscopy are tested on simulants of nuclear waste slurries containing up to 23.2 wt%

(FORENS-01.4)**Online Monitoring of Hydrogen Processing using Hollow Core Waveguide-Based Raman Spectroscopy**

John Kelly, Robert Lascola, *Savannah River National Laboratory,*

We describe the development of an online monitor for hydrogen isotopologues based on commercially available internally reflective hollow core waveguides. The goal is to detect the various H/D/T mixes at concentrations at or below 200 ppm with challenges that include: materials that are resistant to beta radiation; the minimization of spectral interferences from materials used in construction; and the reduction of the instrumentation's footprint to facilitate glove box implementation. We will describe and characterize the performance of our prototype system, identify areas of improvement for future iterations, and outline plans for the eventual implementation in hydrogen processing systems at SRNL.

(FORENS-01.5)**Cascading optical processes and their impacts on spectroscopic characterization of nanoscale or larger materials**

Dongmao Zhang,*Mississippi State University*

The term "cascading optical processes" pertains to a series of consecutive optical phenomena that occur in response to individual excitation photons. Instances of these cascading processes involve the re-scattering of scattered photons, light emission followed by absorption, and the absorption of emitted or scattered photons. During my presentation, I will share several recent research studies that explore cascading optical processes in solutions containing nanoparticles with various optical complexities. These complexities range from pure absorbers or scatterers to light absorbers, scatterers, and emitters. Additionally, I will delve into how these cascading optical processes affect common spectroscopic methods such as UV-vis, scattering, and fluorescence spectroscopy.

23IR04: Nanoscale IR in Bioscience, Sierra 3
Chair: F. Simone Ruggeri

(IR-04.1)**Lipids Uniquely Alter the Secondary Structure and Toxicity of Amyloid beta 1-42 Aggregates.**

Dmitry Kurouski, *Mississippi State University*

Abrupt aggregation of amyloid b1-42 (Ab) peptide is a hallmark of Alzheimer's disease (AD), a severe pathology that affects more than 44 million people worldwide. A growing body of evidence suggests that lipids can uniquely alter rates of Ab1-42 aggregation. However, it remains unclear whether lipids only alter rates of protein aggregation or also uniquely modify the secondary structure and toxicity of Ab1-42 oligomers and fibrils. In my talk, I will discuss the effect of phosphatidylcholine (PC), cardiolipin (CL), and cholesterol (Chol) on Ab1-42 aggregation. We found that PC, CL and Chol strongly accelerated the rate of fibril formation compared to the rate of Ab1-42 aggregation in the lipid-free environment. Furthermore, anionic CL enabled the strongest acceleration of Ab1-42 aggregation compared to zwitterionic PC and uncharged Chol. We also found that PC, CL

and Chol uniquely altered the secondary structure of early-, middle- and late-stage Ab1-42 aggregates. Specifically, CL and Chol drastically increased the amount of parallel b-sheet in Ab1-42 oligomers and fibrils grown in the presence of these lipids. This caused a significant increase in the toxicity of Ab : CL and Ab : Chol compared to the toxicity of Ab : PC and Ab1-42 aggregates formed in the lipid-free environment. These results demonstrate that toxicity of Ab aggregates correlates with the amount of their b-sheet content, which, in turn, is determined by the chemical structure of lipids present at the stage of Ab1-42 aggregation

(IR-04.2)**Effect of Co-Incubation with RNA on the Formation of Alphasynuclein Aggregates**

Antonia Intze, Jakob Rupert, Maria Eleonora Temperini, Raffaella Polito, Elsa Zacco, Gian Gaetano Tartaglia, Michele Ortolani, **Valeria Giliberti,** *Sapienza University Of Rome, Centre for Human Technologies, Istituto Italiano di Tecnologia*

The study of protein aggregation is one of the main biological research fields for which the use of an AFM-assisted infrared (IR) spectroscopy has proved to be of great relevance [Ruggeri et al. Nat. Com. 2015]. Protein aggregation takes place upon protein misfolding, and several efforts are ongoing to gain a deep understanding of this phenomenon also due to the connection that has been identified with many neurodegenerative diseases. In our recent experiments we applied AFM-assisted photothermal expansion IR nanospectroscopy technique (AFM-IR) supported by FTIR microspectroscopy to alphasynuclein protein (αS), identified as the primary component of pathological aggregates found in Parkinson's Disease, with the specific aim to study the effect of the incubation of αS with RNA on the protein aggregation. Recent studies have shown that RNA influences nonlinearly αS in vitro aggregation rate in a RNA concentration- and αS sequence-dependent manner [Rupert et al. bioRxiv 2022]. With the aim to probe possible structural differences between the αS and αS+RNA fibrils, we took advantage of the unique capability of AFM-IR of identifying and selecting - by means of AFM - agglomerates of fibrils within the variety of morphologically diverse protein aggregates, and of obtaining the AFM-IR spectrum of these fibrils. Samples consist of protein aggregates deposited on ultraflat template stripped gold surfaces at different times during the in vitro aggregation. The combined use of ultraflat gold surface and gold-coated AFM tips enabled to exploit the strong field enhancement and confinement in the tip-surface nanogap and increase the signal-to-noise ratio of the AFM-IR spectra [Giliberti et al. Nano Lett. 2019]. Our results show an effect of RNA on the kinetics of aggregation, in agreement with previous studies. In addition, the analysis of AFM and AFM-IR data acquired on fibrils suggests morphological and structural differences among the amyloid fibrils forming either in the presence of RNA or not: the co-incubation with RNA produces amyloid fibrils with a lower mean height, and whose amide-I absorption band shows a spectral weight transfer of the β-sheet component towards low frequencies, suggesting a different conformation in the two cases. Further measurements and analysis are ongoing.

(IR-04.3)**Investigating the bacterial protein quality control system with AFM-IR nanospectroscopy**

Wouter Duverger, Grigoria Tsaka, Katerina Konstantoulea, Ladan Khodaparast, Laleh Khodaparast, Nikolaos Louros, Joost Schymkowitz, Frederic Rousseau, *Catholic University Leuven / Flemish Institute of Biotechnology,*

Protein aggregation is a universal phenomenon occurring, for example, as a response to cellular stress or a consequence of ageing. It is also causally linked to several human neurodegenerative diseases, such as Alzheimer's and Parkinson's.

FTIR spectroscopy has revealed that the secondary structure of aggregated proteins in bacteria depends strongly on the type of stress applied. However, these studies have always been performed in bulk and could not assess the variation within a sample. Therefore, we took advantage of the exceptional resolution of AFM-IR and its sensitivity to mechanical stiffness to directly determine the structural and mechanical heterogeneity of protein aggregates inside bacteria.

We measured the differences between aggregates in situ using this emerging method for nanoscale infrared spectroscopy. We developed an image processing pipeline tailored to AFM-IR data to capture the concentration of misfolded protein (as measured by beta-sheet content) and their mechanical stiffness within the cells and automatically segment images into cells and aggregates. Our results show these parameters are not linked to an aggregate's size or location in the cell, but they do vary depending on the conditions in which the aggregates were formed: aggregates caused by a heat shock are larger, stiffer and more numerous than spontaneously formed ones. Also, aggregates measured after a one-hour recovery period were less stiff yet had a higher concentration of misfolded protein than freshly formed aggregates. We offer evidence for a multi-stage aggregate disassembly process that is most likely caused by the consecutive actions of the bacterial chaperones DnaK and ClpB.

(IR-04.4)**IR nanospectroscopy to investigate biomaterials: where do we stand?**

Ariane Deniset-besseau, Jérémie Mathurin, Margaux Petay, Dominique Bazin, Alexandre Dazzi, *Université Paris-saclay,*

Over the past decade, the AFM-IR technique has gradually evolved from "proof of concept" technique to a more reliable technique, especially for biomaterials. The field of applications is extremely vast and covers fields as diverse as molecular biology, microbiology, medicine, geology and ancient materials. Currently, the AFM-IR measurements is implemented with 3 different AFM modes (contact, tapping, peakforce tapping). More recently, our team have developed a new acquisition mode called 'sensitive surface'. During the oral presentation, several examples on keratinized tissue will be presented to illustrate the capabilities of the AFM-IR technique on biomaterial, in contact, tapping and surface sensitive mode. The main experimental constraints will be discussed. An assessment will be proposed as well as the future challenges to which the technique will have to respond to rise to the level of the other vibrational spectroscopy techniques currently used routinely.

(IR-04.5)**Nanoscale Hyperspectral IR Characterization of Amyloid Proteins via IR PiFM**

Sung Park, **Derek Nowak**, Padraic O'Reilly *Molecular Vista,*

Given the potential linkage between Alzheimer's disease (AD) and the self-assembly of amyloid peptide into aggregates, amyloid fibrils have been characterized via AFM-IR, which couples atomic force microscopy with infrared spectroscopy, to gain a better molecular understanding of its self-assembly. Most of the published results on nanoscale studies of amyloids have been based on Nano-FTIR or photothermal infrared nanospectroscopy (PTIR) forms of AFM-IR. Infrared Photo-induced Force Microscopy (IR PiFM) is another form of AFM-IR, which combines IR spectroscopy with non-contact atomic force microscopy (AFM) to acquire hyperspectral IR data via mechanical detection of attractive dipolar forces acting on the tip in response to IR absorption by the sample. Due to the extreme short-range of the dipolar force, IR PiFM is more surface sensitive than other AFM-IR techniques and offers higher spatial resolution.

For amyloids, amide I and II bands are readily accessible to IR spectroscopy and provide ways to interrogate the molecule's local chemical environment. IR PiFM can provide both high resolution absorption map at a fixed wavenumber and full PiF-IR spectrum (analogue to FTIR spectrum) with a spectral resolution of ~1 to 3 cm-1 (depending on the type of laser used) and spatial resolution of ~5 nm. IR PiFM capability will be demonstrated on amyloid fibrils as individual fibers are coming together to form a larger bundle. With PiF-IR, the contributions of secondary protein structures to the amide I band are clearly visible without having to differentiate the spectrum as is the case with bulk FTIR spectra. A hyperspectral data set consisting of 256 x 256 pixels of full PiF-IR spectra is analyzed via principal component analysis (PCA) and multivariate curve resolution (MCR) technique to visualize the nanoscale chemical make-up of the bundle.

23LIBS01: The New LIBS Generation, Southern Pacific B/C
Chair: Hunter Andrews

(LIBS-01.1)**3D Mapping of Uranium Absorption and Migration in Ex-vivo Human Skin with Femtosecond-LIBS and LA-ICP-MS**

Gregory Hull, Brian Jun, Jennifer Harris, Jeremy Inglis, *Los Alamos National Laboratory,*

The Los Alamos National Laboratory in-vitro bioassay program currently monitors workers from across the lab at occupational risk of radionuclide exposure to plutonium, uranium, americium and tritium. The in-vitro bioassay program is largely based upon monitoring urinary excretion levels which requires an understanding of the solubility and absorption of the radionuclide by the worker and its subsequent excretion. Of particular concern are acute exposures that can occur during serious incidents such as glovebox breaches (leading to inhalation of uranium or plutonium particles) or puncture injuries which can pierce laboratory gloves and skin and embed particles in tissue directly. In these cases, little is still known about the mechanisms and levels of radionuclide absorption in the localized trauma region.
In order to investigate the absorption of radionuclides after an incident, we have exposed a set of living human tissue samples to aqueous and solid uranium contamination. Using femtosecond laser ablation with both LIBS and LA-ICP-MS, we are investigating whether the migration of heavy metals can be tracked as the cells multiply and the tissue grows. Measuring samples daily over a period of weeks enables the spread and migration of analytes through the matrix to be followed in complex biological systems. 3D visualization can be achieved by exploiting the cratering of a sample surface which is inherent in the analytical method. Tracking uranium migration from a puncture site may provide a better understanding of injuries caused by the handling of dangerous metals and how to limit the extent of tissue damage from such injuries.
LA-UR-23-22774

(LIBS-01.2)**Nebulization-assisted LIBS as a potential tool for online analysis of liquids including complex halogen-containing samples**

Cristina Méndez-lópez, Luis Javier Fernández-Menéndez, Cristina González-Gago, Jorge Pisonero, Nerea Bordel, *Department Of Physics, University Of Oviedo,*

The present work deals with the analysis of liquid samples with LIBS, which usually requires relying on instrumental modifications and/or appropriate sampling procedures that increase the complexity of the set-up and the time of analysis [1]. Special focus is made on samples containing halogens, which are indirectly determined via molecular emission [2] rather than using atomic emission either through the resonant lines in the VUV range, which would require a vacuum chamber or, alternatively, the less intense IR lines. Particularly, this problem has been approached with a nebulization-assisted LIBS scheme, in which an auxiliary solid target containing Ca was ablated while nebulizing the liquid sample. A first approach utilizing $CaCO_3$ pellets has been successfully applied to a set of aqueous NaF samples, achieving a LOD of 10 mg/kg and accurately determining the F content of three mouthwash samples [3]. More recently, the methodology was improved by implementing a cheaper and more versatile Ca-containing target, halving the LOD of F to 5 mg/kg. Likewise, the determination of another halogen (Cl) and a metal (Zn) have been explored to test the generality of the method. In addition, the performance of the methodology has been evaluated for the analysis of organofluorines (which can be challenging due to the strong C-F bonds), demonstrating its capability to provide total F content regardless of the molecular bond in which the halogen is present in the sample. This talk will provide a discussion of the developed methodology, both regarding the particularities of nebulization-assisted LIBS and the experimental results of its application for the analysis of liquids.

[1] K. Keerthi et al. (2017) Opt. Laser Technol. 147, 107622.
[2] C. Álvarez-Llamas et al. (2017) J. Anal. At. Spectrom. 32, 162-166.
[3] C. Méndez-López et al. (2023) J. Anal. At. Spectrom., 38, 80-89.

(LIBS-01.3)**Optical Spectroscopy of Laser-Induced Plutonium Plasmas**

Emily Kwapis, Eliel Villa-Aleman, Kyle Hartig, *University Of Florida, Savannah River National Laboratory,*

The development of measurement technologies to monitor nuclear materials remains a consistent interest of the nuclear nonproliferation community to identify undeclared nuclear fuel cycle and weaponization activities. Atomic spectroscopy provides the capability to measure the isotopic composition of materials without sample preparation using a high-powered pulsed laser to produce a luminous micro-plasma via laser ablation. Understanding the underlying physicochemical processes of these highly transient plasmas is especially relevant for the actinide elements, which are characterized by heavily crowded atomic spectra consisting of tens of thousands of transitions. Furthermore, uranium and plutonium readily oxidize in reactive atmospheres such as air, resulting in the formation of simple gas-phase actinide oxides that emit over the same energy range as their atoms. Considerable work has been performed towards characterizing oxidation pathways in laser-induced uranium plasmas to explain the impact of the dynamic plume composition on measured spectroscopic signatures. Yet, comparable investigations on the high-temperature gas-phase plutonium-oxygen system are extremely limited despite marked differences in chemistry between the elements. This work aims to explain the oxidation characteristics of laser-induced plutonium plasmas, beginning with the identification and temporal evolution of PuOx signals at atmospheric pressure in air. Implications of measuring the isotopic composition of plutonium samples from molecular isotope shifts will also be discussed.

(LIBS-01.4)**Overview of LIBS Research at Oak Ridge National Laboratory**

Hunter Andrews, *Oak Ridge National Laboratory*

Laser-induced breakdown spectroscopy (LIBS) is an incredibly robust approach to elemental analysis that is capable of probing solids, liquids, gases, and mixed phases such as aerosols. Additionally, this analysis can be done remotely through fiber optics, providing a pathway for elemental monitoring in hazardous environments such as nuclear reactors or radiological hot cells. Oak Ridge National Laboratory (ORNL) is actively developing LIBS systems for monitoring the off-gases from molten salt reactors and is using the same tools to evaluate treatment options for capturing fission gases during reactor operation. For this application, LIBS is tasked with simultaneously monitoring aerosol particles and noble gases. LIBS is also being used to rapidly map advanced nuclear fuel materials, geological samples, and fly ash. In these applications, LIBS provides an avenue to rapidly evaluate elemental composition and correlations, as well as screen materials to identify regions of interest for a more detailed analysis. LIBS is also being used to investigate environmental samples to aid in carbon capture research. In this application, LIBS is used to rapidly evaluate silicon levels in poplar plant samples to be able to identify genotypes that form phytolith-occluded carbon on the leaf surface. This application aims to better understand the relation between silicon levels, plant genotypes, and plant origins in relation to phytoliths to enable bioengineered plants for optimized carbon retention. This presentation will highlight these examples of LIBS applications at ORNL and provide further updates about many of the projects underway.

23PMA03: Tackling Critical Pharmaceutical Challenges with Advanced Spectral Analyses, Southern Pacific D

Chair: Lydia Breckenridge
Co- Chair: Steve Bouffard

(PMA-03.1)**Transmission Raman Spectroscopy Evaluating Process Induced Phase Transformation at Drug Substance/Drug Product Interface**

MIchelle Raikes, Matthew McKay, Christian Reichardt,*Boehringer Ingelheim Pharmaceuticals,*

Process induced phase transformation (PIPT) of an active pharmaceutical ingredient in a drug product can have significant impact on bioavailability, processability, chemical stability and shelf-life. To meet the increasing demand from regulators for control of critical quality attributes in both drug substance

and drug product has proven challenging by using conventional techniques, such as DSC and XRPD, due to interfering signals from multiple component systems. Transmission Raman spectroscopy (TRS) is an attractive alternative tool due to its nondestructive, high-throughput, and at-line quantification of polymorph and amorphous conversion. We will present quantitative Raman methods developed using multivariate partial least squares (PLS) regression calibration techniques from wt/wt% reference values to evaluate PIPT across the DS and DP processing steps. In addition, we will compare results from ssNMR and from a Raman model developed using ssNMR as the reference method. The results demonstrate that TRS offers a fast, sensitive, and high-throughput (<1 min/tablet) method for quantitating polymorph and amorphous conversion. The multivariate PLS Raman model, developed with ssNMR reference, demonstrated as a viable alternative to XRPD and DSC for regulatory GxP release testing over the life-cycle of the drug to control critical quality attributes.

(PMA-03.2)**From Powder Pediatric In-Use Samples To Multi-Dose Tablets, Method Development And Validation Approaches For TRS In Drug Product**

Greg Doddridge, Yemin Liu, Eddie Hong, David Tan, Anh Nguyen, *Abbvie, The University Of Utah*

Transmission Raman (TRS) has proven to be a valuable tool in the drug product space. At AbbVie, we've used TRS for a multitude of applications. In this talk, we'll cover our development and validation approaches of powder formulations dosed in a soft food to a tablet formulation with three doses. The TRS was established as a quick tool to evaluate pediatric in-use samples for the powder formulations and as a viable alternative to HPLC for content uniformity (CU) of the multi-dose tablet formulation.

For the powder formulation, we designed a calibration with variation in API, water, and milled extrudate particle size. And we used a simplified validation approach to assess accuracy, linearity, selectivity, and robustness across a 0.1% to 1.5% (m/m) range.

For the multi-dose tablet formulation, we utilized a couple of experimental design approaches during method development. The first was to evaluate exposure time and # of accumulations such that we could establish appropriate conditions for each dose to enable one chemometric model. And the second was to augment API variation calibration samples with tablets varying in weight and particle size. The resultant PLS model showed acceptable accuracy on tablets across the three doses manufactured at a commercial site. Additionally, we studied variability by exposing the tablets to different RH conditions and evaluating across instruments to understand if any instrumental or environmental parameters played a critical role during test method transfer. A validation plan was proposed within current FDA and ICH regulatory frameworks.

(PMA-03.3)**Leveraging the Versatility of Atomic Spectroscopy for Pharmaceutical Analysis**

Lydia Breckenridge, Sharla Wood, *Bristol Myers Squibb*

Atomic spectroscopic techniques have long been used to provide data regarding inorganic content of pharmaceutical compounds. However, for the most part, the value of this data has been relegated to product or component quality control (QC) assessments. Currently, the regulatory requirement to assess for Elemental Impurities is the most critical test that typically leverages atomic spectroscopy (e.g., by ICP-MS). This work is often outsourced by pharmaceutical companies or manufacturing organizations to contract QC labs. However, independently or in combination, atomic spectroscopy techniques such as ICP-MS, ICP-AES, Laser Ablation, LIBS and XRF can substantially contribute to pharmaceutical research and, as such, a significant case can be made for maintaining an in-house atomic spectroscopy lab. Such a paradigm allows for greater collaboration between chemists, engineers and analytical scientists, which, in turn, provides data more finely tuned to project needs, whether they be for greater sensitivity, more rapid results or something highly unique.
In the Atomic Spectroscopy Center of Excellence at Bristol Myers Squibb, the generation of QC-related elemental impurities data accounts for less than 10% of the total sample volume; indeed, most of the work could be considered more research-focused, providing data that has been skillfully generated by collaborative subject matter experts to assist an array of critical decisions throughout the

pharmaceutical development. This presentation will provide an overview of the pharmaceutical Atomic Spectroscopy lab with an emphasis on the support it provides to pharmaceutical development from discovery through life-cycle management. It will highlight some of the more innovative pharmaceutical applications of various atomic spectroscopy techniques. Among these include the use of ICP-MS as an essential analytical tool for ensuring stability and efficacy of biologics, the use of ICP-AES to provide residual catalyst quantification for organic synthesis optimization, the application of laser-based solid analysis techniques such as laser ablation and LIBS for foreign matter investigations and packaging assessments, and the establishment of an open access XRF to provide rapid inorganic data for development chemists.

(PMA-03.4)**Advanced Imaging Methods For Studying Structure Morphology And Excipients Solid State Transformations In Pharmaceutical Multiparticulate Formulations**

Elizabeth Legge, Mark Stewart, Lourdes Contreras, Hannah Zhang, Dimitrios Tsikritsis, Natalie Belsey, Mark McAllister, John Richard Murphy, Ken Mingard, Caterina Minelli, *National Physical Laboratory, Pfizer Ltd, U.K.*

The formulation of paediatric medicines faces significant challenges to meet the requirements for safe and accurate administration to children of different age groups, while maintaining a suitable taste. Multiparticulate (MP) formulations have a strong potential to address these challenges because they combine dose flexibility with ease of administration. Understanding of the physical stability of MP formulations over storage as a function of time and environmental parameters such as humidity and temperature is important to manage their commercialization, supply chain and use.
Current techniques for studying MP include scanning electron microscopy (SEM) and confocal laser scanning microscopy, whose resulting chemical information is somewhat limited. We have employed advanced methods such as environmentally-controlled SEM to monitor temperature- and humidity-induced changes in-situ, and a range of Raman spectroscopies including stimulated Raman scattering (SRS) microscopy to identify and map the different ingredients at the surface and inside the multiparticulate formulations.
These techniques allowed us to monitor specific changes to the particulate structure and distribution of individual active ingredients of the formulations due to product aging. We envisage these techniques to be useful in furthering the development of a range of future medicines formulations.

(PMA-03.5)**Use of High-Throughput Raman Spectroscopy for Tablet Coating PAT**

Mark Kemper, Shaun Fraser, Colin Couper, *Tornado Spectral Systems, Inc.*

Solid dosage forms, particularly tablets, remain a very important product genre among marketed pharmaceuticals. The tablet coating step is a critical aspect of the production of these formulations. Tablet coatings are needed for a variety of reasons depending on the product. These reasons potentially include 1) improving marketability (coloring), 2) enhancing dosage compliance (masking bitter taste, easier swallowing), 3) serving a simple functional purpose (protecting active ingredients from oxidation or other degradative reactions), and/or 4) serving a complex functional purpose (facilitating sustained release, containing API in the coating). The coating process is important for solid dosage formulations as it is part of the product's regulatory filing and must be properly controlled.

Raman spectroscopy has become an important and ubiquitous technique for real-time monitoring of pharmaceutical processes, including solid dose coating. The work presented in this paper examines the advantages that Process Raman Spectroscopy provides for tracking tablet coating both qualitatively and quantitatively. Different sampling approaches are explored as is the ability to track different types of coatings. The data presented will feature data analysis options which demonstrate that Raman can often be used without necessarily employing complex modeling. The work will show that Raman can be a potentially valuable PAT tool for tablet coating processes.

23RAM04: SERS 3, Cascade 3
Chair: Sian Sloan-Dennison

Co-Chair: Roy Goodacre

(RAM-04.1)**Point Of Care Detection Of Drug Induced Liver Injury Using SERS-Based Lateral Flow Testing**

Sian Sloan-Dennison, Benjamin Clark, Kathleen Scullion, Paul Fineran, Joanne Mair, Dieter Bingemann, Cicely Rathmell, Jonathan Faircloth, David Creasey, James Dear, Karen Faulds, Duncan Graham, *University of Strathclyde, University of Edinburgh, Wasatch Photonics,*

The bestselling non-prescription drug, acetaminophen, is used by millions of people worldwide as a safe method of pain relief. However, there are many cases of paracetamol overdoses, both accidental and intentional. Due to the increase in toxic metabolites caused by overdose, drug induced liver injury (DILI) occurs which can result in patients requiring a liver transplant or death if not treated fast enough. When a patient presents to hospital following an overdose, the severity of it is determined by taking a blood sample and sending it to a lab within the hospital where the level of alanine aminotransferase (ALT), a biomarker for hepatocyte injury, is measured. Although ALT is an established DILI biomarker, its levels following paracetamol ingestion are slow to rise and the lab test takes several hours to complete. Consequently, DILI diagnosis is often missed. Recently, keratin-18 (K18) has become a candidate for the accurate and early detection of DILI and has US Food and Drug Administration regulatory support. To maximize the benefit of K18 a rapid, quantitative, point of care (POC) test must be developed.

For the rapid detection of K18, a paper-based lateral flow immunoassay (LFIA) combined with surface enhanced Raman scattering (SERS) analysis has been developed. By combining the SERS-LFIA concept with a handheld Raman reader, a POC DILI diagnostic test capable of detecting clinically relevant concentration of K18 in patient serum and capillary blood samples in 20 minutes has been produced. Careful consideration of many parameters such as the SERS active gold nanoparticles, LFIA architecture and combination of device with the Raman reader has produced a device which gives reproducible, sensitivity and quantitative results. To test the usability, reproducibility, sensitivity and selectivity, the device has been used in a blinded, pre-clinical trial of 100 patient samples and achieved favourable results. It is now being used in a clinical trial to assess how well it can predict DILI in patients presenting at accident and emergency departments with suspected paracetamol overdoses. Overall, this POC diagnostic test will lead to better patient stratification and shows the potential of SERS measurements outside the lab.

(RAM-04.2)**Neurotransmitter Sensing With Nanosensors for Dynamic Near Infrared Surface Enhanced Raman Spectroscopy**

Ryma Boudries,*Université de Montréal*

We are exploring the use of near-infrared (NIR) SERS microscopy at laser wavelengths greater than 1 μm for the detection of neurotransmitters in biological samples. This approach combines the benefits of SERS spectroscopy, and deep tissue NIR penetration to enable highly sensitive and specific detection of neurotransmitters. Neurotransmitters are critical signaling molecules that play essential roles in brain function and can cause a range of neurological disorders such as Parkinson's Disease (PD) or Alzheimer's Disease (AD). However, conventional detection methods are limited by their sensitivity and selectivity, as well as by the complexity of sample preparation procedures. Due to exponential decay of the scattering coefficient for brain tissues, longer excitation wavelengths are preferable for Raman microscopy, but commercial microscopes are limited to lower wavelengths. Taking into consideration that biological fluorophores and chromophores are most susceptible to visible excitations, it is preferable to work with an excitation wavelength in the near-IR to minimize the autofluorescence background of complex tissues. Hence, we have constructed a microscope with two near-infrared lasers lines at 1064 nm and 1319 nm and gold nanoparticles coated fibers. Our approach produces SERS spectra that can be used to identify and quantify neurotransmitters with accuracy. One of the key advantages is its ability to detect various neurotransmitters , including dopamine, serotonin, and glutamate in complex biological matrices, such as brain tissue. NIR SERS microscopy will, ultimately, enable real-time monitoring of neurotransmitter release in live cells,

providing critical insights into the dynamic nature of neurotransmitter signaling. Overall, NIR SERS microscopy is an innovative approach that has the potential to improve our understanding of the role of neurotransmitters in the brain and provide new insights into the mechanisms of neurological disorders.

(RAM-04.3)**Ambient Focusing Ion Funnel-assisted Electrospray Deposition (ESD) of Gold Nanoparticles for Uniform and Highly Sensitive Surface-enhanced Raman Scattering (SERS)**

Royston Goodacre, Baris Akbali, Barry Smith, Cedric Boisdon, Boonphop Chaisrikhwun, Kanet Wongravee, Tirayut Vilaivan, Cassio Lima, Chen-Han Huang, Tsan-Yao Chen, Simon Maher, *University of Liverpool, Chulalongkorn University, National Central University, National Tsing Hua University,*

We have developed electrospray deposition (ESD) combined with a highly focusing ambient ion funnel for deposition of high density spherical and rod-shaped gold nanoparticles (AuNPs) to generate large-area, uniform SERS substrates for highly sensitive analysis. These SERS substrates exhibited excellent capture capacity. We exemplify this using model analyte molecules – namely 4-aminothiophenol (4-ATP) and Rhodamine-6G (R6G) – with detection limits in the region of 10^{-11} M and a relative signal standard deviation of < 6% over a large area (500 x 500 μm^2).

We found that the combination of ambient ion focusing with ESD facilitated homogenous and well-dispersed deposition of spherical and rod-shaped AuNPs on the substrate surface. This allowed us to take advantage of the polydisperse colloidal solution of AuNPs, as confirmed by FDTD calculations. Finally, we assessed the quantitative performance of our SERS substrate using the R6G probe molecule. The results demonstrated excellent linearity ($R^2 > 0.99$) over a wide concentration range (10^{-4} M to 10^{-10} M) with a detection limit of just 80 pM.

More details here: https://doi.org/10.1039/D3AN01021J

(RAM-04.4)**Dual SERS/colorimetric - RPA sensing platform for antibiotic resistance diagnosis**

Waleed Hassanain, Christopher Johnson, Karen Faulds, Neil Keegan, Duncan Graham, *University of Strathclyde, Newcastle University,*

Antibiotic resistance is as a major global health threat. It is essential for healthcare practitioners to prescribe effective antibiotics with correct dose to mitigate the bacterial infections in a timely manner. Thus, improving the therapeutic outcomes to the patients and prevent the antibiotic resistance dissemination. Accordingly, there is a need to implement a rapid and sensitive clinical diagnosis to identify the resistant strains of bacteria, as well as to monitor the effect of the used antibiotic. In this work, we applied magnetic scaffold-recombinase polymerase amplification (RPA) technique in combination with colorimetric assay and surface enhanced Raman scattering (SERS) detection to specifically amplify and detect the DNA signature of one of the big five resistant Carbapenemase genes, namely: Verona integron-encoded metallo-beta-lactamase (VIM). Herein, streptavidin-coated magnetic beads were functionalised with biotin-modified forward primers. RPA was performed onto the beads surface and the resulting product was an immobilised duplex amplicon with a single overhang tail. This tail was then hybridised with a HRP probe conjugated to a complementary single stranded oligonucleotide and detected colorimetrically. Additionally, it was hybridised with a similar selective SERS probe and scanned with a handheld Raman spectrometer for its SERS detection. The resultant limits of detection were at sub attomolar level for both assays, which serve the aim of the early diagnosis. Compared to the similar approaches like PCR, RPA is a faster, cheaper and isothermal technique working optimally at 37°C, which eliminates the need for a thermal cycler. Therefore, this novel magnetic scaffold RPA-colorimetry/SERS combination for DNA detection demonstrated a strong potential for the rapid monitoring of antibiotic resistance for points-of-care application, in terms of: sensitivity, portability, speed and cost of analysis.

(RAM-04.5)**Colloidal SERS: The Do's and The Don'ts**

Priyanka Dey, *University of Portsmouth, UK*

Since the SERS phenomenon was discovered, it has been extensively explored in sensing applications for chemicals, biomolecules and now increasingly for medical diagnostics. This often requires a qualitative assessment of the presence or absence of molecules and quantitative assessment of their concentration in the assessed sample. These molecules could either be specific molecules-of-interest for direct SERS sensing or known molecules acting as Raman labels for indirect sensing or tracking of the labelled nanostructures. The nanostructure-molecule system determines its SERS enhancements and its limit of detection (LOD) for qualitative analysis using SERS. Although the past decades have demonstrated tremendous research efforts in the field of SERS, 58,000+ articles in the last decade itself, reproducibility and standardization across different research groups of SERS enhancement factors (EF) have still not been practically feasible.

To this end with almost over a decade of experience in colloidal SERS, I will discuss critical factors to experimentally implement and avoid for allowing maximization of the SERS EFs, obtain an improved LOD and importantly, avoid under/over estimation of the enhancements.

23RAM09: Spatially Offset Raman Spectroscopy, Cascade 1
Chair: Bhavya Sharma

(RAM-09.1)**Imaging and Localisation of Nanoparticles in Tissue Using Surface Enhanced Spatially Offset Raman Spectroscopy**

Karen Faulds, Duncan Graham, Matthew Berry, Samantha McCabe, Sian Sloan-Dennison, Stacey Laing, Neil Shand, *University of Strathclyde,*

In recent years, Raman based techniques have been used extensively in bioanalytical research applications with the ultimate goal of creating platforms for medical diagnostics. Surface enhanced spatially offset Raman spectroscopy (SESORS) is a powerful analytical technique that has emerged in an attempt to combine the signal enhancements offered by surface enhanced Raman scattering (SERS) with the subsurface probing in turbid media offered by spatially offset Raman spectroscopy (SORS). Using SESORS it is possible to non-invasively retrieve subsurface spectra that originate from highly specific biofunctional SERS active nanotags inside diffusely scattering objects such as mammalian tissue.

In this work we explore whether SESORS can be used to determine the location of an object within tissue. To address this question multiple experimental factors pertaining to the optical set-up in imaging experiments using an in-house built point-collection based spatially offset Raman spectroscopy (SORS) system were investigated to determine those critical to the 3-dimensional positioning capability of SESORS. Here we report the effects of the spatial offset magnitude and geometry on locating nanoparticles (NPs) as an imaging target through tissue and outline experimental techniques to allow for the correct interpretation of SESORS images to ascertain the correct location of NPs in the 2-dimensional x, y-imaging plane at depth. Additionally, building on these principles, the concept of 'ratiometric SESORS imaging' is introduced for the location of buried inclusions in 3-dimensions. Together these principles are vital in developing a methodology for the location of SERS active inclusions in 3-dimensions. This approach utilises the relationship between the magnitude of the spatial offset, the probed depth and ratiometric analysis of the NP and tissue Raman intensities, to ultimately image and spatially discriminate between two distinct NP flavours buried at different depths within a 3-dimensional model for the first time. This research demonstrates how to accurately identify multiple objects at depth in tissue and their location using SESORS which addresses a key capability in moving SESORS closer to use in biomedical applications.

(RAM-09.2)**Comparison of resonant and non-resonant reporter for the selection of brightest gold nanoparticles for Surface-enhanced Raman spectroscopy.**

Megha Mehta, *University Of Exeter*

The choice of Raman reporter is a significant aspect for improving the imaging sensitivity and multiplexing capabilities of SERS nanoparticles, particularly when attempting to read out Raman

signals from NPs deeply buried in tissues1-3. In this study, we have investigated the combination of three AuNPs with a range of different Raman reporter molecules. Three resonant reporters, IR-125, IR-820, IR-797 and three non-resonant reporters (2-bi-(4-pyridyl) ethylene (BPE), biphenyl-4-thiol (BPT) and 4-mercaptobenzoic acid (MBA) bound to gold nanoparticles of different morphologies – nanospheres and nano-raspberries. We used commercially available AuNPs and in-house synthesised gold nano-raspberries (AuNRBs) using the green chemistry method4 of reduction of gold ion by 2-[4-(2-hydroxyethyl)-1-piperazyl] ethane sulfonic acid (HEPES). The method carried out limits the need for extensive post-synthesis routines of biofunctionalization to improve sensitivity. The appropriate reporter concentration, and volume ratio of reporter to nanoparticle concentration parameters were analysed to provide a valuable assessment of the reporter molecule that gives maximum SERS enhancement for these AuNPs. We have used 785 nm laser excitation to find the brightest 'Raman reporter – gold nanoparticle' combination for further use in deep Raman multiplexed imaging. We have demonstrated that AuNRBs provide significant SERS enhancement with better sensitivity for Raman resonant reporters due to strong label binding affinity of dye to gold surface as compared to non-resonant dyes. It also explains inherently stronger signals generated by surface-enhanced resonance Raman scattering (SERRS), as opposed to surface-enhanced Raman scattering (SERS). These simple, scalable and tunable size AuNRBs are excellent candidates for predicting which Raman reporters could improve sensitivity and be used for deep Raman multiplexed imaging.

(RAM-09.3)**Detecting changes in tissue hydration across different skin tones: a phantom study**

Trevor Voss, Anita Mahadevan-Jansen, *Vanderbilt University,*

There is a need for an accurate, non-invasive, and real-time measurement of physiological hydration. Current metrics generally seek to measure systemic hydration by measuring the concentration of solutes present in collected fluid samples (e.g., sweat, urine, saliva, blood). Other metrics seek to measure bulk tissue properties in order to back out systemic hydration levels (e.g., skin elasticity, bioelectrical impedance). While the tissue hydration can show a more accurate representation of physiological hydration, the current bulk properties measurements leave far too much variability to be considered accurate.
In order to create a more accurate measurement of tissue hydration, we need to directly measure changes in water content and dynamics in tissue both at the superficial level in the epidermis and deeper into the dermis.
High-wavenumber Raman spectroscopy allows us to directly measure changes in water content and dynamics based on its complex molecular environment through its ability to measure changes in hydrogen bonding states in the OH-stretching region. Further, the use of spatially offset excitation and collection fibers allows for measuring both superficially and a few millimeters deep into tissue, allowing for measurement of both the epidermis and dermis.
Human biology, however, is inherently quite variable, especially in the optical properties of human tissues. Lipid concentration is quite different in the epidermis compared to the dermis and thus changes their respective scattering properties. Different skin tones contain different concentrations of melanin, altering the absorption properties.
Tissue mimicking gelatin-based phantoms with varying levels of intralipid and melanin have been made in order to mirror the optical properties of the epidermis and dermis across various skin tones. Their high-wavenumber Raman spectra have been measured using a SORS probe with the concentric rings offset at 1, 2, and 3mm from the center excitation fiber. The use of the SORS probe allows us to measure changes happening in the dermis phantoms underneath the epidermis phantoms. The experiments that would be presented on here are to determine if we can measure changes happening in the dermis despite what is happening in the epidermis.

(RAM-09.4)**SORS and SESORS how deep can we realistically sample?**

Nick Stone, Benjamin Gardner, Sara Mosca, Priyanka Dey, Megha Mehta, William Skinner, Francesca Palombo, Pavel Matousek, *University of Exeter, Ral, Stfc, Ukri, University of Portsmouth, UK,*

Spatially offset Raman Spectroscopy (SORS) and surface enhanced spatially offset Raman spectroscopy (SESORS) can be used to probe a range of depths below the surface in turbid samples.

Most studies to date have considered the mean depth of sampling achievable when separating the illumination and collection zones. However, when considering inclusions or layers with higher signals to the matrix, depths much greater than expected can be achieved.

Here we will discuss how deep we can we go through scattering materials, particulaly biological tissues. This is particulalry relevant for probing breast calcificaitons and Raman reporter labeled SERS nanoparticles.

23SPSJ03: NIR Spectroscopy (Basic Spectroscopy), Cascade 4
Chair: Akifumi Ikehata
Co-Chair: Krzysztof Bec

(SPSJ-03.1)**Interference Effects On Light-scattering Properties In Dense Colloidal Suspensions**
Hiroyuki Fujii, Hyeonwoo Na, Koyata Nishikawa, Kazumichi Kobayashi, Masao Watanabe, *Hokkaido University,*

Dense colloidal suspensions, whose volume fraction is larger than 5%, encounter in various research fields, such as slurry in chemical engineering, milk in food science, and intravenous fat emulsion in medical pharmacy. It is significant to assess non-destructively the structural properties of the suspensions, such as particle size distribution in the sub-nanometer scale and dispersion degree. Near-infrared spectroscopy has the potential to enable the assessment based on a correlation between light-scattering and structural properties. However, the assessment is still under development because the correlation has not been fully understood yet. In a dense suspension, the interference of the electric fields scattered by the particles strongly influences the light-scattering properties, the so-called interference effect. Since the 1980s, many researchers have studied the interference effect in suspensions at different volume fractions using the dependent scattering theory, which is the electromagnetic theory. However, the mechanism of the effect needs to be clarified because of their complicated dependence on the particle size and its distribution, optical wavelengths, etc. We aim to numerically clarify the mechanism of the interference effect using the density expansion approach. We expand the light-scattering properties with the volume fraction up to the fifth order. The expansion order corresponds to the order of the structural properties, e.g., the zeroth order corresponds to the excluded volume of particles. We show that the odd orders correspond to destructive interference, while the even orders correspond to constructive interference. The superposition of different orders' contributions results in destructive interference dominant over constructive interference. We also show that interference strongly influences the scattering coefficient at a wide size parameter range, corresponding to a photon's inverse mean free path in the ballistic regime. Meanwhile, we show less influence on the reduced scattering coefficient, corresponding to the inverse mean free path in the diffusive regime. Our results are indispensable for developing near-infrared spectroscopy using scattered light.

(SPSJ-03.2)**Sophisticated approach of NIR spectroscopy to agricultural and forest products**
Satoru Tsuchikawa, Te Ma, Tetsuya Inagaki, *Nagoya University,*

The uniformity of quality attributes in agricultural and forest products often necessitates the development of non-destructive and efficient evaluation methods to ensure their quality. Near-infrared spectroscopy (NIRS), which analyzes reflected or transmitted absorbance of electromagnetic energy within the 800-2500 nm range, has been extensively researched for non-invasive measurements of organic materials, including food, agricultural, and forest products. The absorption in the NIR region is primarily attributed to overtones and combinations of vibrational bands involving C-H, O-H, and N-H bonds in the infrared (IR) spectrum.
This presentation highlights scientific and technical studies that employ NIRS for the evaluation of food, agricultural, and forest products. As fundamental spectroscopic research continually advances,

the integration of big data, information technology, and spectral imaging techniques enhances material analysis to optimize performance. The development and production of portable, cost-effective devices have facilitated remote analysis capabilities. Anticipated future advancements are poised to expand NIRS applications in numerous fields, enabling online or at-line quality monitoring.

(SPSJ-03.3)**Combination of Near-infrared Spectroscopy with Other Fast Spectroscopic Methods to Improve Discrimination of Geographical Origins of Agricultural Products**

Hoeil Chung, Seongsoo Jeong, *Hanyang University*

Near-infrared (NIR) spectroscopy has been widely used for classification and discrimination of diverse samples such as agricultural products, since it allows fast analysis with no or minimal sample preparation. Meanwhile, Raman spectroscopy provides complementary molecular compositional information of sample for subsequent discriminant analysis. Also, elemental composition of sample such as obtained by laser-induced breakdown spectroscopy (LIBS) would be also comparably useful for discrimination. Therefore, this study attempted to combine NIR information with either Raman or LIBS information to potentially improve discrimination of geographical origins of agricultural products. For the evaluation, two separate studies were executed: Discrimination of geographical origins of a) red pepper powder samples using NIR and Raman, b) soybean paste samples using NIR and LIBS. In the first study, when hetero-correlation two-dimensional correlation analysis of NIR and Raman spectra was performed, the discrimination accuracy was improved compared to those using either NIR or Raman solely. In the second study, two-trace two-dimensional (2T2D) correlation analysis was separately adopted to recognize minute NIR spectral differences. With using the 1st/2nd principal component (PC) scores of 2T2D slice spectra, accuracy was 95.0%. When the ratios of the areas of C and Ca LIBS peaks and the 2nd PC scores of the NIR spectra were combined together, the accuracy improved to 99.6%. In each study, various strategies for the two data-combination and their characteristics will be discussed.

(SPSJ-03.4)**Chemical Interpretation of Meaningful Variables in Chemometric Models by Theoretical Simulation - The Case of NIR Analysis of Pharmaceuticals**

Krzysztof Bec, Justyna Grabska, Christian Huck, *University of Innsbruck,*

The simulation of near-infrared (NIR) spectra using anharmonic quantum chemical methods has become a practically feasible approach recently. It can provide valuable insights not only in fundamental research but also in designing analytical applications. At the intricate connection plane between the mechanistic understanding of the spectra of overtones and combination bands and the applications of NIR spectroscopy, a deeper understanding of the underlying principles of this ubiquitous analytical technique can be achieved. This understanding enables the optimization of chemometric models and the refinement of analytical methodologies, ultimately leading to improved accuracy and reliability of NIR spectroscopy. Furthermore, this approach can facilitate the identification and quantification of complex molecules, such as pharmaceuticals and biomolecules, in complex mixtures.

In this study, a comprehensive investigation was performed on the predictive capabilities of three NIR spectrometers for a model pharmaceutical formulation. The spectrometers examined in this systematic study were the Hefei SouthNest Technology nanoFTIR, featuring a large-mirror Michelson interferometer; a miniaturized multi-channel spectrometer Viavi MicroNIR 1700ES (USB), utilizing linear varaible filter (LVF) technology coupled with an array detector; and the Büchi NIRflex N-500, a benchtop instrument employing a polarization interferometer.

Regression models based on Partial Least Squares (PLSR), Gaussian Process (GPR), and Artificial Neural Network (ANN) were developed for each instrument and mixture component using sets of data pretreatments optimized for each approach and instrument. Spectral precision, reproducibility, and replicability were determined to evaluate the performance of the instruments. To gain deeper insights into the calibration models, the spectra of pure components and mixtures were chemically interpreted with assistance from quantum chemical spectra simulation. The results showed that all instruments

demonstrated satisfactory performance; however, the interplay between the measured wavelength region, spectral resolution, and absorption features of the quantified chemical components had a notable impact on their analytical performance. As a result, the selection of a sensor tailored to predict a specific compound should consider the unique characteristics of the sample and the instrument used.

(SPSJ-03.5)**Investigation on protein hydration and hydrogen bond network of water molecules induced by the secondary structural changes of proteins using near-infrared spectroscopy**

Mika Ishigaki, *Shimane University*

This study investigated how the secondary structural changes of proteins in aqueous solutions affect their hydration and the hydrogen-bond network of water molecules using near-infrared (NIR) spectroscopy. Heating was used to denature three types of proteins, i.e., ovalbumin, β-lactoglobulin, and bovine serum albumin, from α-helix to β-sheet, and variations of NIR water bands were analyzed in relation to the secondary structural changes. Not only the changes in the hydrogen-bond network due to hydration on the protein surface but also due to the whole of water including it are disclosed. The increase in protein concentration made the hydrogen-bond network stronger due to the hydration between protein and water molecules. The variation patterns of the hydrogen-bond network depended on the type of protein and slightly on the solvent species. Such differences may be caused by the differences in hydrophobic and hydrophilic properties on the surface of protein molecules depending on the protein species and their interaction with solvents. The elucidation of the mechanism of protein hydration and the formation of the hydrogen-bond network of water molecules will afford a comprehensive understanding of the protein functioning and dysfunctioning derived from the structural changes in proteins.

23AES01: Bioanalysis, Southern Pacific F
Chair: Juan Santiago
Co-Chair: Md Nazibul Islam

(AES-01.1)**Candida auris infection detection by dielectrophoresis**

Negar Farhang Doost, Soumya K Srivastava, Tagbo Niepa, *Shimane University, West Virginia University, Niepa*

An emerging fungus, Candida auris, poses a serious threat to global health. The number of Candida auris infections approximately doubled in 2021 according to the US Centers for Disease Control and Prevention (CDC). This fungus is a significant health risk due to several reasons. One of the most important reasons is that it is difficult to detect Candida auris using standard laboratory methods, and it can be misidentified in labs without specific technology. Misidentification may result in improper management and treatment of the disease. The common technique to identify Candida auris is blood culture in microbiology laboratories. However, it is more difficult to detect Candida auris from more common types of Candida. As a result, it is necessary to develop a diagnostic tool that will provide sufficient accuracy and sensitivity to detect Candida auris. Due to lack of specificity in current diagnostic tools, we proposed a novel approach for diagnosis using an electrokinetic technique, dielectrophoresis (DEP). DEP involves the movement of a polarizable particle under a non-uniform electric field. Since different cell types exhibit different dielectric properties depending on cell membrane capacitance and resistance, cytoplasmic conductance, and dielectric constant, DEP can provide a means of manipulating, transporting, and sorting them. We utilize this approach on a microfluidic chip which is made of Polydimethylsiloxane (PDMS) and platinum electrodes to generate the electric field. Biocompatible materials are used in microfluidic chips to avoid cell manipulation during the experiment. The suspension of cells in a low conductivity buffer is prepared and a small amount of suspension is used in a microfluidic chip to study the dielectric properties. , This technique enables researchers to isolate Candida auris in whole blood or other body fluid samples as Candida auris has different dielectric characteristics than other cells such as the first crossover frequencies (fc1) which is dependent on medium conductivity and cell phenotype. This method provides fast and accurate diagnoses, thereby reducing the mortality rate from Candida auris infections worldwide.

(AES-01.2)**Device optimization for electrokinetic separation of microparticles**

Alaleh Vaghef Koodehi, Patricia Cyr, Blanca H. Lapizco-Encinas, *Rochester Institute of Technology,*

There is a growing demand for efficient separation of micron-sized particles, including microorganisms. Insulator-based electrokinetic (iEK) systems could be a viable alternative to more traditional methodologies such as centrifugation and membrane filtration. Optimizing the design of the insulating posts in terms of their shape and arrangement has proven to be an effective approach to achieve better separations. This study aims to enhance the efficiency of iEK systems for separating tertiary samples of microparticles by combining statistical modeling and computational simulation. Minitab was employed to conduct a three-stage design of experiments process, using a general full factorial design to select the optimized iEK design, which was then tested with experimentation. COMSOL Multiphysics® was utilized in parallel with the DOE process to model the performance of the iEK device and estimate the retention time of each type of particles being separated. In the first stage of DOE, circle designs were optimized by adjusting the horizontal and vertical gap of openings between insulator posts to achieve a longer retention time for the particles. Next, the optimal length of the insulating posts was determined while considering the three distinct shapes of the insulating posts: Oval-Diamond, Oval-Oval, and Oval-Rectangle. Additionally, the impact of flipping the shape and its effect on the electric field distribution was investigated. The combination of DOE and modeling results indicated that the Oval-Diamond asymmetrical insulating posts with specific dimensions resulted in optimal particle retention time that allowed for effective separations. Also, flipping the geometry considered in stage three of DOE with a p-value above 0.05 was not a significant parameter in the statistical modeling. Finally, the optimized design was employed to perform charge-based and sized-based separation of tertiary microparticle samples. By effectively combining both linear and nonlinear electrokinetic effects, it becomes possible to distinguish and separate samples with three types of microparticles.

Acknowledgments:
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(AES-01.3)**The electrical properties and morphology of selected Candida strains**

Rodrigo Martinez-duarte, Cora Bisbee, Max Vogel, Emma Barnett, Carly Hammond, Alexandra Smith, Alicia Baldwin, Aaron Toler, Michelle Propp, Shivam Yadav, Erin Henslee, *Clemson University, Wake Forest University*

Almost 10 percent of all nosocomial bloodstream infections are caused by Candida spp., with a mortality rate and length of hospital stay exceeding that of other health-care associated infections. Currently, there are four main techniques used in the detection of Candida spp: microscopic examination, fermentation reaction, blood culturing, and molecular diagnostics. However, the identification of the strain behind suspected infection with Candida, or Candidiasis, currently requires at least 24 hours and can be resource intensive. Hence, there is a need for less resource intensive assays to accurately identify the cause of Candidiasis in a timely manner. This is important to enable the timely administration of the most effective antibiotic, to the obvious benefit of the patient, but also to prevent the use of broad spectrum antifungal drugs before a clear diagnosis, which contributes to the generation of antifungal resistance.

Here, we postulate the method of dielectrophoresis (DEP) to identify different strains of Candida. DEP is an electrokinetic method that allows for the dielectric properties of cells to be exploited for sorting purposes. Hence, specific cells would display a specific response to a non-uniform electric field of varying frequency and magnitude. Presented here is an initial study of selected electrical properties and morphological properties of different Candida strains. We show that different strains indeed show different DEP properties but not significant morphology changes.

(AES-01.4)**Characterization of Human Mesenchymal Stem Cells' Electrical Properties using Light-Induced Dielectrophoresis**

Kiara Lacy, Samuel Salib, Mary Tran, Tunglin Tsai, Rominna Valentine, Herdeline Ardoña, Tayloria N.G. Adams, *University Of California Irvine,*

Human mesenchymal stem cells (hMSCs) are a heterogeneous population of cells that offer a source for conducting mechanistic studies of diseases and several therapeutic applications. Therefore, understanding hMSCs properties, such as electrical behavior at various maturation stages have become more important in recent years. The varied differentiation potential of hMSCs based on the source lends to their inherent heterogeneity and creates challenges for applying hMSCs for therapeutics. Therefore, the characterization and enrichment of hMSCs can improve results for clinical applications. Dielectrophoresis (DEP) is a method that can manipulate cells in a nonuniform electric field, through which information can be obtained about the electrical properties of cells, such as cell membrane capacitance and permittivity. Traditional modes of DEP use metal electrodes, for instance, 3-dimensional electrodes, to characterize the response of cells to DEP. However, due to the limitation of a fixed electrode configuration, we implemented light-induced dielectrophoresis (LiDEP), a novel DEP technique. LiDEP utilizes a microfluidic device with a photoconductive layer, which when illuminated with an electrode configuration, can act as an in situ virtual electrode with readily conformable geometries. The aim is to characterize hMSC behavior using LiDEP through manipulating parameters such as input voltage, electrode color, light intensity, and applied frequency which affect the DEP phenomena. We have previously shown that the DEP response of hMSCs and human embryonic kidney cells (HEK 293) can be controlled through these parameters and help establish protocols for single cell analysis and cell sorting. In addition, having multiple responses of the cells for a set frequency and voltage at one time is significant for understanding the heterogeneity of hMSCs population through their electrical behavior. This is critical for advancing the clinical applications of these cells. In the future, we envision that this platform can pave the way for label-free and real-time technologies capable of characterizing heterogenous populations of hMSCs or other stem cell lines.

(AES-01.5)**Dielectric Characterization of Various Cells under Microgravity**

Sai Deepika Reddy Yaram, Soumya K Srivastava, *West Virginia University,*

Human activities beyond Earth have been increasing gradually over the years. During spaceflight, NASA has discovered that the absence of Earth's gravitational force can cause a reduction in the mineral density of weight-bearing bones, typically ranging from 1-1.5% per month. Even after returning to Earth, rehabilitation efforts may not fully restore. The effects can be observed in the muscles, the neuro vestibular system, the heart, the eyes, and more. Hence, understanding the effects of microgravity on the human body is crucial, but sending samples into space is costly and time-consuming. Therefore, methods of simulating
microgravity on Earth have been developed, such as a clinostat, to simulate microgravity conditions in space. Erythrocytes, or red blood cells, are a suitable biosystem for studying the effects of microgravity. The relationship between microgravity-induced metabolic adaptations and their morphological characteristics is little known. This project investigates the changes associated with morphology due to
microgravity-like conditions. The project will design, fabricate, and test a 3D clinostat device and utilize an electrokinetic technique, dielectrophoresis (DEP), to quantify the changes in morphology under microgravity conditions. The red blood cells will be exposed to AC electric fields to analyze their behavior
yielding dielectric properties such as conductivity and permittivity of the cell membrane and cytoplasm under microgravity conditions. The behavior is analyzed using the DEP crossover technique (where no DEP force exists), where the cells move away or towards a high electric field region at a particular AC frequency. These changes in dielectric properties correspond to the cell membranes' morphological changes and the cytoplasm contents of the microgravity-exposed blood cells. The approach involves designing and testing the 3D clinostat, which can hold about three 1.5 ml centrifuge tubes, to expose cells to microgravity and sampling them at various time points starting from 1-5 hr at different suspending medium conductivities (0.01 – 0.03 S/m). Dielectric characterization of exposed red blood cells will involve utilizing a 3D microwell with two perpendicular electrodes to create non-

uniformities electric fields. The study aims to contribute to a better understanding of the effects of microgravity on blood circulation and advance
research in space health.

23ATOM02: Metallomics Based Applications, Central Pacific A/B/C
Chair: Derrick Qurles

(ATOM-02.1)**Multimodal Approaches for Fully Quantitative Elemental Bioimaging Using Synchrotron Based X-ray Fluorescence Microscopy (SXFM) and LA-ICP-TOF-MS**

Keith Macrenaris, Andrew Crawford, David Zee, Qiaoling Jin, Thomas O'Halloran, *Michigan State University, Northwestern University,Argonne National Laboratory*

Over the past 20 years, quantitative elemental analysis and mapping in biological samples has provided scientists with access to the inorganic phenotypes of a plethora of organisms and tissues. These elemental fingerprints and the subsequent underlying inorganic physiology have been implicated in everything from evolution to the mammalian cell cycle to different diseases such as Alzheimer's and Parkinson's. Until recently, there were many significant hurdles to the wider adoption of LA-ICP-MS for elemental mapping including, but not limited to, long acquisition times, limited resolution, and lack of standardization leading to sub optimal quantification. With the advent of time-of-flight ICP-MS and high repetition rate laser ablation systems, researchers are now able to quantitatively map samples in a fraction of the time with increased resolution removing significant hurdles for elemental mapping. However, quantification has remained a significant challenge due to a dearth of certified reference materials and standards coupled with the complexities of LA-ICP-MS. In order to test and refine quantification for elemental mapping we and others have used Synchrotron-based x-ray fluorescence microscopy (SXFM) for comparison and corroboration. SXFM provides similar (or better resolution) and elemental sensitivity but unlike LA-ICP-MS is non-destructive and absent significant matrix effects providing an excellent complement to LA-ICP-MS allowing for corroboration of elemental concentrations. In this talk, I will discuss recent developments in multimodal elemental mapping of biological tissues via SXFM and LA-ICP-TOF-MS including sample preparation, instrument development, and quantification. In addition, I will discuss new approaches to spectral analysis in SXFM and LA-ICP-TOF-MS that is vital for true element quantification.

(ATOM-02.2)**Elemental Analysis in Biological Material**

Martina Ralle, Sophia Miller, *Oregon Health And Science University*

Our current understanding of disease pathogenesis for disorders that are associated with changes in trace elemental content, distribution, or chemical state is limited by the availability of tools to analyze their distributions. For inborn errors of copper homeostasis such as Wilson and Menkes disease questions of how the subcellular copper distribution might contribute to disease progression is still unclear although the underlying genetic alterations in copper transport have been identified almost 30 years ago.
Our lab uses a diverse array of methods to visualize and analyze trace elemental distributions in cells and tissues.
One such method, X-ray fluorescence microscopy (XFM), has emerged as a powerful tool to quantitatively image total metal distributions in biological specimen and our lab has played an integral role in developing methods and protocols to apply XFM to biological questions. Current resolution for XFM approaches that of electron microscopy and the sensitivity is in the attomolar range. One of the major drawbacks of XFM is its inability of distinguishing cellular or subcellular structures unless they differ in elemental content. We are developing probes that will allow users to identify proteins or subcellular organelles associated with trace metal distributions in one XFM scan. Together with our collaborators we have designed functionalized nanoparticles containing a nickel or a cobalt core for in cellulo and in vitro labeling of organelles and proteins. Our initial nickel prototypes to label mitochondria in mouse embryonic fibroblasts demonstrate good cellular uptake with minimal cellular

toxicity. CoO nanoparticles clicked to a strained bicyclononane-PEG-maleimide which is bound to a COX2 antibody was synthesized and tested for its labeling properties in MEFs in vitro.
As complementary benchtop techniques we routinely use ICPMS and lately spICPMS to determine bulk and single particle concentrations for nanonmaterials. Our current setup for spICPMS includes an Agilent QQQ 8900 with an Elemental Scientific Microfast System for SP analysis. Here, we will present results from our initial measurements using this system.

(ATOM-02.3)**Fluorine mapping in biological and geological specimens by LA-ICP-MS**

Raquel Gonzalez De Vega, David Clases, John Parnell, Jörg Feldmann, *University Of Graz, University of Aberdeen*

Fluorine (F) plays an important role in biology and geology but is hard to analyse and quantify using element-specific analytical techniques. This is related to its high first ionisation potential and analytical methods depending on high energy sources for excitation and/or ionisation of F. Especially ICP-MS was initially found incapable to detect F, however, recent methodological advances, i.e., using ionic Ba-solutions as modifier enables the formation of BaF+ ions in the plasma, which can be targeted instead.
In this work, we demonstrate the adoption of this method for LA-ICP-MS for the mapping of F in geological and biological samples. We mixed the dry laser aerosol with a wet aerosol containing Ba2+ and developed a method targeting BaF+ on m/z 157. For method optimisation, F-based gelatine standards were prepared and subsequently characterised using combustion ion chromatography. Using a single-shot approach, we could gradually improve signal and noise and found best figures of merit when employing an ICP-MS/MS system, where the first quadrupole was operated with a larger mass bandpass, and employing a collision/reaction cell pressurised with H2, O2 and He. Using hard extraction conditions, F analysis in single laser shots was possible and in-house-prepared standards were interrogated to estimated limits of analysis which were in the upper ng/g range. In a proof of concept, the developed method was employed to map the F distribution in two human tooth samples as well as in a Rhynie chert as representative biological and geological samples, respectively. It was possible to interrogate the F distribution in these materials and to generate qualitative F maps.

(ATOM-02.4)**Screening of Bromine Species in Enzymatically Digested DNA Samples Using a Fast and Automated Separation and Quantification IC-ICP-MS Method**

Catharina Erbacher, Nils Flothkötter, Marcel Macke, Derrick Quarles Jr., Michael Sperling, Jens Müller, Uwe Karst, *University of Münster, Elemental Scientific,*

In recent years, there has been a rising interest in utilizing DNA in material sciences, leading to the question of whether DNA is able to transfer electrical charges. Charge transport along DNA could potentially be implemented in nanotechnological applications like molecular wires. Therefore, a fast and automated separation and quantification method for bromide and the artificial nucleoside 5-bromo-2'-deoxyuridine (5-BrdU) via hyphenation of ion exchange chromatography (IC) and inductively coupled plasma mass spectrometry (ICP-MS) is presented in this study. The analysis of these two species is relevant to monitor the transfer of electrons along metal-mediated DNA base pairs. Here, electrons are injected into the base stack using a photosensitive electron donor. The electron acceptor 5-BrdU, which is implemented into the sequence downwards the DNA strand, releases bromide upon one electron reduction after successful electron transfer along the DNA. Concentrations of 5-BrdU and bromide in enzymatically digested DNA samples can therefore be used as a marker for the efficiency of electron transfer along the DNA helix. The developed separation and quantification method utilizes an automated IC system, which enables time-efficient external calibration by inline dilution of a stock solution. Due to the fast separation of the two bromine species in less than 90 s, the developed method is suitable for screening applications with a multitude of samples differing in sequence and electron donor. Despite isobaric interferences and a low degree of ionization for bromine detection via ICP-MS, the method has a limit of detection of 30 ng/L, which is approximately one order of magnitude lower than a comparable method using reversed phase high performance liquid chromatography and ICP-MS.

(ATOM-02.5)**Optimization of Quantification in Single Cell ICP-MS**

Alexander Köhrer, Matthias Elinkmann, C. Derrick Quarles, Michael Sperling, Uwe Karst, *University Of Münster*

The determination of elemental contents in cells is of interest for a large variety of scientific fields, ranging from life to environmental science and beyond. As cells are living entities, prone to natural heterogeneity, determining elemental content in individual cells is key to understand the uptake behavior in cell cultures.
Recently, single cell ICP-MS (sc-ICP-MS) was established as a novel tool to quantify elemental contents on a per-cell level. Herein, cell suspensions are introduced into the plasma without the need for a sophisticated sample preparation. Each cell is atomized and ionized in the process and forms a distinct ion cloud, resulting in a spike in signal intensity. When measured at sufficiently low dwell times, the resulting signal spikes can be separated from the background. The intensity of each spike is proportional to the elemental content in a single cell, enabling quantitative evaluation. Using the method, thousands of cells can be individually analyzed within a few minutes, allowing rapid quantitative determination of elemental distributions in an entire cell population. As the technique is still in the early stage, experimental parameters can be optimized to obtain more reliable results and tackle various challenges. One of these challenges is the drastically varying absolute uptake amount. Depending on cell type as well as both incubation compound and period, cultures ingest substances from low-femtogram level up to hundreds of femtograms. Whereas in standard ICP-MS, the user would counteract the problem by simple dilution, this method is not feasible within sc-ICP-MS as cell contents always arrive in the plasma in one unity.
Therefore, in order to investigate how to overcome this limitation, bacterial cells were incubated with lipophilic organometallic compounds. ICP parameters like gas flow rates and lens settings as well as the experimental setup were optimized for each concentration range to obtain reliable results. Using the optimized parameters, cells with very low (sub-fg per cell) as well as very high (>100 fg per cell) internal elemental concentrations could both be measured and quantified. The cells showed different uptake behavior of the used compounds, which could be shown by quantification.

23AWD05: RSC Joseph Black Award Symposium Honoring Mathew Horrocks, Sierra 5
Chair: Mathew Horrocks

(AWD-05.1)**Using Peptides to Look at Proteins: Developing Peptide-based Imaging Modalities**

Zuzanna Konieczna, Fabio De Moliner, Lorena Mendive-Tapia, Zoe Gidden, Katie Morris, Takeshi Kaizuka, Marc Vendrell, Mathew Horrocks, *University Of Edinburgh*

Fluorescence microscopy is a tool routinely utilised to address biological questions. Direct visualisation of features of interest, confirmation of protein identity or the presence of a post-translational modification, enables researchers to study differences between healthy and pathological phenotypes. As the questions get more complex, more advanced imaging techniques are required – addressing issues of the limit of resolution, probe specificity to target and fluorescent background. Using peptides for imaging applications could address several of these concerns: careful design of the peptide sequence can ensure specific binding to the target of interest; suitable fluorophore could reduce signal-to-noise ratio and enable super-resolution imaging.

To illustrate the potential of peptide imaging, we have looked at a range of sequences targeting the PDZ domain, a common structural motif of anchoring and signaling proteins. We have incorporated a new small fluorescent amino acid, SeNBD (developed by Fabio de Moliner & Marc Vendrell) into the sequences and demonstrated the retained binding to the protein-of-interest. We were then able to use the fluorescently labelled peptides to image the PDZ contained in post-synaptic density proteins in a synaptosome sample, and super-resolve the nanoclusters corroborating literature findings. We looked at incorporation of the dye both externally (N-terminal) and internally (substituting amino acids in the sequence) and found that internal incorporation further enhances the switch-on of the dye and reduces

signal-to-noise ratio. Based on these findings, we are excited to extend this work to sequences targeting proteins implicated in neurodegeneration.

Furthermore, we envisage this approach can be made even more flexible through exploiting a coiled-coil interacting peptide pair: 101A & 101B. By labelling 101A at the N-terminus, we created an imaging strand that binds to 101B peptide, which we co-localised at the target. Whilst synthetic delivery of the targeting sequence proved challenging, expressing 101B attached to the target protein in cellulo produced promising results, and allowed for direct visualisation and super-resolution imaging of mitochondria. .

(AWD-05.2)**Surface enhanced Raman sensors for monitoring responses of tissue models derived from stem cells.**

Colin Campbell, William Skinner, Bilgi Kip, Peter Robinson, Mariska Simpson, Robert Gray, *University Of Edinburgh, University of Exeter, MassCare Ltd,*

3D tissue models are increasingly used in the understanding and treatment of disease because they are more representative of real tissue. When derived from primary cells such as stem cells they can also recapitulate a diversity of genotypes that represents disease phenotype in the wider population. A challenge in the use of such tissue models is monitoring their response to injury and/or therapy in order to understand the processes of repair and regeneration. We have developed substrates that allow the measurement of local pH and redox potential in such tissue models – the measurement of these parameters is important since they are directly affected by metabolic activity. By designing substrates that support and enable healthy cell growth sensors are incorporated within tissue models and probed by measuring the surface-enhanced Raman spectra of reporter molecules on their surface. These sensors have allowed us to make measurements in air-liquid interface (ALI) cultures and in airway organoids derived from basal cells harvested from the nose of healthy volunteers and patients with cystic fibrosis.

(AWD-05.3)**High-Resolution Imaging of CSF Circulation in the Brain Parenchyma and Meninges**

Juan Alberto Varela,*University Of St Andrews*

The circulation of cerebrospinal fluid (CSF) and clearance of protein aggregates from the extracellular space of the brain is a topic of great importance in neurobiology. The extracellular space of the brain is highly complex and shaped at the nano-scale, making conventional fluorescence techniques unsuitable to answer some of the key open questions in the field. Single-molecule imaging and nanoparticle tracking techniques have become powerful tools to study heterogeneous and dynamic species organised at scales below the diffraction limit of light, surpassing the molecular ensemble averages that can be obtained with conventional microscopy.
We show here how a combination of fluorescent nanoparticles and fluorescently labelled proteins allow a detailed study of the clearance from interstitial fluid to the CSF and lymphatic vessels in rodent models. We performed single-nanoparticle tracking in the brain in vivo through a sealed cranial window to evaluate the role of the rheology of the extracellular space of the brain in waste clearance. Our results show how clearance efficiency strongly depends on age, solute size and structural features of the protein aggregates.
This novel combination of nanotechnology and high-resolution microscopy allowed us to provide a quantitative picture of the clearance of protein aggregates from the brain extracellular space at the spatial resolution imposed by the natural organization of the brain.

(AWD-05.4)**Nano Single-molecule Two-color Aggregate Pull-down (Nano-STAPull) Technique: Highly Specific and Sensitive Detection for Early Stage Oligomeric Species in Neurodegenerative Diseases**

Ji-Eun Lee, Rebecca Saleeb, Craig Leighton, Judi O'Shaughnessy, Kiani Jeacock, Alexandre Chappard, Robyn Cumberland, Sarah Ball, Margaret Sunde, David Clarke, Kristin Piché, Jacob

McPhail, Ariel Louwrier, Rachel Angers, Sonia Gandhi, Patrick Downy, Tilo Kunath, Mathew Horrocks, *The University of Edinburgh, The University of Sydney, Stressmarq Biosciences Inc.,UCB Biopharma S.P.R.L., 1) The Francis Crick Institute, 2) UCL Queen Square Institute of Neurology, 3) 8Aligning Science Across Parkinson's (ASA)*

The misfolding and aggregation of protein is a characteristic of many neurodegenerative disorders, including Alzheimer's and Parkinson's disease. The wide range of sizes and structures of oligomers and fibrils generated have previously been studied using single-molecule and super-resolution microscopy. These methods, however, tend to rely on the use of either directly labeled protein, or on the addition of non-specific amyloid stains, such as thioflavin-T (ThT). This has prevented the characterization of protein aggregate composition in complex biological samples. Here, we have developed a single-molecule two-color aggregate pull-down (STAPull) assay combining with super-resolution methods to overcome this challenge by probing immobilized proteins using orthogonally labeled antibodies targeting the same epitope. By looking at colocalized signals, we can eliminate monomeric protein, and specifically quantify aggregated proteins, especially detect early stage oligomeric species which cannot be detected by ThT and are considered more cytotoxic. We demonstrate that this approach can specifically detect aggregates with a limit of detection of 5 pM. Second, by combining DNA-PAINT and using various antibodies, we can also characterize the structural properties of aggregated proteins, such as size, ellipticity and local affinity to the antibodies in a single aggregate, with 20 nm precision. Furthermore, we show that Nano-STAPull can be used in a range of samples, including in human biofluids. Nano-STAPull is generally applicable to protein aggregates from a variety of disorders, and will aid in the identification of biomarkers that are crucial in the effort to diagnose these diseases.

23BIM03: Point-of-Care Technologies for Biomedical Applications, Sierra 2
Chair: Karina Weber

(BIM-03.1)**Novel blood diagnostics using Raman spectroscopy at the point-of-care**

Anja Silge, Anuradha Ramoji, Aikaterini Pistiki, Ute Neugebauer, Iwan Schie, Richard Grohs, Alexander Wiede, Uwe Glaser, Oleg Ryabchykov, Franziska Hornung, Karina Weber, Stefanie Deinhardt-Emmer, Bettina Löffler, Juergen Popp, *Leibniz Institute of Photonic Technology, Institute of Physical Chemistry and Abbe Center of Photonics, Friedrich-Schiller University, Ernst-Abbe-Hochschule, University of Applied Sciences, Fachbereich Medizintechnik und Biotechnologie, Carl-Zeiss-Promena, Jena University Hospital, Institute of Medical Microbiology, and Center for Infectious Diseases and Infection Control*

Blood is a window into both health and disease. The composition and appearance of blood cells reveal vital information about a range of illnesses [1]. Furthermore, bloodstream infections necessitate prompt treatment in case bacteria or fungi are found; else, they can result in sepsis. Selecting an effective anti-infective agent requires knowledge of the type of infection, the organism, and its resistance profile [2]. Blood diagnostics utilizing biophotonic techniques is a highly innovative field of study. Since the combination of Raman spectroscopy and microscopy has significantly increased the applicability of biophotonics to many bioanalytical questions, work has begun to transfer these concepts to POCT diagnostics [3]. Starting with customized sample preparation, intelligent measurement workflows, functional bioassays, and high-throughput devices, the POCT method is rounded out by machine learning pipelines that automatically evaluate spectroscopic data and present the results as uncomplicated medical diagnostics.
This presentation will focus on Raman spectroscopic approaches for point-of-care blood diagnostics.

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(BIM-03.2)**Visible-, Near-Infrared and Raman Spectroscopic Imaging Data Fusion for Point-of-Care Diagnostics**

Christian Huck*University Of Innsbruck, Institute Of Analytical Chemistry*

Visible-, near-infrared (NIR), and Raman spectroscopic imaging data fusion can be a powerful approach for point-of-care diagnostics. Each of these spectroscopic techniques provides unique information about the chemical composition and molecular structure of a sample, and by combining them, we can obtain more comprehensive and accurate information.

Visible spectroscopy is commonly used for qualitative and quantitative analysis of samples in the 400-700 nm range. It is particularly effective in identifying colored compounds and assessing the concentration of chromophores.
Near-infrared spectroscopy, exploits the region between the visible and mid-infrared regions (typically 700-2500 nm). NIRS is sensitive to the overtones and combinations of molecular vibrations and can provide information about the functional groups and chemical bonds present in a sample.

Raman spectroscopy is a scattering-based technique that provides information about the vibrational modes of molecules. Raman spectroscopy can identify and characterize chemical compounds, including organic and inorganic materials, and it is highly sensitive to molecular structure and conformational changes.

By combining visible, NIR, and Raman spectroscopic imaging data, we can leverage the complementary strengths of each technique. The fusion of these data sets allows for a more comprehensive analysis of the sample, providing a holistic view of its chemical composition and molecular structure. This can be particularly valuable in point-of-care diagnostics, where rapid and accurate assessments are crucial.

For example, in biomedical applications, the fusion of spectroscopic imaging data can aid in the identification of disease markers, monitoring of therapeutic responses, and detection of abnormal tissues. The combination of visible, NIR, and Raman spectroscopy can enable the identification of specific biomolecules, such as proteins, nucleic acids, and metabolites, providing insights into disease progression and treatment efficacy.

Furthermore, the integration of spectroscopic imaging can enhance spatial resolution and enable the visualization of molecular distributions within a sample.

The development of portable and user-friendly devices capable of acquiring and analyzing multiple spectroscopic modalities is an active area of research to enable the translation of this technology into clinical practice.

Overall, the fusion of visible, NIR, and Raman spectroscopic imaging data holds significant promise for point-of-care diagnostics, offering comprehensive and accurate chemical analysis of samples.

(BIM-03.3)**AI-enabled detector array for deeper vision and sensing beyond the limit**

Saif Islam, Laura Marcu, *Univ of California Davis,*

This presentation will introduce a novel sensing technology that utilizes nanostructure-enabled ultra-fast sensors. These sensors operate by manipulating photons, employing deep learning techniques and computational imaging. Using micro and nanoscale surface structures, we will showcase a technique that redirects incident light beams along the plane of semiconductor films and slows them down. This approach enhances light absorption efficiency, makes the devices fast, and opens up possibilities for applications in data center communication, advanced bioimaging, LIDAR, and highly efficient solar cells. These sensors can potentially diagnose tissue during surgery and monitor molecular activity in plant cells for autonomous nutrient management.

(BIM-03.4)**Towards Determining Amyloid Fibril Structures Using Experimental Constraints from Raman Spectroscopy**

Madeline Harper, Uma Nudurupati, Riley Workman, Taras Lakoba, Delaney Nelson, Nicholas Perez, Yangguang Ou, David Punihaole, *University of California Davis, University of Vermont, Sealy Center for Structural Biology and Molecular Biophysics, University of Texas Medical Branch,*

We demonstrate that dihedral and bond angles of peptides measured by Raman spectroscopy can be used as experimental constraints to determine the structure of amyloid fibrils formed by amylin20−29 (sequence SNNFGAILSS) and amyloid-β(Aβ)25−35 (sequence GSNKGAIIGLM). Using both polarized and non-polarized Raman measurements, we determine the distribution of peptide backbone amide C=O and CN bond orientations, as well as Ramachandran ψ dihedral angles, adopted by amylin20−29 and Aβ25−35 fibrils. We then use these experimentally measured distributions as structural constraints to guide molecular dynamics (MD) simulations of the fibril structures. The resulting structure indicates that amylin20−29 fibrils are comprised of extended β-strands that are arranged into an antiparallel β-sheet architecture in excellent agreement with some previously published solid-state NMR (ssNMR) studies. In contrast, our resulting structure for Aβ25−35 indicates extended β-strands that are arranged into a parallel β-sheet architecture. Overall, our work lays the foundation for using data from Raman spectroscopy as structural constraints in MD simulations to determine the three-dimensional molecular structural models of amyloid fibrils in a manner that could complement analogous gold-standard protein structural determination approaches such as NMR.

(BIM-03.5)**Rapid diagnosis of fibromyalgia disorder and other rheumatologic syndromes by portable mid-infrared spectroscopy combined with chemometric analysis**

Shreya Nuguri, Luis Rodriguez-saona, *The Ohio State University,*

Fibromyalgia (FM) is the second most common rheumatic disorder after Osteoarthritis (OA) affecting 2-4% of population. It is a chronic centralized pain syndrome characterized by many symptoms including fatigue and cognitive disorders. The criteria for FM classification have been based on tender points, however, due to a fine line between pain and tenderness perception, the case often remains ambiguous. Moreover, overlapping pain conditions with other rhematic disorders like rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), and OA make the diagnosis more challenging. Early identification is thus essential therapeutically, avoiding unnecessary opioid consumption and high healthcare expenses. We aim to develop reliable and robust classification algorithms using unique spectral profiles of portable FT-MIR that can be deployed for real-time, in-clinic screening of FM disorder. A total of 275 blood samples of patients with different disorders (FM = 178, OA = 18, SLE = 33, RA = 37) collected on neoteryx sticks were analyzed over the years 2020-23. Physical extraction

using filter membranes (10k Da) washed with water was used in this study. Samples were sonicated in millipore water and centrifuged in washed filter membranes; the obtained low molecular fraction was dried to pellet. The pellet was redissolved in 6ul water, 1ul of this solution was placed on FTIR for spectra acquisition. OPLS-DA statistical tool was used to generate classification algorithm. FM samples were designated to class 1 while other category diseases were assigned to class 2. Calibration models showed good performance with correlation coefficient >0.97. Seven factors contributing to 70% total variation were used. Accuracy, sensitivity, and specificity of the validation set were 88%, 93.6% and 74% respectively. Inclusion of more samples in class 2 may improve the specificity of the model. The proposed research explores the potential application of portable mid-infrared for inexpensive rapid diagnosis of Fibromyalgia. It fosters the motto of Precision Medicine (right treatment at right time) improving not only the health but also socio-economic status and living quality of affected individuals.
Keywords: Rheumatic diseases, fibromyalgia, FTIR, OPLS-DA

23IR09: Two-Dimensional Correlation Spectroscopy, Sierra 3
Chair: Young Mee Jung
Co-Chair: Isao Noda

(IR-09.1)**Enhanced Spectral Resolution and 2D-COS**

Isao Noda, *University of Delaware*

One of the beneficial features of two-dimensional correlation spectroscopy (2D-COS) is the apparent enhancement of spectral resolution. Highly overlapped adjacent bands often encountered in the one-dimensional IR or Raman spectra may be effectively differentiated and identified by spreading the peaks along the second dimension. This differentiating feature is especially prominent in asynchronous spectra, where even a slight difference in the variation patterns of overlapped bands in response to a given perturbation results in the generation of cross peaks. In contrast, synchronous spectra may not provide strong differentiating feature for overlapped bands, unless the relative directions of spectral intensity changes are in the opposite directions, in which case distinctive negative cross peaks are generated. Positive synchronous correlation intensity by itself does not guarantee that signals observed at different spectral coordinates are completely synchronized, so there is a risk of false positive assessment or lack of sufficient selectivity. Moderately synchronized signals may be mistaken as originating from the same species or within a single peak profile of the same mode. While cross peaks in asynchronous spectra can identify signals originating from different moieties or bands, they cannot effectively specify which regions of spectra actually share the same origin, such as common species in a mixture. Only when the so-called SACP (systematic absence of cross peaks) condition is observed, where the signals are completely synchronized, asynchronous spectra can identify the common origin of the spectral signals. However, any interfering signals from overlapped bands or noise will eliminate SACP condition. Thus, the overreliance on asynchronous spectra alone risks the potential false negative assessment or lack of sufficient specificity, leading to the failure of classifying signals into a reasonable finite set of component groups. The combined use of both synchronous and asynchronous spectra coupled with the effective scaling of signal coherence and a robust line shape narrowing technique may provide a means to achieve both selectivity and specificity for resolution-enhanced spectral signals in 2D-COS. Examples of such methods are explored.

(IR-09.2)**Characterization of Silicone Rubber under Electrical, Chemical, and Thermal Stress**

Kavin Darshan, Aruna Kumarasiri, Mosfeq Uddin, Harpreet Kaur, Rajkumar Padmawar, **Dennis Hore,** *ASAsoft, University of Victoria,*

Composite polymer insulators are next-generation devices for global power distribution networks, replacing aging glass and ceramic components on the electrical grid. Silicone materials are particularly attractive for high voltage applications as the insulator surfaces have prolonged hydrophobicity, especially under harsh environmental conditions such as contamination by de-icing salts and algae. To

date, measurements of the water contact angle has been used as an indicator of insulator performance and lifetime, as loss of hydrophobicity is associated with eventual component failure. It is therefore of significant interest to have a more quantitative, reliable, and chemically-specific marker of insulator performance to provide better correlation with predicted service life. Here we explore the use of vibrational spectroscopy, specifically ATR-IR spectroscopy, in order to achieve this objective. A tracking wheel is used to expose 15 kV rating composite polymer insulators to concentrated salt solutions while 12 kV at 60 Hz is applied across the insulators, alternating chemical and electrical stress for tens of thousands of cycles. Subsequently, the heating-cooling hysteresis of the polymer vibrational bands is investigated with ATR-IR. Two-dimensional correlation analysis (2D-COS) is then used to reveal patterns accompanying density changes that can be traced to material degradation due to aging.

(IR-09.3)**Investigation of bread staling by handheld NIR spectroscopy in tandem with 2D-COS and MCR-ALS analysis**

Heinz Siesler, Marina De Gea Neves, Isao Noda, *Department of Physical Chemistry, University Duisburg-Essen, University of Delaware*

The presentation reports investigations, that were carried out using the technique of handheld near-infrared (NIR) spectroscopy for time-dependent diffuse-reflection measurements of the cut face of a fresh baguette. The critical structural factors in the staling process of bread are the crystallization of amylopectin in starch and the decrease of water content by evaporation and diffusion from core to crust. To monitor the synchronicity and sequence of these changes, Two-Dimensional Correlation Spectroscopy (2D-COS) was applied. Due to the significant variation of spectral changes at different stages of the aging process, the spectra were split into two sets of 0 – 6 and 6 – 48 hr. 2D-COS analysis allowed to differentiate the detailed sequence of the following structural events for these time intervals: crystallization of amylopectin/evaporation of weakly and strongly hydrogen-bonded water/reorganization of starch OH-functionalities. Furthermore, Multivariate Curve Resolution-Alternating Least Squares (MCR-ALS) was used to investigate the changes in the spectra profile as a function of aging time.

(IR-09.4)**Quantum Cascade Laser Microscopy And Two-Dimensional Correlation Algorithms: A Powerful Combination For The Characterization Of Therapeutic Proteins**

Belinda Pastrana, Elizabeth Culyba, Sherly Nieves, Steve Sazinsky, Eduardo Canto, Isao Noda, *Protein Dynamic Solutions, Inc.Verseau Therapeutics, Inc**,** Auxilio Mutuo Hospital, University of Delaware*

Therapeutic proteins are complex and represent the fastest growing drug class for the treatment of a wide variety of diseases. The complexity of this class of drug is not only limited to their size and structure, but also the modifications therapeutic proteins possess. This leads to a list of critical quality attributes (CQA's) for which therapeutic proteins must be evaluated. The results obtained for these CQA's are used to assess therapeutic protein's manufacturability, safety, efficacy and regulatory approval. One such CQA is known as asparagine or glutamine deamidation, which introduces a negative charge in the protein's amino acid sequence and may potentially impact other CQA's such as: efficacy, stability and aggregation. Furthermore, aggregation may induce an unwanted immune response in a patient. Herein we present, a breakthrough platform technology comprised of a combination of innovative components: a quantum cascade laser microscope equipped with an unique slide cell array that allows for the comparability assessment of intact proteins in solution under accurate thermal control and software that enables multi-attribute assessment. Real-time hyperspectral images were acquired for 8 clinical antibodies under control and forced degraded conditions at set temperatures during their thermal perturbation. The spectral data was then subject to two-dimensional correlation and co-distribution analyses for the determination of deamidation, aggregation and stability during thermal stress. Two-dimensional co-distribution spectroscopy (2D-CDS) provided evidence of the impact of deamidation that led to the aggregation of the therapeutic protein. While, two-dimensional correlation spectroscopy (2D-COS) asynchronous plots enabled the monitoring of key

signature peaks during the deamidation process. 2D-COS also allowed for the spatial and temporal description of the molecular dynamics of the therapeutic protein in solution. More importantly, the results were: (1) orthogonal to other analytical techniques without the need for sample preparative steps, (2) crucial to the determination of both asparagine or glutamine deamidation, (3) key to aggregate formation assessment and (4) used for the evaluation of stability of an array of therapeutic proteins. This novel approach is a promising transformative solution for the Biopharma industry.

(IR-09.5)**Polarized Raman Microscope Studies of Spherulites in Thin PHA Films as a Means to Further Characterize Crystallization Mechanisms**

Fran Adar, Isao Noda, *HORIBA Scientific*, *University of Delaware*

Raman microscope spectra of thin annealed films of polyhydroxyalkanoate are measured in crystalline spherulites exhibited by illuminating the films between crossed polars. By measuring from the periphery of the spherulite to the center, and selecting polarization conditions, it is possible to follow the rotation of the twisting lkamellae along a direction normal to the radius. The spectra are analyzed with two-dimensional correlation spectroscopy (2D-COS) to further elucidate the crystallization mechanisms. A previous 2D-COS study of isothermal crystallization indicated how the condensation of the molecular chains in the unit cell can be monitored. The present measurements will indicate how this condensation is localized in the spherulite. That is, is it related to the distance from the center of the spherulite or the polarization conditions of the rotating micro-crystallites?

23MASS03: 50 Years in Mass Spectrometry, Southern Pacific A/G
Chair: Benjamin Garcia

(MASS-03.1)**Development of new tools for RNA modification analysis by MS**

Benjamin Garcia, *Washington University School Of Medicine In St. Louis*

Post-transcriptional modifications of RNA are associated with fundamental biological processes such as RNA splicing, translation, and degradation, as well as human physiology and disease such as cancer or viral infection. Over 140 modifications have been identified across species and RNA types, with the highest density and diversity of modifications found in tRNA. Nevertheless, the analysis of ribonucleoside modifications is hampered by the hydrophilicity of the ribonucleoside molecules, and difficulty to fully characterize and quantify them by mass spectrometry (MS) based methods. To improve both mononucleoside and oligonucleotide analyses, we have developed a variety of chemical, sample prep, MS and chromatography and computational advances. We have adapted chemical derivatization approaches to improve the retention of RNA mononucleosides on C18 based columns and enhance quantification. Additionally, we have developed data independent acquisition (DIA) methodology which has allowed us to quantify over 70 RNA modification types. For oligonucleotide analysis, we have combined two orthogonal modes of RNA ion separation before MS quantification: high-field asymmetric ion mobility separation (FAIMS) and electrochemically modulated liquid chromatography (EMLC). FAIMS RNA MS increases the depth of coverage and throughput, while the EMLC LC-MS orthogonally separates RNA of different length and charge. These two methods combined offer a broadly applicable platform to improve length and depth of MS-based RNA sequencing while providing contextual access to the analysis of RNA modifications. Overall, these approaches will significantly improve the characterization of RNA molecules by MS.

(MASS-03.2)**The glycomics of food**

Carlito Lebrilla, *University Of California, Davis*

Carbohydrates are a significant challenge in food analysis, as current analytical methods are deficient in their ability to accurately characterize monosaccharides, linkages, and polysaccharide structures. This shortcoming has resulted in poor sensitivities and limited quantitative capabilities. In this presentation, novel approaches for rapidly and accurately characterizing carbohydrates are detailed. Over 1000 foods have been subjected to monosaccharide and linkage analyses, creating a

comprehensive database of food carbohydrates. In addition, quantitative analysis of polysaccharide mixtures has enabled the identification of absolute abundances of constituent polysaccharides in common foods. This research is having a notable impact on a variety of fields, including bioactive foods and investigations into the gut microbiome.

(MASS-03.3)**Unraveling the Spatial Lipidome Using Gas-phase Ion/ion Reactions**

Boone Prentice, *University Of Florida*

Imaging mass spectrometry is a powerful analytical technique for analyzing the spatial lipidome. This technology enables the visualization of molecular pathology directly in tissues by combining the specificity of mass spectrometry with the spatial fidelity of microscopic imaging. This label-free methodology has proven exceptionally useful in research areas such as cancer diagnosis, diabetes, and infectious disease. However, state-of-the-art experiments stress the limits of current analytical technologies, necessitating improvements in molecular specificity and sensitivity in order to answer increasingly complicated biological and clinical hypotheses. For example, the diverse array of lipids present in tissue samples results in many isobaric (i.e., same nominal mass) and isomeric (i.e., same exact mass) compounds in imaging mass spectrometry experiments. Adequate separation and identification of these compounds is necessary to ensure accurate analyte annotation and avoid composite images comprised of multiple compounds. Conventional technologies such as high-resolution accurate mass (HRAM) measurements, collision induced dissociation (CID), and ion mobility-mass spectrometry (IM-MS) are able to resolve these compounds in some instances, but are not always successful in isomer separation and de novo structural characterization. Alternatively, our lab develops instrumentation and novel gas-phase ion/ion reactions to provide high levels of chemical resolution. These gas-phase transformations are fast, efficient, and specific, making them ideally suited for implementation into imaging mass spectrometry workflows. These workflows have enabled the identification of multiple sn-positional phosphatidylcholine (PC) isomers, the separation of isobaric phosphatidylserines and sulfatides, and the identification of fatty acid (FA) double bond isomers using a variety of charge transfer and covalent ion/ion reactions. For example, a charge inversion ion/ion reaction between protonated PCs and a 1,4-phenylenedipropionic acid (PDPA) reagent dianion was used to convert protonated PCs to anionic ion types and reveal up to five sn-positional isomers for each sum composition lipid, with the relative abundance of these isomers varying in abundance throughout rat brain tissue. Working with biologists and clinicians, we leverage these novel imaging technologies to understand the molecular events associated with important problems in human health, including infectious disease, diabetes, and neurodegenerative diseases.

(MASS-03.4)**Functional Group-selective Ion-molecule Reactions in Structural Characterization of Drug Metabolites and Degradation Products by Tandem Mass Spectrometry**

Hilkka Kenttamaa, *Purdue University*

In tandem mass spectrometric efforts to identify the structures of unknown analytes in complex mixtures, gas-phase ion-molecule reactions have proven to be more sensitive to differences in the structures of isomeric ions that the traditionally used collision-activated dissociation (CAD) method. Therefore, ion-molecule reactions often allow the distinction of isomeric ions that yield identical CAD mass spectra. Further-more, diagnostic ion-molecule reactions can frequently be used to rapidly identify specific functionalities in unknown analytes. Automation of these experiments enables the screening of nine or more functionalities in analytes directly in a mixture as they elute from an HPLC by using pulsed valves to rapidly introduce the neutral reagents into the mass spectrometer. Several examples are discussed, including the identification of potentially harmful drug metabolites and impurities. Further, the on-going development of coupling machine learning to the automated HPLC/tandem mass spectrometry platform based on diagnostic gas-phase ion-molecule reactions is discussed. Machine learning can be used to optimize the experimental conditions, identify functional groups in the analytes with no human bias, and select useful neutral reagents for new types of analytes.

23PAT05: PAT Coblentz: Machine Learning, Southern Pacific E

Chair: Jim Rydzak
Co-Chair: Barry Wise

(PAT-05.1)**Getting "lean" on monitoring your process: the value proposition of "machine learning" for early-phased development**

Zhenqi (Pete) Shi, *Senior Principal Scientist*

With the recent success of deploying process analytical technology (PAT) in continuous manufacturing process on drug product (CM DP), it is fair to say that PAT adoption in pharmaceutical industry is at its second climax since FDA's issuance of PAT guideline back in 2004. It is commonly acknowledged that PAT requires upfront investments on infrastructure and more so on robust and representative calibration datasets, which is often a luxury to have for early-phased development. This presentation is intended to showcase those PAT and spectroscopy deployments with "lean" and phase-appropriate calibration models, spanning from lipid nanoparticle (LNP) formulation screening to traditional batch process development and to characterization of decay curve in Roche's mini-batch continuous process on drug product. The machine learning approaches used in these case studies includes locally weighted regression (LWR), classical least squares (CLS) and prediction augmented classical least squares (PACLS).

(PAT-05.2)**Evaluation of Machine Learning Techniques for PAT applications**

Larry McDermott, Gregory McLaughlin, Meredith Brown, Massoud Ghasemzadeh-Barvarz, Rajesh Morampudi, Sean Daughtry, *Vertex Pharmaceuticals*

Various chemometric approaches have played a key part in enabling process analytical technology applications in the pharmaceutical and other industries. Approaches such as partial least squares regression (PLS-R) and partial least squares discriminant analysis (PLS-DA) have become the standard approaches for quantitative and categorical analysis of spectral data. The field of machine learning has seen rapid growth with numerous algorithms seeing adoption for analyzing diverse data sets. In this presentation we will compare results for both quantitative and categorical analysis of a large sample set collected over three years using conventional techniques and machine language approaches that have been published. We will compare supervised, unsupervised and ensemble learning approaches and compare the performance on typical data and will evaluate the robustness of the evaluated techniques to known process changes.

(PAT-05.3)**Machine Learning Based Vision System For Tablet Elegance**

Yong Mei, *Pfizer*

Functional or cosmetic coatings are applied in the pharmaceutical industry to aid performance or appearance of tableted products and the application of machine vision for automated inspection has been gaining attention with recent advances in computing power and high-resolution sensors.

Traditional manual inspection is both time and resource intensive for large batches. Defects on tablet coating are not only an indication of product quality, but also of paramount importance to process understanding. Machine vision utilizing deep learning techniques can provide an automatic and fast tool to facilitate both inspection and diversion from the good product stream based on defined visual CQA's (Critical Quality Attributes). The goal of the vision system currently under development is to become an alternative option for labor intensive visual AQL (Acceptable Quality Limit) testing for tablet elegance and, by examining a larger proportion of the batch, extract more information from the types of defects and their frequency of occurrence.

Two approaches to machine vision of tablet inspection will be compared. The first is supervised training that requires labeling defects in training datasets/images and can generate defect specific information, which can show advantages in commercial manufacturing with large batch and relatively

fixed formulas. The second is unsupervised training that uses only defect-free tablets, leading to an efficient method development. The advantages and considerations of each approach will be discussed along with a case study to demonstrate the results from both scenarios. Finally, the presentation will discuss the workflow, modeling approaches of the vision system, and its benefits for continuous manufacturing.

(PAT-05.4)**Locally Weighted Regression Revisited**

Barry M Wise, Sean Roginski, Lyle W Lawrence, Donal O'Sullivan, Bob Roginski, *Eigenvector Research, Inc.*

Locally Weighted Regression (LWR) first appeared in the statistical literature in 1988 and was introduced to the chemometrics community in 1990. Numerous improvements were made to the technique in the following decade and then interest in the method apparently languished. Meanwhile, chemometric practitioners were quietly using the technique with great success. Recently, perhaps due to the increased attention to non-linear machine learning methods, interest in LWR has increased. LWR has some advantages over other non-linear methods in that it is conceptually simple, straightforward to optimize, accommodates a wide array of non-linearities, includes integrated prediction outlier statistics, and is easy to update. On the downside, LWR is actually a procedure rather than a model per se, and as such is difficult to interpret. In this talk we review the method, demonstrate optimization of the meta-parameters (number of neighbors, number of factors, local regression method and weights, y-distance, etc.). “Model” interpretation is shown through use of Shapley values as well as more traditional variable sensitivity and robustness tests. Finally, possibilities for automatic calibration outlier detection and removal are considered.

23PMA07: Vibrational Spectroscopy in Devoloping Biologics & Cell and Gene Therapy, Southern Pacific D
Chair: Kevin Dahl

(PMA-07.1)**Investigating The Physicochemical Properties Of Lipid Nanoparticles In Protein Containing Media**

Rand Abdulrahman, Panida Punnabhum, Callum Davidson, Karim Daramy, Yvonne Perrie, Zahra Rattray, *University Of Strathclyde*

Lipid nanoparticles (LNPs) are emerging new modalities for mRNA therapeutics and have been in the spotlight over the past few years. Upon administration of the formulation intravenously, LNPs interact with constituents in the biological fluid forming nanoparticle-protein corona complexes [1]. Despite the success of nanotechnology, there is still a gap in understanding how nanoparticles change under physiologically-relevant conditions, hence the physical properties of the LNP differs from its complexes following exposure to biomolecules [2]. The aim of this study was to investigate the changes in LNP physical parameters in physiologically-relevant media. PolyA LNPs were manufactured using 1,2-dioleoyl-3-trimethylammonium-propane (DOTAP): DSPC (1,2-distearoyl-sn-glycero-3-phosphocholine): Cholesterol: 1,2-dimyristoyl-rac-glycero-3-methoxypolyethylene glycol-2000 (DMG-PEG2000) at a molar ratio of 50:10:38.5:1.5 mol%. LNP diameter was measured using Dynamic Light Scattering (DLS) and Nanoparticle Tracking Analysis (NTA), and encapsulation efficiency (EE) and mass balance (MB) were quantified using the Ribogreen Assay. Key attributes were also measured for PolyA LNP formulation (1mg/mL) following incubation with Bovine Serum Albumin (BSA) at physiologically-relevant temperature (37oC). Following 24 hour incubation of LNPs with PBS, DLS size measured by the intensity distribution contained a peak at 98 ± 49.04 nm corresponding to the PolyA LNPs. A 24-hour incubation with BSA, this peak occurred at 140.1 ± 64.90 nm, indicating that BSA interacted with LNPs causing a shift in particle size. Corresponding NTA data showed a multimodal distribution with a higher LNP distribution span of 0.61 and 0.69 for LNP-PBS and LNP-BSA respectively arising from NP-protein and protein-protein interactions. Taken together, this work demonstrates changes in LNP properties in biologically relevant conditions, consistent with protein surface adsorption. Incorporating the early assessment of LNP interactions with biomolecules can support a more fundamental understanding of the biological fate of LNPs. Further

work is underway to investigate the role of lipid composition in LNP biological stability in complex biological media (serum) and under physiologically-relevant flow conditions.

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2. Chan, C., et al., Computational and Experimental Approaches to Investigate Lipid Nanoparticles as Drug and Gene Delivery Systems. Current Topics in Medicinal Chemistry, 2021. 21(2): p. 92-114.

(PMA-07.2)**Microbiology in minutes for the Pharmaceutical Industry: Could Raman spectroscopy be the solution to this challenge ?**

Markus Lankers, Oliver Valet, *MIBIC GmbH & Co KG,*

There are many fields in the food and pharmaceutical industries that could benefit from detection and identification of microbiological contamination within 1-3 hours:
o rapid sterility testing - especially in the field of personalized medicine such as cell therapies.
o bioburden testing
o incoming material testing
o manufacturing support and root cause analysis - if something goes wrong
o water systems monitoring
All of these areas can benefit from a rapid microbiology technique that is not growth-based.
Raman spectroscopy is a promising label-free tool for the detection of microorganisms at the single cell level and thus could gain great importance as a future culture-free method for sterility testing in the pharmaceutical industry. In particular, the ability to identify individual bacteria by their spectra opens up the possibility of a cultivation-free sterility test that even allows rapid identification and risk assessment of any germs that may occur. This could be of particular importance for individualized cell therapies.
However, due to the weak bacterial Raman signal, it is difficult to achieve small detection limits while maintaining high accuracy. Other challenges in the use of Raman spectroscopy include fluorescence interference, Raman-appropriate sample preparation and database setup.
In a feasibility study for a sterility test in cell therapy we were able to detect a contamination level of less than 10 cells / ml. For this purpose CHO cell suspensions were spiked with different bacterial or fungal concentrations. Subsequently, the samples were lysed and filtered. Image analysis of microscopic images of the filter delivered the coordinates of the potential germs. The microorganisms were identified by Raman spectroscopy. The identification and differentiation of dead and live germs is achieved using a PLS-LDA model. The results were correlated with the corresponding plate counts. Our case study discusses the correct sample preparation but also the problems and limitations of the methods.
Our results indicate the great potential of this fast and cost-effective approach for quality control in the pharmaceutical industry. However, it also reveals some challenges that still need to be addressed.

(PMA-07.3)**Real-time Monitoring of Protein Capture Chromatography Load Phase and Break-through Detection using High Throughput Process Raman Spectroscopy**

Shaun Fraser, Mark Kemper, Colin Couper, *Tornado Spectral Systems*

High-throughput Process Raman Spectroscopy can be extremely valuable for real-time monitoring of the elution profile from columns in capture chromatography schemes used in downstream bioprocessing. This capability facilitates value-added contributions from Raman for characterizing the effectiveness of separation media. It also allows for effective and reliable monitoring of breakthrough, thus enabling purification efficiency. The use of Raman spectroscopy allows molecular characterization of the proteins and other materials eluting from a column, which in turn provides specific actionable information to optimize the downstream operations critical to the purification

process. The work shown in this presentation use two model proteins as proxies to demonstrate the capability of the Tornado Raman for this application. The data show that Raman analysis using the Tornado HyperFlux™ PRO Plus (HFPP) can be used to detect and quantify breakthrough of individual proteins with a high degree of specificity during the loading phase in capture chromatography processes. This quantitative analysis was successfully applied to monitor the breakthrough of Human Serum Albumin in the presence of excess amounts of Lysozyme. This work demonstrates that the HFPP can be a key tool to significantly enhance process control for continuous capture chromatography.

(PMA-07.4)**The Intricacy of In-line Monitoring of Tween 80 in Protein Formulations**

Gregory Webster, *Abbvie*

Polysorbates (also known as "Tween") are a common component in protein formulations used to minimize protein adsorption and stabilize the protein. Protein formulations with Tween-80 are commonly found to be in the concentration range of 0.005 to 0.05%. As nonionic surfactants, polysorbates are heterogenous mixtures of fatty acids with a complex reversed phase profile due to the inhomogeneity of the polymers present. In addition, these polymers contain no chromophore for detection.

The routine analysis of polysorbates in protein formulations was greatly improved through the introduction of online solid phase extraction (SPE) to simplify the polysorbate profile for quantification. Learning from these SPE applications has enabled inline measurement of polysorbates during the manufacturing process. The challenges of monitoring polysorbates inline and the approaches taken in our laboratory will be presented.

(PMA-07.5)**Opportunities and Challenges of Process Analytical Technology (PAT) in Bioprocessing 4.0**

Dhanuka Wasalathanthri, *Bristol-Myers Squibb*

Bioprocessing 4.0 enables agile, sustainable, and smart process development of biopharmaceuticals with the integration of Cyber Physical Systems, Internet of Things (IoT), Process Analytical Technology (PAT) and Automation tools. PAT plays a crucial role in extracting analytical information from the bioprocess to allow real time control of critical quality attributes and process parameters, which enables continuous and/or intensified bioprocesses. However, the implementation of PAT tools in bioprocess poses unique opportunities as well as challenges. The talk features some of the opportunities and challenges in implementing PAT for bioprocessing 4.0.

23RAM06: SERS – 50th Anniversary, Cascade 3
Chair: Duncan Graham

(RAM-06.1)**50 years of SERS and Scix**

Duncan Graham, *University Of Strathclyde*

This presentation will look back at some SERS papers presented at Scix over the years and highlight areas of significance that have been viewed as first in the field. There will also be a commentary on the emergence of SERS and the people who work in the community and engage via Scix.

(RAM-06.2)**What We Learn from SERS**

Janina Kneipp, *Humboldt Universität Zu Berlin*

SERS has provided Raman spectroscopy with extreme sensitivity. At the same time, it can introduce a level of selectivity to a spectroscopy experiment that requires approaches to the experiment itself as well as to data interpretation that are very different from other types of vibrational spectroscopy. SERS

data always give information about the molecules that are probed together with the plasmonic nanostructures that enable the enhancement, rather than just about the molecules. In this way, they can be used for many specific purposes in molecule-material characterization. The talk will discuss examples that illustrate the special character of SERS spectra when used in the detection and in the structural characterization of organic molecules and their interactions and transformations in chemistry and biochemistry.

(RAM-06.3)**SERS as a Healthcare Diagnostic Tool: Are We There Yet?**

Marc Porter, *University Of Utah*

It is somewhat difficult to believe that is has been 50 years since the first publication on what is now referred to as Surface-Enhanced Raman Scattering or SERS. This discovery triggered the development of the field of plasmonics and an amazing number of offshoots, including approaches to measure biomarkers and other components in body fluids that signal the onset of cancer, infectious disease and other human and animal maladies. This presentation will describe and discuss work from our and other laboratories on approaches to measure such markers from whole blood, plasma, serum, saliva, and other body fluids by using a variety of strategies designed to take advantage of the remarkably large signal strength and molecular level signatures generated by SERS. It will also briefly examine: (1) the evolution of the instrumentation used since the early days of SERS (e.g., photon hotels) to today's smart phone sized devices; (2) the status of the efforts to translate diagnostic tests based on SERS from the laboratory research bench to the patient's bedside; and (3) the often-overlooked impact of sample preparation on the clinical accuracy of today's testing formats.

(RAM-06.4)**In vivo SERS biosensing for human health monitoring**

Bhavya Sharma, *University Of Tennessee*

Human health and performance monitoring is a field that is rapidly evolving through the development of point of care, wearable, and electrochemical sensors. While conventional medicine relies on physical exams, patients' response to questions, blood tests, and imaging, recent evidence demonstrates that real-time detection of molecules correlated with changes in physiological conditions provides greater insight to human health on a personalized level. Both non-invasive and minimally invasive sensors have been developed, including smart watches and fitness devices, that can monitor human health conditions through the skin, and in non-invasively collected biofluids such as urine, saliva, tear fluid, and sweat. Surface-enhanced Raman spectroscopy (SERS) has shown great promise in the field of human health monitoring through detection of biomarkers in cells, tissue, and biofluids. Further, through the combination of SERS with spatially-offset Raman spectroscopy (termed SESORS), great strides have been made in human health monitoring. Major breakthroughs over the last (almost) 20 years and future directions for in vivo SERS biosensing will be discussed.

23RAM12: Raman Spectroscopy for Security and Forensics Purposes, Cascade 1
Chair: Igor Lednev
Co-Chair: Lamyaa Almehmadi

(RAM-12.1)**Spectroscopic Analysis of Liquids and Airborne Particles**

Karina Weber, Dana Cialla-May, Juergen Popp, *Leibniz Institute Of Photonic Technology,*

Raman spectroscopy is known to provide fingerprint specific information to analyze the chemical composition of gaseous, liquid or solid substances and therefore became an important analytical technique within the last decades. Within this contribution the broad band of Raman applications focused on presentation of the entire analysis chain, from sample preparation till the result, will be introduced. As an example, fungal spores, which are associated with causing respiratory diseases, were investigated by means of UV-Raman spectroscopy. [1] The health hazard of those spores is due to the presence of allergens and mycotoxins in varying amounts between the species and their fast identification is of tremendous interest. Thus, UV-Raman spectra were recorded and by applying

innovative chemometric tools, a classification on the genus, species and strain level was achieved. Moreover, Raman imaging was applied in the analysis of the biochemical composition of birch pollen. [2] Thus, the layered structure of these grains was visualized and by applying chemometric algorithms, components within the grain wall as well as inner core were identified and quantified. Finally, Raman spectroscopy was applied for the detection of the antibiotic ciprofloxacin in pharmaceutical formulations. [3] In the case of low background matrices such as NaCl infusion solution, the estimation of the concentration can be performed without further sample preparation. However, in the case of a pharmaceutical composition with high Raman background such as dextrose infusion solution, the Raman marker mode of the antibiotic is not identifiable. Therefore, the sample was mixed with water in a ratio 1:5000 and surface enhanced Raman spectroscopy (SERS) is applied to enhance preferentially the Raman modes of ciprofloxacin due to its high affinity towards the metallic SERS-active sensor surface.

References:
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(RAM-12.2)**Raman Spectroscopy as a Universal test for Body Fluid Identification: Method Validation vs. False Positives**

Luis Perez Almodovar, Igor Lednev, Lenka Halamkova, Marisia Fikiet, *University at Albany, SUNY, Texas Tech University, University of New Haven*

Identification of body fluids at crime scenes is vital in forensic investigations as they provide critical DNA evidence for resolving criminal cases. However, the identification of body fluids can be challenging due to ubiquitous substances that may resemble the appearance of a specific body fluid and result in a false positive. This study combined Raman spectroscopy with advanced statistical analysis, specifically Random Forest for body fluids identification, a proven powerful tool for forensic purposes. To overcome potential environmental interferences (EI), substances were examined that might resemble body fluid stains at a crime scene or yield a false positive result when conventional biochemical tests are used. The random forest algorithm was used to differentiate between body fluids and EIs, achieving complete separation of classes with a 70% classification probability threshold, demonstrating the potential of Raman spectroscopy for rapid and non-destructive identification of body fluids traces at a crime scene.

(RAM-12.3)**Application of Raman Spectroscopy to the Forensic Analysis of Drugs**

Sergey Mamedov, *HORIBA Scientific*

Raman microspectroscopy is very applicable in the field of forensics. It covers a wide spectral range from 10 cm-1 to 4000 cm-1, making the technique ideal for identifying organic and inorganic substances, including fibers, drugs, pharmaceuticals, explosives, inks, paint, etc. Only a small amount of sample is required, and little or no sample preparation is necessary.
This presentation aims to demonstrate some of the forensic applications of Raman spectroscopy, particularly the capability of confocal micro-spectroscopy to identify substances directly through transparent evidence bags and packaging such as glass and plastics. Besides, the spectra of substances with slight differences in the chemical structure show a variation of vibrational bands and, therefore, play a vital role in helping to determine when drugs, for example, have been illegally manufactured. To illustrate the above-mentioned, spectra of the two main forms of cocaine (cocaine hydrochloride and cocaine base) will be highlighted in this presentation. Raman spectra of the particles trapped on the inner surface of the plastic bag (case material) and far from the surface were collected, and the method of analysis was described. Despite the presence of spectral bands arising from plastic bag,

confocal Raman micro-spectroscopy allows one to identify cocaine by its characteristic Raman bands. The effect of the side group on the vibrational spectra of a few controlled substances (amphetamines) becomes an essential tool for drug identification.
Method development, including statistical analysis and advanced software packages, allow quickly identifying materials whose spectra have been collected in a library or by comparing the spectra of samples suspected to be similar.

(RAM-12.4)**Effect of Temperature and Time on the Stability of Drugs Contaminated Fingermarks Probed by Raman Spectroscopy**

Mohamed O. Amin, **Entesar Al-hetlani**, Igor Lednev, *Kuwait Unversity, University at Albany, SUNY*

For over a decade, chemical analysis of fingermarks (FMs), with particular reference to "touch chemistry," has offered additional intelligence to the forensics community. Therefore, understanding the FM degradation trends in the presence of exogenous contaminants is vital. The current study aimed to investigate the underlying changes in drugs in contaminated FMs upon exposure to different temperatures and times since deposition using Raman spectroscopy. For this purpose, five non-steroidal anti-inflammatory drugs (NSAIDs) were used to produce contaminated FMs, and their change at various temperature and times was studied. We found that the Raman signal of aspirin, diclofenac, and ibuprofen in contaminated FMs were significantly reduced at high temperatures, whereas naproxen and ketoprofen were more stable at the studied temperatures. Furthermore, the chemical/physical changes of these drugs in FM samples were monitored for 40 days post-deposition. The Raman spectra of the FM contaminated with aspirin exhibited monotonic changes over time, whereas the diclofenac-, ibuprofen-, ketoprofen-, and naproxen-contaminated FMs showed little change. Additionally, the intensity of the Raman bands assigned to individual drugs showed a monoexponential decrease with time since FM deposition, and the characteristic decay time of each drug varied between 5 and 600 h. The present study advances the use of Raman spectroscopy to study the stability of drugs in FM samples exposed to high temperatures and aged up to 40 days.

23SPR01: Early Career Plasmonics Researchers, Cascade 4
Chair: Jean-Francois Masson

(SPR-01.1)**Plasmonic Heating of Metallic Nanostructures: Comparing Bulk and Surface Temperatures**

Gregory Wallace, Jodie Fergusson, Amritpal Singh, Ewen Smith, Tell Tuttle, Karen Faulds, Duncan Graham, *University of Strathclyde*

An important property of metallic nanostructures is that upon illumination, heat is generated at the metal surface through non-radiative decay processes. This generation of heat has enabled metallic nanostructures, such as nanoshells, to play an emerging role in photothermal therapies. When coupled with plasmon-enhanced spectroscopies, notably surface-enhanced Raman scattering (SERS), these structures can be used in theranostic applications where the Raman spectrum provides insight into the distribution of the nanoparticles within a biological sample (i.e. tumour), and the photothermal heating the therapeutic aspect. Determining the therapeutic potential of the nanostructures requires understanding how much heat is being generated. This is typically done using infrared imaging or inserting a temperature probe into the colloidal solution. However, this raises an important question: what is the temperature at the surface of the nanostructure compared to the bulk temperature?

In this presentation, we examine two different ways of determining the temperature at the surface of a nanoparticle using SERS: (i) a temperature-sensitive analyte, and (ii) comparing the anti-Stokes to Stokes ratio. The use of temperature-sensitive analytes, such as phenyl isocyanides, offer relatively straightforward means of determining surface temperatures. As the temperature increases, changes in the spectra can be observed. Analyzing the anti-Stokes to Stokes ratio requires more specialized experimental set-ups, and complicated data analysis due to the presence of the localized surface plasmon resonance. Given that nanostructures can be prepared with different optical, and physical

properties, we explore which method(s) should be used for different structures. We then compare the readily measured bulk temperature to the determined surface temperature to demonstrate how much of a temperature difference there truly is. By better understanding the temperature at the surface of the nanostructures as opposed to the bulk, the hope is that more structures can potentially be used for theranostics and photothermal therapies.

(SPR-01.2)**Using Plasmonic Fibre Nanosensors to Probe Chemical Dynamics Essential for Cell Physiology**

Malama Chisanga, Haiyan Wu, Jean-Francois Masson, *University of Montreal*

Understanding cell physiology at the molecular level is crucial for unequivocal diagnosis and treatment of infections, which necessitates the development of high-resolution analytical tools. Existing chemical profiling techniques, mainly based on mass spectrometry and imaging tools, are challenging to apply for spatiotemporal investigation of cellular activity within the biological context in situ. Plasmonic nanofibres have emerged as easy-to-use optical tools for real-time characterisation of site-specific chemical trends in single cells. We have developed polymer-coated curved glass fibres decorated with nanoparticle arrays (so-called nanosensors) with sub-excitation wavelength dimensions to fingerprint metabolites and ions in the regions of a cell that are inaccessible by conventional tools. The main goal of this talk is to demonstrate the applicability of custom-designed miniaturised nanosensors for rapid, label-free, and non-destructive surface-enhanced Raman scattering (SERS) fingerprinting of metabolite changes in cells under physiological conditions and in response to stress. When conjugated with 4-mercaptobenzoic acid (4-MBA) reporter molecule, the SERS-active nanosensor detected pH gradients in the proximity of a cell in real time. Plasmonic fibre nanosensors hold potential for cell biochemistry assessment and medical diagnostics, especially along the lines of intracellular metabolite elucidation to unravel metabolic pathways underlying various diseases and therapeutics.

(SPR-01.3)**From Water to Whisky: Golden Opportunities in Analysis with Plasmonic Particles**

Jennifer Gracie, Justin R Sperling, Alasdair W Clark, **William Peveler,** *University of Glasgow*

Localised surface plasmon resonance, the strong interaction between electrons in nanoscale particles of metal, and light, results in the observed strong colour of nanoscale colloidal gold, silver etc. Such plasmonic colour is exquisitely sensitive to the material, size and shape of the colloidal particles as well as their local dielectric environment – what is bound to the particle surface.

I will demonstrate that by using varied surface chemistries, inspired by mammalian taste or smell, we can control the interactions between gold nanomaterials and many different components in an analytical sample, and thus generate hyperspectral ‘chemical tongues’ as cross-reactive sensing units. By processing the combined colour change output of the plasmonic array a wide variety of samples can be studied or identified [1].

I will also discuss how we can utilise certain samples to actually generate colloidal gold nanoparticles in-situ. Through examination of the rate of formation, shape, size and surface chemistry of the resulting nanoparticles and correlating this with measured chemistry in a library of samples, we can generate a rapid and informative chemical test for quality control in a range of food and beverage products [2].

[1] Macias, G. et al. Whisky tasting using a bimetallic nanoplasmonic tongue. Nanoscale 11, 15216–15223 (2019).

[2] Gracie, J., Zamberlan, F., Andrews, I. B., Smith, B. O. & Peveler, W. J. Growth of Plasmonic Nanoparticles for Aging Cask-Matured Whisky. ACS Appl Nano Mater 5, 15362–15368 (2022).

(SPR-01.4)**Highly ordered nanostars-based solid substrate for ultrasensitive SERS**

Alexandre Chícharo, Alexandra Teixeira, Maria Relvas, Marta Aranda-Palomer, Jérôme Borme, Lorena Diéguez, Sara Abalde-Cela, *INL - International Iberian Nanotechnology Laboratory*

Surface Enhanced Raman Spectroscopy (SERS) is a highly promising analytical tool for non-destructive molecular analysis in various fields, including medical diagnostics, environmental monitoring, and food safety. It allows for the detection of molecules near metal nanostructures upon laser irradiation, eliminating the need for labeling. Traditionally, chemical methods have been employed to produce metallic nanoparticles with diverse morphologies like nanospheres, nanorods, nanotriangles, or nanocubes. Recent advancements have led to the development of sharper and rougher morphologies such as nanoflowers and nanostars, which create localized hot spots for enhanced signal intensity. These hot spots can also be generated at the gaps between nanoparticles in assemblies or arrays. However, effectively controlling these hot spots remains a challenge, impeding the progress of SERS sensing technologies.

Alternatively, top-down approaches utilizing micro- and nanofabricated substrates have emerged to create surfaces with well-ordered arrays of metallic nanostructures. These substrates enable precise control of interparticle distances, resulting in more predictable enhancement factors, high signal homogeneity, and a unique focal plane for analysis. In this study, a high-density SERS array was developed using electron-beam lithography. The array comprised gold nanodisks with varying diameters (ranging from 20 to 650 nm) and interparticle distances or pitches (ranging from 200 to 700 nm). To achieve ultrasensitive SERS detection, a chemical process was employed to transform the nanodisks into nanostars, which possess multiple hot spots at their tips, leading to highly intense SERS signals. To evaluate the SERS efficiency of these substrates, experiments were conducted using a standard Raman Reporter molecule, 1-Naphthalenethiol (1NAT). Additionally, the novel substrate was utilized for the detection of tryptophan, a metabolite expressed by certain tumors, serving as a proof of concept. Metabolites play crucial roles in cellular physiology, including cell communication, receptor activation, and energy cycles. Thus, these SERS substrates hold the potential for uncovering metabolic processes associated with cancer development, intercellular communication, and microenvironmental conditions supporting cancer replication.

[1] Abalde-Cela S. et al., Adv Opt Mater. 9, 2001171 (2021).
[2] Abalde-Cela S. et al., Adv Colloid Interface Sci. 233, 255 70 (2016).
[3] Pavlova N.N. et al., Cell Metab. 23, 27-47 (2016).

(SPR-01.5)**Characterization of Mycobacterium Tuberculosis – Bacillus Calmette-Guerin Using Surface Enhanced Raman Spectroscopy**

Timothy Yokley, Andrea Locke, *Vanderbilt University*

Tuberculosis (TB) is the second leading cause of death by an infectious disease as defined by the World Health Organization (WHO). While TB is curable, only 5-10% of people infected with TB will develop symptoms, which can lead to untimely or misdiagnosis of the disease. Moreover, people with weakened immune systems, such as the cases with HIV, latent TB is difficult to detect due to the inaccuracy of the immuno-based assays. While rapid diagnostic TB tests exist, accuracy across all types of TB infections lack sensitivity. With this in mind, the WHO has committed to ending the TB epidemic by 2030, thus; the development of culture-free, rapid diagnostic/prescreening tests are needed. Herein, this study demonstrates the ability to detect biochemical features of Mycobacterium Tuberculosis – Bacillus Calmette-Guerin (BCG), an attenuated form of TB, by ways of surface enhanced Raman spectroscopy (SERS). SERS measurements were compared using citrate-capped (AuNPs) and thioglucose-capped (AuNPs-TG) gold nanoparticles to evaluate biochemical features of the cell wall. Preliminary results show significant enhancement in signal using AuNP-TG, the limit of detection (LOD) for detecting BCG, and increased sensitivity versus their AuNPs counterpart. The platform has the potential to translate into an innovative technology to screen for TB at the point-of-care.

Poster Presentations

Tuesday Poster Sessions

(Tues-P1)**Optimization of cell recovery for label-free dielectrophoretic cell sorting in microfluidic systems**

Syleah Manns, Ella Fitzgerald, Dean Thomas, KATHERINE DEGEN, Ridi Barua, **Alexandra Hyler**

Background: Biological samples spanning biopsies, blood, and tissue are incredibly heterogenous. In order to better understand cell behavior and interaction, sorting cell subpopulations is a critical tool in understanding various disease processes. Microfluidics are a tool used to identify and sort subpopulations on samples with low cell numbers. Dielectrophoresis (DEP), one such method, exerts a force on cells using non-uniform electric fields which depends on their biophysical properties. By characterizing the response for a particular cell type, label-free sorting of cell subpopulations is possible. In order to reach sorting goals, high cell recovery is required. In gold standard methods such as flow cytometry recovery rates are often < 60% and can be as low as 10%, thus we aim to reach 80% recovery rates. Here, we test, design, and optimize a variety of strategies to achieve high cell recovery rates.
Methods: Initial investigations identified syringe leurs and tubing to be major sources of loss within the system. A variety of surface coatings were prepared in CytoBufferTM and applied to all fluidic components and soaked for 30 minutes prior to use. Low dead volume syringes and Darwin coupling systems were also tested in order to assess their impact on cell loss. Finally, two new connection designs that eliminate leur and tubing connections, Direct Injection and Reservoir Method, were evaluated to assess cell recovery.
Results: The 0.5% Pluronic F-127 showed a 1.3-fold increase in cell recovery compared to the standard setup, and the Darwin coupling system showed a 2.15-fold increase in recovery compared to the current system. The Direct Injection design, wherein the sample syringe is directly connected via nozzle to the microfluidic chips, drastically improved cell recovery and achieved recovery rates nearing 100%. However, the Direct Injection design resulted in unstable flow rates, so a final pressurized Reservoir Method was developed. Initial tests of the Reservoir Method suggest the flow profile scales linearly and is within the parameters required for dielectric cell sorting. Furthermore, cell recovery in the Reservoir Method was in the target range achieving 82%. Further work will validate these capabilities across additional applications of cell sorting.

(Tues-P2)**[FeFe] and [NiFe] Hydrogenase Stability at Low Temperatures in Cryosolvents: An Alternate Approach for Capturing Fleeting Intermediates**

Alexander Jackson, James A. Birrell, Olaf Rüdiger, Sagie Katz, Kushal Sengupta, Nina L. Breuer, Ingo Zebger, Simon J. George, Serena DeBeer, Stephen P. Cramer

Nature processes molecular hydrogen, H2, with remarkable efficiency via metalloenzymes known as hydrogenases (H2ases), which have a [FeFe] or [NiFe] metal center as the heart of their active sites. To fully describe the mechanism of H2ases it is necessary to study how the metal center binds and reacts with H2, and how the surrounding ligands guide catalysis. We obtain this information by trapping intermediate states and characterizing them with a battery of spectroscopic techniques, such as infrared (IR) spectroscopy or the synchrotron x-ray techniques nuclear resonant vibrational scattering (NRVS) and extended x-ray absorption fine structure (EXAFS). However, the experimental constraints of current methods prevent us from identifying and stabilizing key short lived catalytic intermediates (ns or μs).

In this poster we describe a novel photoinduced cryokinetic approach to isolate and characterize key initial catalytic intermediates by employing low temperatures, below 0°C and down to -83 °C. We describe how we plan to study the bound-H2 intermediate, so-called (Hox)H2, a likely first step in the

H2-splitting reaction and key to understanding the mode of initial H2 interaction at the catalytic site in both prototypical [FeFe] and regulatory [NiFe] hydrogenases. This approach requires precisely and efficiently delivering H2 coupled with cryobiochemistry; the use of aqueous-organic solvent mixtures to both prevent freezing and preclude irreversible protein alterations that may affect catalytic integrity. For H2 delivery a photocatalyst is coupled with the H2 generating compound ammonia borane so that near-infrared irradiation will cause a controlled release of H2 into the experimental system.

So far, we have performed an extensive feasibility study for this approach using IR spectroscopy, which offers an exquisite probe of the active site, the H-cluster, via its carbonyl and cyanide ligands that offer characteristic signatures of the different catalytic states. These measurements show that the enzyme is essentially unaffected by the use of cryosolvents. Additionally, we have used bioelectrochemistry to study enzyme activity under cryocooled conditions, and we find the expected consistent reduction in rate with temperature, with a linear Arrhenius plot showing a well-behaved system. These results, together with our progress on trapping (Hox)H2 are discussed in this poster.

(Tues-P3)**Analyzing Stomach Cancer Tissue using Convolutional Neural Networks (CNN) and Autofluorescence**

Jin Il Jang, Hyung Min Kim

Autofluorescence is a non-invasive analysis method which is used to diagnosing for in vivo and ex vivo tissues. Normally, Autofluorescence appears at a wavelength of 400 nm to 700nm and includes various biometric information. Among them Nicotinamide dinucleotide (NADH) and Flavin adenine dinucleotide (FAD) are co-enzymes used in metabolism and respirations, which are strongly associated with pathological symptoms. In addition, various components such as Lipofuscin PPIX and vitamins show fluorescence, and the change in composition appears as a change in fluorescence spectrum.
In this study, we make centimeter-scale hyperspectral fluorescence images of tissue sections using a point mapping method and performed semantic segmentation using a 3D Convolutional Neural Networks model. Hyperspectral imaging, a multi-channel imaging technique incorporating spatial and spectral information, facilitates detailed analysis of tissue characteristics at a microscopic level, and a 3D CNN model was employed to analyze and classify the spatial correlations within these images, with the categorized data being subsequently visualized and validated through Hematoxylin and Eosin (H&E) stained images. Utilizing this method, we segmented the tissue into four types - mucosa, submucosa, muscle, and cancer, which showed high similarity with the results from pathological analysis.

(Tues-P4)**Determining the Effects of Glycocalyx Modification on Human Mesenchymal Stem Cells' Electrophysiology**

Sydney Joseph, Rominna Valentine, Lexi Crowell, Stephany Alonso, Tayloria N.G. Adams

Dielectrophoresis (DEP) is a novel cell analysis tool used to analyze and manipulate biological cells. DEP allows for label-free measurement of cells' electrophysical properties (membrane capacitance and cytoplasm conductivity) which may serve as numerical indicators of cell health and stem cell fate. While many advancements have been made in the field of DEP, little is known about the role the cell surface plays in DEP polarization. The objective of this project is to explore how modifying the cell surface of hMSCs affects DEP polarization. Recently human mesenchymal stem cells (hMSCs) have been an important factor in advancing clinical trials due to their multipotent differentiation characteristics. Due to the multipotency of hMSCs (which lends to their heterogeneity) and a lack of unique biomarkers, it is challenging for researchers to select one cell type for use in clinical studies. One way to better understand hMSCs and distinguish cell types is through investigating the cell's glycocalyx. The glycocalyx is a membrane of dense, charged macromolecules covering the surface of all cells, including the hMSCs. The glycocalyx protects the cell from chemical injury, enables the immune system to identify hostile cells, aids in cell adhesion and cell communication. It is unknown how the polarization of hMSCs is affected when the glycocalyx surface area is increased or decreased.

. In this study the glycocalyx of adipose derived hMSCs was modified using N-Acetylglucosamine(N-GlcNAc), which increases the thickness of the glycocalyx and Kifunensine(Kifu.), which decreases the glycocalyx thickness. Cells were treated with N-GlcNAc and Kifu for 5 days before they underwent DEP analysis with the 3DEP. Results from the 3DEP were further verified using scanning electron microscopy and transmission electron microscopy imaging. DEP analysis found changes between the modified glycocalyxes of the treated hMSCs, showing that DEP is a useful label free tool able to identify differences in cell biomarkers.

(Tues-P5)**(O-PTIR Spectral Imaging of Human Bone Biopsies Embedded in Thick Blocks at Sub-Micron Resolution**

Sofia Mehmood, Isha Dev, Yana Bronfman, Edward DiCarlo, Nancy Pleshko, William Querido

Osteoporosis is a degenerative bone disease that affects millions worldwide. Currently, there is a lack of understanding on how tissue-level properties are associated with disease development and progression. Historically, infrared (IR) spectroscopy has been used to assess tissue composition, but traditional modalities cannot reach high enough spatial resolution to assess the basic building blocks of bone tissue at the sub-micron level. Recently, the new modality optical photothermal infrared (O-PTIR) spectroscopy was developed, which may allow sub-micron resolution assessment of bone composition in thick samples without the need to cut thin sections. The goal of this research is to establish the application of O-PTIR spectroscopy to assess tissue-level composition of clinical human bone biopsies. Human samples were iliac crest biopsies embedded in thick polymethyl methacrylate (PMMA) blocks (22 mm by 21 mm). O-PTIR data collection was carried out in the mIRage Sub-Micron IR Spectrometer (Phototermal Spectroscopy Corp) to assess bone tissue composition at 500 nm spatial resolution. Protocol establishment involved tuning the laser and probe power to optimal settings and collecting both point spectra and single wavenumber images at peaks of interest, including for Amide I (collagen), PO4 (mineral), and PMMA components. To fit the thick samples into mIRage microscope stage, we designed and 3D printed a custom sample holder using SolidWorks. Scanning electron microscopy (SEM) was carried out in the FEI Quanta 450 FEG to visualize bone structure. Cortical and trabecular bone tissues embedded in thick PMMA blocks can be clearly visualized in brightfield mode using the mIRage. O-PTIR spectra of embedded bone show typical peaks of matrix components (mineral, collagen), with little to no influence of PMMA. O-PTIR spectral imaging illustrates differences in distribution of PMMA and bone tissue components. In particular, the ratio between the mineral and PMMA images reveal bone tissue porosity associated with osteon Haversian canals and osteocyte lacunae, as well as lamellar structure. Additionally, overlaying O-PTIR and SEM images allows visualizing the association between bone structure and composition. O-PTIR spectral imaging has proven to be an efficient and effective method to assess tissue-level composition of human bone biopsies embedded in thick blocks at sub-micron resolution.

(Tues-P6)**Design of Multi Wavelength and Output Laser system for Biomedical Applications**

Youngmin Moon, **Junyoung Seo**, Hwangryol Ryu, Kisung You, Jonghyun Woo, Chang-Su Na

Many biomedical applications require different wavelengths and multi-output configurations to serve their specific purposes. However, a common problem with laser diodes frequently used in biomedical applications is the temperature increase over extended usage, leading to a decrease in laser output. This issue becomes more pronounced when multiple outputs are used. Especially, stable laser output power is one of the most important conditions for biomedical applications. To solve this problem, we have designed a method to control the temperature of the laser diode by using a feedback system composed of a Peltier element and a temperature sensor. Also, we have designed the optical system to enable multiple wavelengths and outputs using a minimal number of laser diodes.

(Tues-P7)**Analytical Methods for Implantable Medical Devices: Bioprosthetic Heart Valves and Hip Implants**

Chady Stephan, Ken Neubauer, Ewa Pruszkowski, Bin Yuan, Kamal Yadav

According to the World Health Organization, an estimated 2 million different types of medical devices are now present on the global market. As the population distribution is shifting towards older ages with the increase of chronic and age-related medical conditions, this number is expected to grow at the same pace hitting the medical device industry with unique demand. For every innovative medical device that reaches market, comes a collateral concern for that device's safety that can be assessed by precise analytical characterization. In this poster, two of the most used implants are investigated: bioprosthetic heart valves and hip implants. In the two following sections, accurate and reliable analytical methods are presented to evaluate their biocompatibility and durability.
In the first part, glutaraldehyde (GA) analysis in bioprosthetic heart valve is carried out by LC-UV. A xenograft heart valve is a type of artificial bioprosthetic valve obtained from an animal source (porcine or bovine) and largely used in the over 300,000 heart valve surgeries conducted every year. Crosslinking with GA renders a cardiac xenograft inert, nonbiodegradable, and non-antigenic. However, GA crosslinking does not guarantee complete biocompatibility, and paradoxically causes dystrophic calcification due to phospholipids, free aldehyde groups and residual antigenicity. In this study, a robust and reliable LC-UV workflow method is presented for the assay determination of GA.
In the second part, total and single particle analysis of Titanium (Ti) in Serum and Hip Aspirates is carried out by ICP-MS.
Ti is a metal of choice in all kinds of prosthetics, such as artificial hip joints, knee, dental implants. The artificial joints are made from various Ti alloys, usually with a small addition of aluminum and vanadium. Because the hip ball-and-socket joint undergoes extensive use, Ti could slowly wear away over time and enter the blood stream. Even though Ti is nontoxic, its levels can give medical providers information on the level of implant degradation. In this work, total and single particle analysis of Ti in serum and hip aspirates from a patient with artificial hip was conducted using ICP-MS showing that Ti was present in both the dissolved and particulate forms.

(Tues-P8) **Optimization of Approach for Differentiation of Saline and Hydrated Joint Tissues During Vis-NIR Arthroscopic Fiber Optic Assessment**

Amanda Spurri, William Querido, Mohammed Shahriar Arefin, Chetan Patil, **Nancy Pleshko**

Assessment of musculoskeletal tissues using visible-near infrared (VNIR) fiber optic spectroscopy is a nondestructive approach for compositional analysis, with the potential for in vivo application. However, there are several challenges for collecting and evaluating spectra obtained during arthroscopy, including spatial limitations of the joint anatomy and presence of irrigation fluid (saline) throughout the procedure. To address the spatial challenges of arthroscopic data collection, a fiber optic probe was designed with a 90 degree bend at the tip, allowing for direct contact with the tissues of interest, tibial and femoral cartilage that overly bone. Here, we determine optimized spectral collection parameters to differentiate tissue compositional water from the irrigation fluid.
Methods: Tissue samples were extracted from porcine patella using a 4 mm biopsy punch and included cartilage, bone, and cartilage on top of bone (osteochondral) plugs. Spectra were collected under several conditions, simulating potential situations that could occur during in vivo arthroscopic data collection. These conditions included: placing the probe in direct contact with the tissues, establishing an offset from the probe tip and the tissue surface, and introducing saline to the space between the probe tip and tissue surface. Spectra were collected with 50 co-added scans using an ASD VNIR spectrometer with a diffuse reflectance fiber optic probe with quartz fibers. A Spectralon standard was used for baseline collection and as the background for spectra acquisition. Raw spectra were processed using a second derivative Savitzky-Golay filter.
Results and Discussion: A peak shift at ~7100 cm-1, a frequency associated with free water, was observed when saline was present between the osteochondral samples and the probe tip, which could indicate the presence of irrigation fluid. This difference was also evident in principal component analysis (PCA). Additionally, PCA was utilized to evaluate contributions of environmental hydration while probe contact was maintained during spectra collection. The spectra obtained from samples with additional saline with probe in contact with tissues were not distinguishable from those of the initial tissue hydration state. Further methodology development to optimize use of this VNIR probe in an in vivo setting will focus on sample contact to minimize artifacts.

(Tues-P9) **A SERS-based substrate for a rapid and efficient detection of mutations in acute myeloid leukemia**

Alexandra Teixeira, Maria Sousa-Silva, Alexandre Chícharo, Ahmed Mahmoud, Sara Abalde-Cela, Lorena Diéguez

Acute myeloid leukemia (AML) is the most common form of acute leukemia in adults and is frequently associated with poor outcomes to conventional therapies in the most affected population[1,2]. After treatment with chemotherapy, even patients that clinically achieve complete remission (CR) can relapse through the persistence of minimal residual disease (MRD), with fatal consequences. Current diagnostic tools are not enough for early detection of MRD[2,3]. An accurate and early diagnosis of MRD would allow the application of appropriate therapy, improving the prognosis of patients. Nanotechnology, surface-enhanced Raman scattering (SERS) spectroscopy and microfluidics are some of the key enabling technologies (KETs) that have remarkably flourished in the recent years and demonstrated to be powerful tools for cancer diagnosis[4,5].
The main goal of this work is to create a non-invasive diagnostic tool for the early and non-invasive detection of specific mutations. For this purpose we designed, fabricated, tested and optimized a SERS sensor for efficient detection of AML mutations. For this, some substrates were developed, some using gold nanostars synthesised using different strategies and immobilized on different materials (glass or paper). On other type of substrates using silicon wafers the nanoparticles were grown in situ using an innovative strategy. Afterwards, immobilization of molecular beacons was performed and the performance of the substrates was tested using synthetic targets. In parallel, microfluidic devices for the isolation and concentration of leukemic blasts from peripheral blood, was done, in order to apply the SERS substrates with better performance. This will promote a multiplex and quantitative detection of specific AML mutations in blasts isolated from patient samples, enabling the application of personalized therapy and consequently improving the prognosis of patients.

(Tues-P10) **Towards imaging millisecond neural computations with an electro-optical two-photon microscope**

Harishankar Jayakumar, Deano Farinella, **Chris Warkentin**, Samuel Stanek, Jacob Gable, Zachary Newman, Sakal Singla, Biswanath Chakraborty, Aaron Kerlin

The precise timing of neuronal activity carries unique sensory information, correlates with perceptual decisions, and powerfully influences synaptic plasticity. To understand the circuits that generate behavior, we must interrogate signaling in vivo at millisecond timescales. However, current microscopy technologies limit the targeted recording of fast indicators of activity to a small number of sites. To overcome this barrier, we leverage the unparalleled speed of light deflection through electro-optical crystals. In proof-of-principle experiments, we developed a small field-of-view (12 μm by 6 μm) electro-optical two-photon microscope using commercial potassium tantalate niobate (KTN) deflectors. We observed minimal nonlinear distortion of femtosecond pulses (100 fs duration) by the deflectors when using energies typical of two-photon microscopy (15 nJ). By implementing a novel two-dimensional KTN-based deflection module, we achieved ultrafast (14.7 kHz frame rate, 560 kHz line rate) and high resolution (0.5 μm lateral, 4 μm axial) in vivo imaging of action potentials using a genetically encoded voltage indicator (ASAP3). Based on interferometric and thermal imaging of KTN crystal, we are designing, building and testing new electro-optical deflectors for random-access imaging over fields-of-view similar to current acousto-optical systems, but with access times up to 50 times faster.

(Tues-P11) **Study on the Global Oncology Challenges Using Biotechnology**

Xianfang YUE

Background: Oncology symptoms often rarely appear until a patient's disease is at an advanced stage, especially for some unconventional examples'cancer. Treatment options for cancer may include surgical resection, chemotherapy or a combination of different approaches. However, if a patient's

cancer is extensive, the likelihood of such therapeutic approaches being effective declines. Even when cancer is detected at its earliest stage, it almost always has shed cells throughout the body, and the cancer returns. Objective: By understanding the mechanism how blood metabolism and neuromodulation can lead to discoveries that improve patient health and wellness, particularly for patients who have cancer. Methods: Using new digital RNA profiling, identifying specific areas could be dug deeper into that demonstrated high- and low-grade areas of abnormal cell growth, discovering new biomarkers and identifying these unique markers which could be more accurate predictors of cancer and should be identified that could help clinicians to predict cancer situations, which will have effect on the next generation of drug development and precision medicine. As promising therapeutic targets for cancer therapy, cancer stem cells (CsCs) have self-renewal capacity and differentiation potential and contribute to multiple tumor malignancies.
Results: Understanding the molecular and cellular regulatory mechanism of dynamic cellular events along the cancer progression in a heterogeneous nature, can provide significant impact on CSC-specific therapeutics development, so as to achieve the healing effect shuttling cancer immunotherapies only to tumor cells, sparing healthy ones and limiting side effects. Conclusion: As promising therapeutic targets for cancer therapy, CsCs have self-renewal capacity and differentiation potential and contribute to multiple tumor malignancies. To reduce the risk of human error, improve outcomes, and cut costs, the machine-learning technology could assist pathologists in making more accurate diagnoses treatment by algorithms to filter, organize and search for patterns in aspects of patient care and help big datasets, predictive analytics, and informed decisions. In addition, machine learning could lower the barrier in pharmaceutical sciences and in drug formulation.

(Tues-P12) **Analytical Method Development for Quantification of Quartz Using Raman Spectroscopy**

Elizabeth Ashley, Vasileia Vogiazi, Chen Wang, Pramod Kulkarni

Raman spectroscopy method for quantification of α-quartz in workplace aerosols for inclusion is presented. A laboratory Raman spectroscopy-based method was recently developed for the measurement of trace quantities of respirable crystalline silica using a microconcentration technique. The analysis of a dried spot (~2 mm diameter) obtained by redepositing the particulate sample, after low-temperature plasma ashing of the polyvinyl chloride (PVC) filter sample, has yielded detection limits ranging from 0.016µg –3.26 µg. These detection limits are two to three orders of magnitude lower compared to those attainable using current standardized X-ray diffraction and infrared spectroscopy methods. This work investigates the application of different laser power levels, additional sample scanning and spectra acquisition methodologies (such as rastering), and estimations on the recovery of redeposited samples. The silica samples deposited on filters were analyzed using both a portable probe-based Raman spectrometer and an analytical-grade confocal Raman microscope. The analysis was conducted at various integration times and laser power levels, which are dependent on the mass loading of the samples. The signal intensity for α-quartz was determined by measuring the peak height at a Raman shift of approximately 465 cm-1 after applying baseline correction. For the probe-based Raman method, spectra were obtained from three randomly selected locations (using a non-rastering method) and the average of peak heights at the characteristic peak of a-quartz. On the other hand, for the rastering method using a confocal Raman microscope, spectra were acquired from 164 locations across the redeposited sample. The summation of the peak heights for the Raman shift of ~465 cm-1 was calculated for each sample using this rastering method. Finally, redeposited sample recoveries were estimated for 37 mm and 25 mm spiked PVC filter samples. To quantify loss or recovery during the sample preparation steps, both Raman and gravimetric measurements were utilized. Analytical figures of the merit of the method were determined and will be presented.

(Tues-P13) **A SERS-based Galactose Sensor using Molecular Reporter-Immobilized Ag Shell-Au Satellite Hetero-Nanostructure**

Hyejin Chang, Eun Hae Heo

Increased galactose levels in the blood and urine of newborns indicate galactosemia, and accumulation of galactose in the body can lead to liver disease, brain damage, and sometimes death. Therefore, it is

necessary to develop a sensitive, fast, and reliable galactose diagnostic system that can enable early detection of galactose. In this presentation, we report a surface enhancement Raman scattering (SERS) based capillary sensor for sensitive, fast and simple detection. The developed SERS sensor is fabricated by decorating inner walls of capillary with the heterostructures of silver nanoshells and gold nanoparticles for efficient nanogap formations. In our experimental design, 4-mercaptophenylboronic acid (4-MPBA) as a molecular Raman reporter was introduced between the nanogap, and 4-MPBA was converted to 4-mercaptophenol (4-MPhOH) by hydrogen peroxide (H_2O_2) produced from catalyzed reaction of galactose with galactose oxidase (GOx). The reaction was monitored through the SERS signal shifts. After optimization processes, a selective galactose detection was successfully achieved. These results indicate that the developed capillary SERS sensor has great potential for early diagnosis of galactosemia. Details of the results will be discussed in the presentation.

(Tues-P14) **Measuring Glass Transition Kinetics of Polymers with Low-Frequency Raman**

Robert Chimenti, James Carriere, Danielle D'Ascoli, Jamison Engelhardt, Alyssa Sepcic, Kayla Bensley, Alexandra Lehman-Chong, Joseph F. Stanzione, III, Samuel Lofland

Glass transition is one of the most essential kinetic processes for describing the behavior of polymeric materials. It is critical to understanding the structure-property-processing relationships of bulk polymers, films, and fibers. Many tools are available for monitoring this process, such as differential scanning calorimetry, dynamic mechanical analysis, rheology, and infrared spectroscopy, but none of these techniques are well suited for in situ or microanalysis. Raman spectroscopy is an attractive alternative due to the high spatial resolution and inherent non-contact nature of the technique. Traditionally, Raman-based kinetic studies have focused on the fingerprint region where chemical changes alter the peak intensities, or steric changes shift or broaden the peaks. However, amorphous materials also display low-frequency Raman features related to the phonon density of states resulting in a broad disorder band below 100 cm-1. This band includes the Boson peak and a shoulder, which is dominated by the van Hove peak, and additional contribution from quasi-elastic Rayleigh scattering.

When a material goes through a glass transition, conformational entropy increases, which affects the phonon density of states. Using a first principles-based modeling approach, we have demonstrated that the van Hove peak, which is primarily responsible for the apparent shoulder in the disorder band, is relatively independent of the material kinetics. In contrast, the Boson peak and QERS, which form the peak in the disorder band, are highly sensitive to structural changes. In this work, we have demonstrated that the temperature dependence of the ratio of the integrated intensity in the proximity of the Boson peak to that of the van Hove peak shows a kink near the glass transition temperature as determined by differential scanning calorimetry for various thermoplastics (polystyrene, polylactic acid, and polymethyl methacrylate). Careful analysis of the Raman spectra confirms that this is related to a change in the phonon density of states at the glass transition temperature. This makes low-frequency Raman a promising technique for the thermal characterization of polymers because this technique is chemically agnostic and contactless and does not require either intensity calibration or advanced spectral analysis.

(Tues-P15)**Optimization of Nano-materials for Surfaced Enhancement Raman Spectroscopy Lateral Flow Assays**

Alexander Cikanek, Lyndsay Kissell, Daewoo Han, Andrew Steckl, Pietro Strobbia

Traditional lateral flow assays (LFAs) use colorimetric detection: tags attach to a test line and the operator receives a diagnostic response for a specific pathogen or biomarker by observing the line color. This method of detection has several disadvantages, including limited quantification capabilities and low sensitivity. By using surface-enhanced Raman scattering (SERS) tags in lateral flow assays (SERS-LFAs), we can overcome these disadvantages at the cost of adding a Raman reader. In this work, various nanomaterials were prepared and characterized to identify the optimal material for SERS-LFAs. We tested gold nanospheres, gold nanostars and silver coated gold nanostars to identify

particles with ideal plasmonic properties, stability in solution, flow within the assay's paper, and SERS signal. To ensure the consistency of these SERS-LFAs, the nanoparticle concentration, Raman reporter concentration, and stability of the nanoparticles must be predictable. To establish a reliable concentration-optical density relationship, UV-Vis spectroscopy and nanoparticle tracking analysis were employed. Once concentration could be reliably predicted, the nanoparticles were functionalized with a Raman reporter molecule and characterized. Finally, the nanomaterials were tested on LFA's using a lab-built Raman reader to scan the LFA strip. Our work provides the basis for understanding the role of different plasmonic particles in SERS-LFAs.

(Tues-P16) **Lego™ Blocks as Evaluation Samples For Raman Spectrometers**

Richard Crocombe, Brooke Kammrath, Pauline Leary

Sample fluorescence is a longstanding and well-known problem in Raman spectroscopy, and a large number of schemes have been proposed, and are available, to avoid or mitigate those effects. This is a particularly important problem for handheld Raman instruments used in the field because of the limited opportunity for data examination and manipulation by the operator. Therefore, samples previously used to show the efficacy of these schemes are closely related to field work, and include items like sesame seed oil and dark rum. However, these are not reproducible samples, and therefore it is difficult to make meaningful comparisons.

This work proposes the use of Lego blocks as 'standard samples' for this purpose, having these properties: rugged, low-cost, easily obtained, non-hazardous, solid, and easily-transportable. A standard set of Lego blocks comes in white, yellow, red, blue, gray and black, presenting challenges for Raman spectrometers. The underlying material is stated on the Lego web site to be an acrylonitrile-butadiene-styrene (ABS) resin, and ABS reference Raman spectra are available.

In this work, laboratory reference spectra have been acquired using 532nm, 638nm, 785nm and 1064nm excitation, and these demonstrate a variety of challenges including sample heating, fluorescence and likely resonance Raman signals from the pigments. In addition, spectra have been acquired from some portable instruments. These results will be compared, and conclusions drawn on the suitability of these Lego blocks as evaluation samples.

(Tues-P17) **Screening and Differentiation of Virus-like-particles Using Vibrational Spectroscopy**

Ankit Dodla, Magdalena Giergiel, Aaron Mclean, Linda Earnest, Melissa Barrow, Julie McAuley, Dale Godfrey, Damian Purcell, Joseph Torresi, Bayden Wood

The COVID-19 pandemic has presented the most significant global health crisis in the past century, resulting in rapid development of vaccines ahead of the anticipated timelines (7-8 years). Viruses exhibit multiple mutations, thereby lowering the potency of vaccines. Virus-like particles (VLPs) that are structurally very similar to viruses but lacking genomic material, have been exploited as candidates for vaccine development. The characterization of these VLPs is crucial to ensure similarity with the original virus and that no transfer of genomic material occurs during immunization. Different techniques such as PCR, RP-HPLC, SDS-PAGE, and microscopy are extensively used to characterize the virus-like particles, which are laborious, time-consuming, and resource intensive.

Vibrational spectroscopic techniques like infrared absorption and Raman scattering, are high-precision, label-free analytical techniques that work in the chemical fingerprint region of molecules to increase the specificity. Vibrational spectroscopy-based studies for VLPs are underexplored. Here we apply FTIR and Raman spectroscopy to study the chemical composition of virus-like particles in combination with multivariate data analysis methods to improve the differentiation and classification of VLPs. We observed prominent peaks for RNA at 1242, 1110, 782, 723 and 670 cm^{-1} missing in Raman spectra of β strain and Omicron strains SARS-CoV VLPs which were present in the SARS-

CoV 2 virions. These studies will result in the development and optimization of safe and effective vaccines in the future.

(Tues-P18) **Non-Invasive Screening of Otitis Media with In vivo Raman Spectroscopy**

Sean Fitzgerald, Alexander Ho, Guillermo Monroy, Jay Werkhaven, Kevin Mason, Alistair Harrison, Stephen Boppart, Anita Mahadevan-Jansen

The otoscope is the standard clinical diagnostic tool to observe the middle ear for diagnosis of Otitis Media (OM). However, even pneumatic otoscopy has limited contrast to detect signs of infection, such as clearly identifying and characterizing middle ear fluid or biofilms that accumulate within the middle ear during OM. Likewise, invasive sampling of every patient's ear fluid is not clinically indicated nor practical. As a result, pediatrician accuracy in distinguishing different infection states with otoscopy can widely vary from 47–93%. There is a clear need to noninvasively collect accurate diagnostic factors in order for clinicians to deliver a precise OM diagnosis and effective treatment regimen. To address this need, the presented work outlines recent progress in applying Raman Spectroscopy (RS) for optical characterization of middle ear fluids during OM. First, RS measurements of middle ear tissues were collected to investigate their independent Raman spectral signatures, and to address design challenges in developing a system specific for clinical use. A prototype non-contact RS handheld probe was then built to measure RS signatures from middle ear fluid through a standard ear speculum, which was tested on ex vivo tissues to verify device performance. Finally, this device was tested in vivo in a cohort of human subjects that were scheduled for bilateral myringotomy and tympanostomy tube placement. RS demonstrates the ability to optically distinguish various forms of OM and associated ear fluid, which offers a new diagnostic avenue for rapid and noninvasive light-based screening of OM.

(Tues-P19) **Characterization of multi-photon polymerized microstructures without photoinitiator by using in situ Raman and Brillouin spectroscopy and biocompatibility analysis**

Atsushi Nakayama, Yasuaki Kumamoto, Menglu Li, Teng Li, Meiling Zheng, **Katsumasa Fujita**

Photoinitiators used in photolithography are known to be cytotoxic, and the biocompatibility of structures fabricated by photopolymerization has been an issue. We have developed a technique that enables multiphoton microfabrication without the use of initiators via two-photon absorption of visible light. By incorporating Raman and Brillouin scattering spectroscopic optics into the photofabrication system, we have developed a system that allows in-situ evaluation of changes in molecular structure and elastic modulus induced by photopolymerization. Using the developed system, we measured PEGda polymerized under different light exposure conditions and confirmed a strong correlation between the shift of Brillouin scattering peaks and the intensity change of Raman scattering peaks. We also cured collagen without photoinitiator and analyzed the structural changes of collagen from the dependence of its Raman scattering spectra on different polymerization intensities. Cell adhesion on the collagen structures and spheroid formation in the structure of bovine serum albumin (BSA) were observed to confirm the biocompatibility of the photofabricated structures without photoinitiators.

(Tues-P20) **Raman Spectroscopy Characterization of Antibody-ligand Association at Supported Phospholipid Bilayers**

Julia Clista Galecki, Grant Myres, Jay Kitt, Joel Harris

Antibodies are proteins generated by the immune response to target pathogens through their specific binding to proteins or other ligands on cell membranes. Two fundamental questions being addressed in this research are the following: What is the stoichiometry of ligand-to-antibody binding and how does the accessibility of ligands at supported-lipid bilayers (models of cell membranes) vary with ligand coverage, thereby impacting their association with antibodies in solution? We detect the binding of

proteins by measuring inelastic light scattering from their characteristic molecular vibrations using Raman microscopy, which allows label-free and quantitative analysis of antibody-to-ligand binding. We are able to determine the concentration of antibody that has accumulated on the surfaces of supported-lipid bilayers. Raman spectroscopy can also detect changes in the binding state of the targeted ligand, understanding of which is crucial in assessing how ligand accessibility may influence its interaction with solution-phase antibodies. To achieve these results, we prepare lipid bilayers on the interior surfaces of porous silica particles, whose high surface area provides a sufficient internal concentration of both ligand and its bound antibody to allow experiments with quite modest mol-fractions (2-mol%) of ligand-modified (2,4-dinitrophenylated) lipid. Capture of antibodies is selective, requiring the presence of ligand in the bilayer, where lipid bilayers prepared without dinitrophenylated-lipid produce no detectable signal from the antibody. By varying the dinitrophenylated-lipid density in the bilayer, the surface concentration of captured antibody increases proportionally to a level that is limited by antibody size and its packing density at the lipid-bilayer surface. Carrying out the same experiment with FAB fragments of the antibody is utilized to determine the stoichiometry of ligand-to-antibody binding.

(Tues-Pg21) **Monitoring of Environmental Pollutants using Quantitative Surface Enhanced Raman Spectroscopy**

Il Han, Wei Yu, Melissa Gelwicks, Micheal Allen

Conventional methods for assessing water quality, such as chromatography, mass spectrometry, and electrochemistry, suffer from challenges related to sample preparation complexity, lengthy measurement time, and high operational costs. In this study, we explore the application of ink-jet printed Surface-Enhanced Raman Scattering (P-SERS) substrates as a promising approach for detecting trace contaminants, specifically pesticide residues, in water. The P-SERS substrates consist of chromatography paper embedded with gold or silver nanoparticles. Pesticides were dissolved in their respective solvents and diluted to 0.0 – 20.0 μM. Diluted samples were applied to the P-SERS substrate and measured by Raman spectroscopy. Comparative analysis revealed that P-SERS significantly enhanced the limit of detection for pesticides, improving it by over a thousand-fold compared to the normal Raman methods. Consequently, P-SERS enabled the detection of pesticide concentrations as low as ~1 μM. Furthermore, the development of calibration and validation models using the partial least square method yielded high R^2 values (~0.99) and small standard errors (SE) (~0.7 μM), highlighting the potential for quantitative SERS analysis. P-SERS offers significant advantages over traditional techniques, including rapid measurements completed within seconds, minimal consumables, and reduced waste generation. These findings demonstrate the promising potential of ink-jet printed SERS substrates for quantitative analysis of pesticides and other contaminants in water.

(Tues-P22) **Phase transitions in low dimensional materials characterized by Raman spectroscopy**

Shayne Harrel, Adam Wise, Antoine Varagnat

We report how Raman spectroscopy is used to characterize a lithium intercalation induced phase transition in the Weyl semimetal WTe2. Low frequency Raman spectra, sensitive to inter-layer vibrational modes, are presented and show a splitting of the shear mode at 8 cm-1 into two peaks at 6 cm-1 and 13 cm-1, indicating a reduction of symmetry upon lithiation. Angular-resolved Raman spectroscopy reveals a change in the symmetry of the Raman modes around 210 cm-1 from four-fold to two-fold for the non-lithiated and lithiated samples respectively, consistent with a Td to Td' phase transition in the crystal structure of the sample. The discovery of this new phase enriches the phase diagram of WTe2 and provides a platform to study the interplay between superconductivity and charge density waves (CDW.) It is expected that the lithium intercalation approach to electron doping will introduce similar phase transitions resulting in CDW states in a wider family of 2D transition metal dichalcogenide (TMD) systems that have been previously inaccessible, stimulating research for novel quantum phases such as topological superconductivity in these types of materials.

(Tues-P23) **The Role of Race/Ethnicity, Sex, and Age in Surface-Enhanced Raman Spectroscopy- and Infrared Spectroscopy-Based Analysis of Artificial Colorants on Hair**

Aidan Holman, Dmitry Kurouski

Forensic microscopy has been used in forensic hair analysis to determine the racial origin of hair samples. However, this technique is subjective and often inconclusive. Although, to a large extent, this problem can be solved with the use of DNA analysis, which is capable of identifying the genetic code, biological sex, and racial origin from a strand of hair, this PCR-based analysis of hair is time- and labor-consuming. Infrared (IR) spectroscopy and surface-enhanced Raman spectroscopy (SERS) are emerging analytical techniques that can be used to advance the forensic analysis of hair by enabling confirmatory identification of hair colorants. Having said that, it remains unclear whether the race/ethnicity, sex, and age of individuals should be considered upon IR spectroscopy- and SERS-based analysis of hair. Our results showed that both techniques enabled robust and reliable analyses of hair of different races/ethnicities, sexes, and age groups colored using four different permanent and semipermanent colorants. We also found that SERS could be used to identify the race/ethnicity, sex, and age of the individuals via spectroscopic analysis of colored hair, whereas IR spectroscopy was capable of accurately revealing this important anthropological information only from uncolored hair. These results outlined some advantages and limitations of both vibrational techniques in the forensic examination of hair samples.

(Tues-P24) **Sensitive Detection of Exosomes as Cancer Biomarkers using SERS Immunoassay**

Sila Jin, Woojeong Lim, Eungyeong Park, Ahhyun Woo, Igor Lednev, Jongmin Park, Young Mee Jung

Exosomes, abundantly present in biofluids, have significant potential as cancer biomarkers due to their reflective nature originating from cells. Despite their diagnostic value, rapid, accurate, and sensitive detection method for small amounts of exosome remains a challenge. Surface-enhanced Raman spectroscopy (SERS) is a powerful technique for sensitive analysis of chemical and biological materials.

In this study, we utilized Au-crossed nanowires chips as a substrate for SERS immunoassay. The junctions of Au nanowires create stronger hot-spots compared to flat Au substrate. In addition, the Cassie-Baxter-like structure of Au-crossed nanowires forms a hydrophobic surface, which reduces the contact area and allows the capture a large number of exosomes per unit area.

We used this method to detect cancer biomarkers in various exosomes from cancer patients and healthy controls. The Au-crossed nanowires chip exhibited excellent performance in detecting cancer-specific biomarkers. The potential of this technique for sensitive and accurate exosome-based cancer diagnostics for early cancer diagnosis will be discussed in this presentation.

(Tues-P25) **Enhancing Plasmonic Activity of Graphene through Femtosecond Laser-Assisted Selective Metallization.**

Sarika Joshi, Gaurav Pratap Singh, Ankit Dodla, Donald McNaughton, Bayden R. Wood, Sumit Saxena, Shobha Shukla

Surface Enhanced Raman Spectroscopy (SERS) is a powerful technique that significantly enhances the sensitivity of detecting chemical compounds. There is a need for rapid, reliable, and sensitive detection of compounds with practical applications in medical diagnostics and chemical analysis. Recently, there is growing interest in using graphene networks as SERS substrates due to immense potential for tunability and selectivity towards analyte through functionalisation. Towards this end, site-specific

control upon 2D graphene sheets is important to control optoelectronic and plasmonic behaviour along with uniform spectral acquisition.
In this study, we present a novel approach to enhance the plasmonic activity of graphene oxide (GO) for SERS applications through femtosecond laser-assisted selective metallization and photoreduction. The design and fabrication of silver nanostructures on 2D templates like graphene networks offer site-specific modification capabilities, making them of particular interest for SERS applications. We demonstrate controlled reduction of silver salt over the graphene oxide template using femtosecond laser irradiation. The 800nm femtosecond laser, with a pulse width of 140 fs at fixed power, is employed to simultaneously reduce the silver nitrate and graphene oxide and to deposit silver onto graphene framework. We identified the optimum silver loading to obtain maximum intensity of Raman signals. The Raman spectra obtained at these optimized conditions exhibited over one order of magnitude increase in signal intensity.
The reduced structures showed excellent uniformity and repeatability which are essential for reliable sensing applications. Overall, This work paves way for the creation of ultra-sensitive sensing platforms, with potential applications in diverse fields such as environmental monitoring, medical diagnostics, and security applications.

(Tues-P26) **Time-Resolved Confocal Raman Microscopy of Post-Melting Crystallization in Linear Polyethylenimine**

Miharu Koh, Jay Kitt, Carol Korzeniewski, Joel Harris, Shelley Minteer

Linear poly(ethyleneimine) (LPEI) is a hygroscopic polymer widely used for cellular transfection in gene delivery, polymer electrolytes in rechargeable batteries, and redox polymers in biosensor applications. Previous X-ray structural analyses have revealed that anhydrous crystalline LPEI exists in a double-stranded helix conformation due to intra-chain hydrogen bonding (N-H…H) between secondary amines on adjacent chains. Despite the broad applications of LPEI, the kinetics and conformational changes during the transition of the polymer from melted to crystalline states remain poorly understood. Herein, we employ real-time in-situ confocal Raman microscopy coupled with self-modeling curve resolution (SMCR) analysis to elucidate the relative contributions of time-dependent spectral changes that report structural and conformational changes during the crystallization of LPEI. The Raman spectra reveal vibrations corresponding to CH_2 rocking and NH bending modes that are especially sensitive to the degree of crystallinity. In particular, the formation of sharp peaks near 807 cm-1 and 855 cm-1 suggests the presence of crystalline or helical order. Additionally, the shift in the NH stretching frequency from 3303 to 3215 cm-1 further supports the formation of helical chains, owing to the increase in the strength of intra-chain hydrogen bonding interactions during the crystallization process. Efforts are made to elucidate from the SMCR analysis the transitional behavior to gain insights into the molecular mechanism of and intermediate structures formed during LPEI crystallization. The approach aims to unravel the evolution of time-dependent structural and conformational ordering during LPEI crystallization from the melt.

(Tues-P27) **Lyophilizing SERS Sensors for point-of-care (POC) Diagnostics**

Lutfun Naher, Steven Quarin, Pietro Strobbia

Pathogen detection is a critical aspect of combating infectious diseases. While point-of-care diagnostics have been successful in detecting viruses like COVID-19, there is an urgent need to improve the sensitivity of these tests for more accurate results. Reagentless surface-enhanced Raman spectroscopy (SERS) sensors are emerging as a solution for on-site detection of infectious diseases. The sensing mechanism of these sensors offers seamless detection without the need for additional processing steps. However, a limitation arises in remote areas due to sensor stability over time in the solution. To tackle this issue, we employed lyophilization, converting liquid sensors into stable powder form, ensuring improved long-term stability. The reagentless nature of the sensors also permits to use them by simply adding the sample over the sensor in powder form. This study shows the effect of lyophilization on the stability of SERS sensors at different freezing temperatures using different concentrations of cryoprotectant. The lyophilization of a DNA-catalysis SERS sensor was also

investigated. Our research findings show that silver-coated gold nanostars undergo irreversible aggregation during freeze-drying in the absence of the cryoprotectant, as supported by TEM imaging and NTA data. We demonstrated and optimized a protocol for the lyophilization of sensors to increase their shelf-life. Lyophilizing sensors are also important for the easy transport of sensors to the point of need. In conclusion, our work demonstrates the potential of lyophilization in significantly enhancing the stability of SERS sensors, thus enabling the deployment of these diagnostic assays in resource-limited and remote areas.

(Tues-P28) **Nanomaterials Platform for Surface-Enhanced Raman Spectroscopic Chemical Fingerprinting of Cancer-Associated Volatile Organic Compounds**

Hannah O'Toole, Ambarish Kulkarni, Marie Heffern, Randy Carney

Introduction: Early-stage cancer detection can drastically enhance patient outcomes and improve 5-year survival rates. However, traditional medical imaging modalities such as CT, MRI, and PET are limited in terms of early-stage detection accuracy, and tumor biopsies are invasive. Liquid and breath biopsy present promising non-invasive means of cancer detection, but we are not there yet. This project lays the groundwork for a non-invasive "e-nose" platform for the detection of volatile organic compounds (VOCs). VOCs are low molecular weight metabolic by-products released in breath and biofluids due to pathophysiological changes like in cancer. To pare down the complexity of the breath volatilome and detect low concentration (ppmV to pptV) cancer-associated VOCs from ubiquitous VOCs, we have engineered a combinatorial nanoplasmonic sensor array for multiplexed adsorption of VOCs. The dual-stage platform features (1) an engineered array of selectively sorbent core-shell nanostructures consisting of plasmonic nanoparticle cores encapsulated by tunable metal-organic frameworks (MOFs) for VOC capture, followed by, (2) ultrasensitive readout via surface-enhanced Raman spectroscopy (SERS), i.e., a "SERS-MOF" nanomaterial sensing array. Tunable MOF coatings selectively adsorb relevant VOCs at the near-field of encapsulated gold nanourchins (AuNUs), enabling specificity.

Materials/Methods: Synthesis of a lead SERS-MOF ZIF-8@AuNU was performed using self-assembly of the MOF ZIF-8 around AuNU cores. Rigorous nanomaterial characterization was performed using UV-Vis, DLS, TEM, SEM, and EDS of ZIF-8@AuNU drop-cast onto substrates to form the VOC sensor. VOC analytical standards were measured by Raman and SERS at 535nm and 785nm for the construction of an open-source spectral library. An in-house spectral processing software was used for spectral analysis.

Results/Conclusions: Proof-of-concept testing of one lead SERS-MOF formulation for VOC detection was demonstrated. Synthetic parameters to control MOF shell thickness and purification methods have been optimized. We have developed a novel open-source spontaneous Raman and SERS spectral library of VOCs ubiquitous to breath and those associated with cancer for use in testing analytical standards of a biomimetic breath volatilome. Together, this work paves the way for breath testing of clinical samples. Successful engineering of an array of SERS-MOF formulations could enable sensitive and specific characterization of the breath volatilome associated with cancer.

(Tues-P29) **Fillings of Single-Walled Carbon Nanotubes**

Dale L Perry, Nataliya Kalashnyk, Eric Faulques, Victor G Ivanov, Charlotte Slade, Jean-Luc Duvail, Stéphane Cordier, Ann Sanchez, Jeremy Sloan

This study investigated the properties of molybdenum clusters, potassium iodide, and tin selenide (SnSe) nanowires encapsulated in single-walled carbon nanotubes (SWCNTs). Techniques used included high-resolution transmission electron microscopy (HR-TEM), Raman spectroscopy, and density functional theory (DFT) calculations. Mo6Br142- clusters formed semi-ordered arrays in wider SWCNTs and 1D structures in narrower tubes via confinement-induced polymerization. DFT characterized Raman fingerprints of polymerized clusters. 1D KI crystals in SWNTs had high filling fractions. Calculations confirmed stable KI nanowires, matching Raman lines in KI@SCWNT

systems. SnSe encapsulation revealed two 1D types: tetragonal Sn4Se4 units and hexagonal Sn6Se6 motifs. Raman data supported specific modes for Sn4Se4 and Sn6Se6 1D crystals. Calculations demonstrated a 1.5 eV electronic gap for Sn4Se4 nanowires and a semi-metallic character for Sn6Se6 nanowires. Raman spectra of cluster@SWCNT and SnSe@SWCNT hybrid samples showed suppression of a radial breathing mode, indicating SWCNT interaction with encapsulated compounds. This research was partly supported by the French-Bulgarian PHC RILA Project N° 38661ZF 'EOPEN' and the Distributed Research Infrastructure INFRAMAT, funded by the Bulgarian Ministry of Education and Science.

(Tues-P31) **Confocal Raman Microscopy Investigations of the Two-Step Functionalization of Porous Chromatographic Silica**

Maran Sardoni, Joel Harris, Jay Kitt

Mesoporous silica supports have a variety of applications in chemistry, including catalysis, separations, biosensing, and extractions, thanks to their open pore network facilitating rapid reactant transport to the extensive within-particle surface area. The prevalence of mesoporous silica arises from the development of silane chemistry, enabling straightforward surface modification. (3-Aminopropyl) triethoxysilane (APTES) is commonly employed in porous silica salinization due to the reactivity of its terminal amine, allowing further functionalization via various chemistries. However, surface coverage and degree of polymerization in the APTES layer can impact subsequent functionalizations, necessitating a better understanding of initial salinization chemistry for optimized multi-step processes. Few analytical techniques can probe inside porous silica, resulting in chemistry often being inferred from planar models. In this work, Raman microscopy is used to quantitatively probe APTES functionalized porous silica chromatographic particles. The near-matched refractive index of silica and water, along with reduced mie-scattering effect from small silica structures within pores, enables interior particle probing. Vapor- and solution-phase depositions of APTES are carried out following existing literature methods. Raman spectral modes of C-C, C-O and C-H stretching are used to determine APTES density, polymerization degree, and examine particle-to-particle consistency. The Raman spectra indicate that solution-phase deposition, compared to vapor-phase deposition, resulted in greater surface coverage, confirmed through elemental analysis, with results of 4.6980 μmol/m2 and 0.9771 μmol/m2, respectively. To test the viability of a secondary functionalization, APTES layers were reacted with an NHS-ester-alkyne. Successful reactivity of both solution- and vapor-phase is observed demonstrated by an increase in alkyne stretching mode at 2130 cm-1 in the Raman spectra. Carbon analysis yields reaction efficiencies of 91.8% and 81.02% for vapor- and solution-phase depositions respectively. Initial experiments are underway to immobilize redox-active naphthoquinone to the silica surface through the same NHS-ester chemistry, allowing electron shuttling through porous silica for spectroelectrochemistry.

(Tues-P32) **SERS-based Kinetic Monitoring of the Pt-catalyzed Reduction of the Three Nitrothiophenol Isomers on Gold Nanorods**

Daniel Schäfer, Jesil Jose, Roland Grzeschik, Sebastian Schlücker

4-nitrothiophenol (4-NTP) has been widely used in surface-enhanced Raman scattering (SERS)-based reduction studies for testing the catalytic performance of a variety of noble metal nanoparticles using hydride or hydrogen as reducing agent.[1,2] The catalytic conversion of 4-NTP to 4-aminothiophenol (4-ATP) proceeds via a Langmuir-Hinshelwood mechanism, involving the formation of active Pt-H species. Two alternative reaction pathways with respect to the transferred hydrogen species are in principle possible. The first possible pathway describes the direct transfer of a hydrogen radical or hydride to the molecule (hypothesis 1), while the second pathway describes an electron transfer from the platinum to the 4-NTP followed by a proton transfer from the active species (hypothesis 2). To falsify one of these two contradicting hypotheses, we varied the position of the nitro group relative to the thiol groups and thereby the distance of the nitro group to the metal surface. The reduction kinetics of the three isomers of NTP (2-NTP, 3-NTP, and 4-NTP) on platinum-coated gold nanorods was monitored by SERS with molecular hydrogen as the reducing agent. Based on the experimental SERS kinetic data giving first-order rate constants in the order k2-NTP > k3-NTP > k4-NTP, the conclusion

is that the reduction does not proceed via an electron transfer mechanism, but via hydrogen/hydride transfer.[3]

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(Tues-P33) **Design of Aptamer-based Reagentless SERS Sensors for Environmental Analysis**

Manisha Sheokand, Lyndsay N. Kissell, Pietro Strobbia

Reagentless surface-enhanced Raman scattering (SERS) sensors could have a significant impact in environmental analysis because of their sensing mechanism, which makes them ideal for in-situ analysis and continuous monitoring of environmental pollutants. These sensors works autonomously and require no processing steps for target detection. We are working on integrating aptamer sequences in reagentless SERS sensors for the detection of small molecules (e.g., pollutants). Specifically, we use a duplex aptamer mechanism to make the mechanism adaptable. Although there have been few examples of SERS duplex aptamer, the design rules for this type of sensors have not been explored. To take full advantage of these sensors in environmental analysis and target multiple pollutants, we need to understand design rules and a design protocol. Here, we discussed a protocol for the design of aptamer-based reagentless SERS sensors. Initially, we highlighted the non-equilibrium nature of these sensors and identified the optimal length of the complementary element required for them to work. We applied this protocol on ATP which is a well characterized aptamer and translate this on an aptamer for estradiol (E2), a common water contaminant. In this work, we established the design protocol for a short aptamer (i.e., ATP) and analyzed the issues and remedies associated with extending this protocol to longer aptamer sequences (e.g., E2). Finally, we will show the use of this protocol on an updated E2 design.

(Tues-P35) **Optimizing Label-Free Exosome Analysis with Surface-Enhanced Raman Scattering (SERS) and Machine Learning for Non-Invasive Cancer Diagnostics**

Der Vang, Maria S. Kelly, Manisha Sheokand, Manju Sharma, Leyla Esfandiari, Ruxandra I. Dima, Pietro Strobbia

Current cancer diagnostic techniques are not suitable for broad screenings because they are costly, invasive, and burdensome. Liquid biopsies are a promising alternative that can be used as a non-invasive method to detect and monitor cancer through the detection of biomarkers. Circulating exosomes are emerging as powerful biomarkers for cancer diagnostics. However, exosomes have similar compositions and are difficult to differentiate with standard separation and labeling techniques. Herein, we used surface-enhanced Raman scattering (SERS) to classify exosomes based on their vibrational spectra. We examined chemical and spectral differences of cell line-derived exosomes via SERS and built a predictive tool to classify exosomes via machine learning. We optimized different methods and selected the classification algorithm with the highest prediction accuracy. These algorithms were also used to identify what Raman bands significantly impacted the classification. The optimized algorithm was further applied to SERS spectra of exosomes isolated from kidney cancer patients' blood via nanopipette dielectrophoresis. We report a model predictive of cancer from isolated exosomes with a sensitivity of >92% and specificity of >92%. This research suggests that machine learning applied to SERS spectra has great potential for exosome analysis for non-invasive cancer diagnostics.

(Tues-P36) **Non-Negative Matrix Factorization with Raman Hyperspectral Imaging for Immobilized Biocatalyst Analysis**

Hong Wei, Joseph Smith

Biocatalysis is an established technology that has significant applications in the pharmaceutical industry. Immobilization of enzymes is essential for commercial and practical purposes to enhance the stability and recyclability of biocatalysts. Determination of the spatial and chemical distributions of immobilized enzymes on solid support materials is critical for optimal catalytic performance. However, current analytical methodologies often fall short to rapidly identify and characterize immobilized enzyme systems. Herein, we present a new analytical methodology that combines non-negative matrix factorization (NMF) – an unsupervised machine learning tool – with Raman hyperspectral imaging to simultaneously resolve the spatial and spectral characteristics of all the individual species involved in enzyme immobilization. Our novel approach facilitates the optimal selection of an NMF model that can fully resolve all chemical species present, offering a robust analytical methodology for analyzing immobilized enzymes. Specifically, we demonstrate the ability of NMF with Raman hyperspectral imaging to resolve the spatial and spectral profiles of an engineered pantothenate kinase immobilized onto two different commercial microporous resins. Our results demonstrate that this approach can accurately identify and spatially resolve all species within this enzyme immobilization process. To the best of our knowledge, this is the first report of NMF within hyperspectral imaging for enzyme immobilization analysis, and as such, our methodology can now provide a new powerful tool to streamline biocatalytic process development within the pharmaceutical industry.

(Tues-P37) **Visual Time-Temperature Indicators of Biospecimen Exposure to Thawed Conditions**

Jorvani Cruz Villarreal, Emil Ljungberg, Aaron Gabriel Uy, Chad Borges

Many biological analytes of interest to biomedical research can show limited stability when the biospecimens in which they reside are exposed to thawed or partially frozen conditions. Although specific guidelines are available for proper pre-analytical sample handling and storage of biospecimens, improprieties and inconsistencies can occur unwittingly, leading directly to costly false leads in biomedical research. Implementing a system to track the exposure of biospecimens to thawed conditions or improper storage is necessary to avoid using compromised samples. Toward this end, we are developing an inexpensive visual time-temperature indicator (TTI) that can be used in biospecimens at the individual aliquot level to track exposure to thawed or non-ideal frozen conditions.
The proposed TTIs are based on the kinetic control of the autocatalytic permanganate/oxalate redox reaction, which goes from an intense pink solution to a colorless solution, providing a visual colorimetric indicator. Controlling the reaction kinetics allows the design of TTIs for specific time-temperature intervals, depending on specific handling or storage needs. With the help of a MATLAB script, the reaction kinetics can be simulated, facilitating the design of the TTIs for specific times at room temperature. We have demonstrated that the reaction time exhibits a dependency on temperature and can be adjusted to run between a few seconds and 2 h at room temperature, matching with the simulated data. Additionally, eutectic perchlorate solutions can be used for depressing the system's freezing and melting point (m.p.), allowing TTIs to remain active at subzero temperatures warmer than the proper biospecimen storage and handling guidelines indicate, e.g., when storage at -20 °C or -40 °C is unacceptable. We have characterized LiClO4 (m.p. -18 °C), NaClO4 (m.p. -37 °C), and Mg(ClO4)2 (m.p. -67 °C) as antifreeze solutions, showing the potential of keeping the reaction active at subzero temperatures, while maintaining kinetic control. Currently, TTIs for specific applications in biomedical research are being designed. We expect that their implementation can contribute to avoiding the use of unstable biospecimens, helping to minimize false discoveries.

(Tues-P39) **Synchrotron-infrared microspectroscopy of live Leishmania major infected macrophages and isolated promastigotes and amastigotes**

Thulya Chakkumpulakkal Puthan Veettil, Rebekah Duffin, Supti Roy, Jitraporn Vongsvivut, Mark Tobin, Miguela Martin, John Adegoke, Philip Andrews, Bayden Wood

Neglected tropical diseases (NTDs) result in devastating health, social and economic consequences for more than one billion people. Leishmaniasis is a vector-borne NTD associated with a spectrum of clinical manifestations and now endemic in over 90 tropical and sub-tropical low socioeconomic countries. Current diagnosis for this disease involves serological assessment of infected tissue by either light microscopy, anti-body tests or culturing via in vitro inoculation or in vivo animal inoculation. Furthermore, co-infection by other pathogens can make it difficult to accurately determine Leishmania infection with light microscopy. Herein, for the first time, we demonstrate the potential of combining synchrotron FTIR microspectroscopy with powerful discrimination tools such as partial least squares – discriminant analysis (PLS-DA), support vector machine – discriminant analysis (SVM-DA), and k-nearest neighbors (KNN), to characterize the parasitic forms of Leishmania major both isolated and within infected macrophages. To date no spectroscopic studies investigating biochemical fingerprints of the intra and extra cellular leishmania parasitic forms have been performed. Nor have any studies investigated Leishmania infected macrophages paving the way for a spectroscopic based approach to diagnosing leishmania infection. For measurements performed on functional infected and uninfected macrophages in physiological solutions the sensitivity from PLS-DA, SVM-DA, KNN classification methods was found to be 0.923, 0.981, and 0.989, while the specificity was 0.897, 1.00, and 0.975, respectively. Cross-validated PLS-DA models on live amastigotes and promastigotes showed a sensitivity and specificity of 0.98 in the lipid region, whilst a specificity and sensitivity of 1.00 was achieved in the fingerprint region. The study demonstrates the potential of the FTIR technique to identify unique diagnostic bands and utilize them to generate machine learning models to predict leishmania infection.

(Tues-P40) **Smart Polymer Analysis using Raman Two-Dimensional Correlation Spectroscopy**

Julian Hniopek, Josefine Meurer, Robin Kampes, Stefan Zechel, Martin Hager, Michael Schmitt, Juergen Popp

Smart polymers are a special class of polymers that can adapt to changes in their environment and providing additional functionalities besides structural support. This class includes for example self-healing, shape-memory and surface-responsive polymers that have applications in the fields of green materials, biomedicine and high-tech industries such as aviation. Although these polymers have been extensively studied over the past decades, much of their molecular working principle is still poorly understood in many cases.

Raman spectroscopy is a technique with molecular specificity, which can generally be applied without sample preparation and, crucially, in situ. This makes it possible to observe smart polymers while they undergo their stimuli-response, e.g. introduced by changes in temperature or pH-value, with high temporal and spatial resolution. Pairing these measurements with two-dimensional correlation spectroscopy (2DCOS), enables the direct observation of the molecular mechanism behind the smart functionality.

We have employed Raman-2DCOS to investigate the molecular mechanisms of shape-memory and self-healing polymers based on metal-ligand and halogen bond interactions.[1,2] Here, Raman spectroscopy was able to directly confirm proposed mechanisms based on the dynamic exchange of bonds at elevated temperatures for the first time.

Furthermore, we investigated surface-responsive polymers based on reversible imine groups using confocal Raman microscopy.[3] Utilizing isotope labeling and a novel global phase angle based 2DCOS approach, it was possible to quantify the surface-response as well as the diffusion behavior inside the polymer in a spatio-temporally resolved manner.

In summary, in situ Raman-2DCOS is a unique method that provides direct observation of molecular mechanisms in smart polymers and will allow polymer scientist to move from a trial-and-error based development approach to targeted synthesis based on these results. This will enable the production of

tailored polymers for specific applications and significantly accelerate the overall development of this field.

Acknowledgements:
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References:
[1] Meurer et. al., Adv. Mater, 2021, 33, 2006655
[2] Meurer et. al., Adv. Func. Mater, 2022, 32, 2207313
[3] Hniopek et. al., Chem. Sci., under review

(Tues-P42) **Molecular Compositional Study of Lipid Droplets During De Novo Lipogenesis in Hyperglycaemic Live Hepatic Cells Using Confocal Raman Micro-spectroscopy**

Pradjna Novedya Paramitha, Bibin Bintang Andriana, Kosuke Hashimoto, Hidetoshi Sato

The present study aims to develop a non-invasive method that can be applied to assess the health status of non-alcoholic fatty liver disease (NAFLD) patients based on the fat composition accumulated in the liver. Previous studies have been conducted to observe the fatty chain composition of lipid droplets in HepG2 cells, a model human hepatic cell line, exposed to a high concentration of fatty acids. The study shows that hepatic cells have a lower uptake for saturated fatty acids i.e., palmitic and stearic acid, and high uptake for unsaturated fatty acids i.e., oleic and linoleic acid. However, saturated fatty acids and linoleic acid cause hepatic cell death, while oleic acid does not. The result suggested that the types of fatty acids play the important role that induces the liver, irrespective of their total fat accumulation rate. Consequently, it is important to investigate the composition of fat accumulated in the hepatic cells caused by possible stimulations which may accelerate or induce hepatic cell death. In the present study, a high glucose concentration (hyperglycaemia) is applied to HepG2 cells, to investigate the composition of fat that are synthesized by the hepatic cells. Hyperglycaemia is one of the main characteristics of diabetic patients. The high blood glucose content may cause excessive fat accumulation in the liver. Raman micro-spectroscopy and chemometrics were applied to analyse the fatty chain content in the lipid droplets. Fat accumulation was observed on days 1, 4, and 7 of cultivation with the hyperglycaemic medium. The results showed that lipid droplets accumulation in cells cultivated with hyperglycaemic medium occurred slower than that of cells cultured with high fatty acids concentration. Furthermore, Raman analysis indicated that over 7 days of cultivation, an increment of saturated fatty chain content in the lipid droplets occurred. However, a significant cell death was not observed. Those results suggest that although hyperglycaemia resulted in a high accumulation of saturated fat in hepatic cells, the condition is rather safe for the hepatic cells as compared to exposure to high concentrations of saturated fatty acid and linoleic acid.

(Tues-P43) **Photostability and Excited-State Features of Vulpinic Acid, a Natural Ultraviolet-Screening Compound**

Derek Moore, Tanzil Mahmud, Christopher Jeffrey, Matthew Tucker

Environments that are subjected to significant amounts of ultraviolet radiation, such as the surface of Earth prior to the formation of the ozone layer and the current surface of Mars, are inhospitable to most organisms. However, certain species of lichens have been uncovered that are able to survive in areas with elevated UV exposure. We hypothesize that photoprotective compounds found within the upper cortex of these lichens interact with other compounds, including a polysaccharide sheath, to effectively dissipate the energy from absorbed UV light. Several spectroscopic techniques were utilized to evaluate the photostability and excited-state features of one of these natural products, vulpinic acid, isolated from wolf lichen. This compound was investigated under environmental conditions mimicking those found in nature and its spectral response was compared to the isolated

form. Significant increases in photostability were observed in vulpinic acid when it was in environments rich with hydrogen bonding potential, and features of the transient absorption spectrum differed from those of the isolated compound. This suggests that intermolecular interactions between UV-absorbing compounds and other localized compounds within the organism are responsible for protecting lichens from harmful wavelengths of light.

(Tues-P45) **The CytoR1TM: Optimization of a Commercial Dielectrophoretic Label-Free Cell Sorting Platform**

KATHERINE DEGEN, **Alexandra Hyler**, Dean Thomas, Kyle Brown, Ridi Barua

Dielectrophoresis (DEP) utilizes non-uniform electric fields to exert a force on particles and cells. Cells respond to these fields in a manner dependent on their biophysical properties. By characterizing the frequency response to a given field, label-free sorting of cell subpopulations is possible. A large body of literature has investigated these parameters with unique setups and cell varieties. The technical challenge of the technique coupled with the uncertainty in the breadth of data has limited the commercialization and widespread adoption of DEP for biological research applications. A user-friendly DEP system for biologists is required for further advancement of this unique biophysical sorting technology. The CytoR1 integrates a custom generator design that produces a wide range of frequencies and voltages, precision fluidic systems, and a flexible chip interfacing system with an easy to use software. Various configurations of the chip interfacing system were tested for usability and repeatability. User feedback was incorporated to create a plug and play interfacing system that can be setup quickly and repeatably. Additionally, various electrode materials were assessed to optimize both usability and functionality. Liquid electrodes proved difficult for the user and resulted in lower field strength at higher frequencies. Utilization of a fusible alloy improved chip stability, repeatability, and performance. Cell recovery is another important variable for end users. A variety of surface coatings and tubing connections were also tested in order to assess their impact on cell loss. 0.5% Pluronic F-127 showed a 1.3-fold increase in cell recovery compared to the standard setup, and the Darwin coupling system showed a 2.15-fold increase in recovery compared to the current system. By optimizing these components, recovery was increased from 30-40% to 70-90% depending on the cell type and conditions. These elements led to the CytoR1 Platform which was then used to test multiple cell types for DEP response in order to database the frequencies and voltages most useful for enriching each cell type in flow. Cross over frequencies for each cell type were calculated for comparisons of characterizations to existing platforms like the 3DEP.

Wednesday, October 11, 2023

Oral Presentations

23ART02: Analysis of Exotic Materials from Mummies to Mars, Southern Pacific B/C
Chair: Mary kate Donais

(ART02.1)**Unravelling the Secrets of Egyptian Mummification using Vibrational Spectroscopy**

Bayden Wood, Janet Davey, Callum Gassner, Ankit Dodla, Magdalena Giergiel, *Monash University, Victorian Institute of Forensic Science,*

Vibrational spectroscopy is a powerful tool for analyzing Egyptian mummies. Using FTIR and Raman spectroscopy, we investigated the chemical composition of skin, hair and anointments of Graeco-Roman Egyptian mummies, shedding light on these ancient tissue preservation and embalming processes.

Through our analysis of FTIR images and Raman spectra obtained from the Warrington mummy and tissues provided by the Australian Institute of Archaeology, we identified several biomolecules, including calcium soap, free fatty acids, triglycerides, collagen, and hemoglobin. We performed FTIR imaging on 4 μm thick sections of formalin-fixed dewaxed tissue and cryosectioned tissue. Notably,

we discovered a 150 μm layer of highly pure crystallized calcium soap complex in the formalin-fixed tissue. This layer results from the saponification of fat tissue after death, converting body fat into a substance called adipocere or grave wax, due to microorganism activity. The presence of specific bands at 1575 cm^{-1} (associated with unidentate carboxylate coordination with the Ca^{2+} ion) and 1539 cm^{-1} (associated with bidentate coordination) suggests the presence of three-dimensional calcium soap crystal structures. The spectrum also exhibited pronounced σCH_2 progression bands indicative of C16 and/or C18 calcium soap crystals, as well as evidence of triglycerides. Cryosectioned tissue from the Warrington mummy displayed evidence of free fatty acids and calcium soap. Notably, the adipocytes in the formalin-fixed xylene washed tissue were highly preserved and birefringent, unlike previous studies on mummy adipocere or adipocere found in soil samples from animal remains. Additionally, regions adjacent to the calcified adipose tissue from the Warrington mummy showed evidence of aspergillus fungi.

In addition to the calcium soap bands, our Raman spectroscopy analysis revealed spectra characteristic of hemoglobin, although we have not yet detected any red blood cells. These findings underscore the exceptional preservation of biomolecules in Graeco-Roman mummies, serving as compelling evidence of the remarkably sophisticated mummification practices employed during the Graeco-Roman period.

(ART02.2)**Exotic Hardwood Species Classification using Network Guided Classification Schemes**

William Gilbraith, Karl S. Booksh, Caelin Celani, *Savannah River National Laboratory, University of Delaware*

Exotic hardwoods, such as genus Dalbergia, are lucrative trade goods and as such have many have been overharvested and face conservation problems. Current species determination relies on experts to identify morphological traits in these exotic hardwoods. However, a considerable push has been made to develop analytical based methods to determine species without human interaction. One such method is trace element analysis by ICP-OES and ICP-MS and chemometric modeling for proper classification. In this work, four different preprocessing techniques and three different classification models were evaluated for minimal element viability for classification purposes. A set of 239 samples across 20 classes were classified via flat classification, then network visualization was used to develop binary, success based decision schemes for increased classification success. A 15 element data set resulted in >0.92 Cohen's kappa while only 5 elements were required for Cohen's kappa of 0.9.

(ART02.3)**Making GANEs in Wine Detection for Archaeological Ceramics**

Vernon Stafford, Rachel Sparks, David Jenkins, *University of Tennessee, Knoxville*

Chemical analysis of organic residues absorbed into archaeological pottery is an increasingly popular method of investigating prehistoric societies. The identification of compounds related to foods and drinks can provide direct scientific evidence that certain foodstuffs were consumed at a site. Wine was an important commodity in many ancient cultures, and thus its identification is of particular importance to archaeologists and anthropologists. Nevertheless, current methods of determining the presence of wine lack specificity because they focus almost exclusively on the presence of tartaric acid, and thus can lead to inaccurate conclusions regarding a pottery vessel’s use. Furthermore, current methods are generally only available to those with powerful instruments (e.g., LC-MS/MS) and specialized training. We are developing a method of accurately determining the presence of wine residues by analyzing a suite of biomarkers that together can only be generated by grape wine. These markers include tartaric and malic acid, the two most abundant organic acids in grapes, and ethyl hydrogen succinate (EHS), a product of fermentation. By employing a combined extraction/derivatization with neopentyl alcohol, these compounds can be extracted directly from the ceramic matrix and converted to their neopentyl esters in a single step. The creation of these Grape Acid Neopentyl Esters (GANEs) enables their detection by GC-MS, which is a far more accessible technique than prevailing methods. Neopentanol is a crucial reagent for this process, as it is sufficiently sterically hindered to prevent significant transesterification of the ethyl ester moiety of EHS, while also being reactive enough to diesterify both tartaric and malic acid. Our novel method can

offer fast, reliable determination of wine residues in archaeological ceramics and can be implemented effectively by a wide range of researchers who do not have access to more specialized instrumentation.

23AES06: AES Lifetime Achievement Award Symposium Honoring James Landers, Southern Pacific F
Chair: Christopher Easley

(AES-06.1)**High resolution DNA separation as the backbone for a portable genotyping system for human identification.**

James Landers, *UVA*

Since its inception more than three decades ago, micro-total analysis systems (μTAS) 'promised' to deliver a radically new platform for chemical and biochemical analysis, bolstered by the ability to allow multistep sample preparation processes to be seamlessly integrated with a variety of analytical techniques. By the early 2000's, it was predicted that microfluidics would revolutionize chemical and biochemical analytical processes over a broad array of scientific disciplines, and would make its way into clinical, pharmaceutical, environmental and forensic testing, not only in the form of lab-based technologies, but as portable, point-of-need systems. With the rudimentary 'unit operations' being well defined for aliquoting, mobilizing, mixing, storing and interrogating fluids in microfluidic systems, fulfilment of that 'promise' seemed inevitable, but translation to real-world applications was constrained by macro-to-micro interfacing and challenges with cost-effective manufacturing. Case-in-point - we described an etched glass microdevice in 2006 that was capable of DNA extraction, amplification, electrophoresis and fluorescence detection – it could accept a nasal swab and diagnose pertussis infection with a sub 30-min analytical time. While a critical proof-of-concept at the time, cost-effective fabrication would be difficult, and the cumulative hardware for the multistep process would be the size of a photocopy machine. New manufacture-friendly materials and novel approaches for microdevice fabrication and fluid flow control have evolved the field, and this has allowed for the types of application-driven developments envisioned by Manz and Widmer 35 years ago. In this presentation, we will discuss the genotyping functionality described above, but with a platform cored on centrifugal microfluidics. We will focus on the electrophoresis and fluorescence detection domains of the disc, and demonstrate that DNA separation polymer can be introduced into the separation channel solely through centrifugal force, show that the fluorescence detection optics must 'find' dead-center of the separation channel, and illustrate that high resolution separation of DNA out to 325 base pairs was possible in ~400 seconds.

(AES-06.2)**Using Electrical Circuit Analogies to Design Plug-and-Play 3D-Printed Pneumatic Logic Gates and Oscillators for Microfluidic Flow Control**

Christopher Easley, Joanne Seow, Md Mohibullah, *Auburn University*

In the early days of microfluidics research, it was recognized that electrical circuit components could be used to model microfluidic circuits. Modeling of pneumatic, valve-control circuits has also leveraged such analogies, although the compressibility of air must be carefully considered. In practice, however, a major challenge has been the relatively time-consuming photolithographic methods used for microdevice fabrication, limiting a user's ability to rapidly iterate circuit designs. Here, we show that rapid prototyping with 3D printing can be used to better exploit the analogy between pneumatics and electronics.

Exquisite fluidic volume control, in the picoliter to nanoliter range, can be achieved with microfluidic valves driven by pneumatic circuits. With an affordable 3D printer (US $600), we printed circuit elements such as resistors and capacitors fully in place, while logic gates were printed in two layers then assembled with a flexible polydimethylsiloxane (PDMS) membrane sandwiched in between. These elements could be connected and disconnected easily, allowing "plug-and-play" operation akin to a solderless breadboard in electrical circuits. Pneumatic inverters (NOT gates) were sequentially connected in odd numbers to act as an oscillator, with only a single vacuum input.

Oscillator frequencies over two orders of magnitude, from 0.8 Hz to 112 Hz, were demonstrated by simply changing connected pneumatic capacitors and resistors.

To validate their use in microfluidics, a 5-ring pneumatic oscillator was used to drive a valve-based pump at up to 6 μL/min. The control system was run with only one vacuum input, allowing multiple reagents to be pumped, mixed, and added to electrochemical (EC) immunosensors. We also demonstrated a completely pneumatic system that can automate a four-step droplet sampling sequence ((sample, probe), oil, (reference, probe), oil) in a recurring manner without requiring any electronic power or control.

In summary, we show that rapid prototyping of pneumatic circuits via 3D printing can enable complex valving functions to be achieved on microfluidic devices with minimal pneumatic inputs and even without electrical control, using pneumatic oscillators. We envision that many other applications, e.g. autonomous control of tissue-on-a-chip microdevices, could be made accessible to non-experts in this way.

(AES-06.3)**Microfluidic Analytical Systems For Assaying Dynamic Cellular Secretions**

Michael Roper, *Florida State University*

Islets of Langerhans are the endocrine portion of the pancreas and are composed of several cell types that release peptide hormones into the bloodstream for regulating glucose levels. Proper control of blood glucose is dependent on the amounts and dynamics of hormones released from these cells. Because the dynamic profiles of hormone secretion are essential to proper glucose control, examining secretion from single or small groups of islets are essential, necessitating analytical tools with high sensitivity. Microfluidic systems are an ideal platform to interrogate islets as they reduce dilution of the secreted components and can be used to deliver complex glucose profiles like those observed in vivo. We have developed a number of analytical approaches that use microfluidic systems to measure hormone and small molecule secretions from single or small groups of islets of Langerhans. Initially, microfluidic electrophoretic immunoassays were used to measure insulin secretion from single islets. While highly sensitive, the microfluidic systems were difficult to use due to the shallow channels that were required to limit Joule heating. As such, a homogeneous fluorescence anisotropy competitive immunoassay for insulin was developed which allowed for microfluidic systems with larger channels. To increase the throughput of the assay and make it more amenable to screening applications, a fluorescence anisotropy imaging system was employed for measurement of insulin secretion from 12 groups of islets in parallel with minimal fluidic inputs. To automate the system further and allow for smooth operation, approaches to integrate microfluidic valves and other microfluidic elements will be discussed. Finally, the application of antibody-free assays providing multi-analyte monitoring will be described. The use of microfluidic systems to hold islets and couple with liquid chromatography or solid phase extraction and mass spectrometry detection will be described. These systems offer the potential for combining the benefits of microfluidics with high information content detection.

(AES-06.4)**Use of Electroosmotic Flow in Brain Tissue for Biochemical Measurements**

Stephen Weber, Tingyuan Xu, *University Of Pittsburgh,*

We determined about 15 years ago that the zeta potential of brain tissue, specifically rat hippocampal tissue, was significant - about -23 mV. We have used this property to pass solutions containing peptides through tissue to measure reaction rates of peptide hydrolysis by peptidases online. When we began to think about doing these measurements, we (my group and I) knew we needed help. James Landers to the rescue. James and his group, and especially Jerome Ferrance and Kerui Xu, understood our research problem and created microfluidic devices for us. Juanfang Wu in my group at the time made significant discoveries related to glutathione and coenzyme A (CoA) in hippocampus using the Landers groups' devices.

Since that time, we have developed a sampling probe using two-photon polymerization (Nanoscribe) for in vitro and in vivo work. Using current-induced electroosmotic flow, our system passes substrate (we now focus on enkephalins) and an internal standard through tissue at flow rates in the range of

about 8-24 nL/min. While many peptidases are membrane-bound, there are soluble peptidases in the extracellular space. In order to remove these from the sample as it travels to an LC-MS² system to prevent ex-vivo hydrolysis, we sample the samples with a microdialysis probe that whisks (and dilutes) the sample at 500 nL/min to the measurement system.

The major challenges relate to our trying to estimate rates based on a steady-state measurement of product/remaining substrate at a certain substrate infusion rate through tissue. Remember, the Michaelis Menten equation is an expression predicting an observed rate, dP/dt. We must use the integrated form of the equation. In addition, diffusion both dilutes substrate and affects substrate's time-in-tissue. These complexities require simulations for determining rate parameters. Experimentally, we have completely redesigned the valve-based fluidic system used for sample preparation and analysis. While yet to be tested, this redesign should decrease the analysis time from about 40 min per measurement to 20 min per measurement.

(AES-06.5)**Bioanalytical applications of microchip electrophoresis**

Susan Lunte, *University Of Kansas*

Oxidative stress is involved in many neurological diseases including Alzheimer's Disease and traumatic brain injury. New tools capable of monitoring biomarkers of oxidative stress in vivo and in vitro can be used to investigate the cause and progression of these disease states. Microchip electrophoresis is a powerful tool for the analysis of biological samples. In particular, its ability to perform fast, efficient separations of multiple analytes in a single run makes it possible to monitor several biomarkers in a single sample with high temporal resolution. In addition, the small dimensions of the channels in the chip are compatible with the analysis of microdialysis samples and single cells. In this presentation, two applications of microchip electrophoresis (ME) for biochemical investigations will be presented. The first involves the development of ME-based methods for the detection of reactive nitrogen and oxygen species (RNOS) in macrophages and immune cells. This includes direct amperometric detection of RNOS as well as the evaluation of fluorescent reagents used for specific species. The second application involves the combination of microdialysis with microchip electrophoresis for near real-time continuous in vivo monitoring of biogenic amines and biomarkers of inflammation. The ultimate goal is to use these tools to investigate the role of oxidative stress in neurodegenerative disease.

23ATOM06: Common Strategies for LA-ICP-MS and LIBS, Central Pacific A/B/C
Chair: Matthieu Baudelet
Co-Chair: Mauro Martinez

(ATOM-06.1)**LIBS and Visible/Near Infrared Spectroscopy on Mars**

Valerie Payre *University of Iowa*

Because human cannot easily access Mars to explore its history, spacecraft and rover missions have been sent to Mars to study its geology and climate, and to determine whether life ever existed. Instrumental analyses onboard spacecrafts can nearly cover the whole planet with a low spatial resolution, between a few meters to several hundreds of kilometers, while data from rovers are localized at a few specific locations on Mars with high resolution analyses (from tens of μm). The combination of the two datasets thus enlightens our geological understanding of the planet.
Since 2006, the Compact Reconnaissance Imaging Spectrometer for Mars (CRISM) onboard the Mars Reconnaissance Orbiter is exploring the mineralogy of the surface of Mars acquiring visible/near infrared (VNIR) reflectance spectra with a spatial and spectral resolution of 15 – 19 m/pixel and 6.55 nm/channel, respectively. Laser Induced Breakdown Spectrometer (LIBS) is onboard two rovers as part of the ChemCam suite aboard the Curiosity rover that landed in 2012 in Gale crater and SuperCam suite aboard the Perseverance rover that landed in 2021 in Jezero crater. Both ChemCam and SuperCam enable the analyses of major, minor, and a few trace elements of rock and soils at a distance between 2 and 7 meters away the rover. Three spectrometers receive LIBS signal, separating

emission into the ultraviolet (ChemCam: 240 – 340 nm), violet (ChemCam: 382 – 469 nm), and VNIR (4 ChemCam: 73 – 906 nm).
Both CRISM and ChemCam LIBS identified feldspar-rich igneous rocks with evolved compositions (SiO_2 > 53 wt.%) compared to a basalt, suggesting that Mars crust is not basaltic only, but could be covered by basaltic flows hiding a more evolved crust underneath. The talk will review orbital VNIR CRISM observations and LIBS measurements that raise questions about the nature of the martian crust, thus questioning the formation of Mars crust.

(ATOM-06.2)**New approaches of laser induced breakdown spectroscopy (LIBS) for imaging in medicine.**

Mauro Martinez, Manish Arora, Christine Austin, *Icahn School Of Medicine At Mount Sinai,*

Fluoride exposure has been associated with significant neuro and renal toxicity, necessitating the study of its health effects and the accurate capture of exposure timing. Teeth, characterized by incremental growth and a strong affinity between fluoride and calcium, serve as an excellent matrix for studying fluoride exposure. However, the longitudinal measurement of fluoride in teeth poses challenges due to the high detection limits and difficult for measurement associated with most imaging methods. In this work, we propose a novel method for quantifying fluoride in teeth using laser-induced breakdown spectroscopy (LIBS) and the CaF molecular emission. By leveraging the high Ca content found in teeth, the formation of the CaF molecule produces distinct emissions at approximately 530 nm (orange) and 600 nm (green) when analyzed using a basic LIBS setup with optimized plasma conditions. To achieve quantitative imaging of fluoride in teeth, the development of a series of matrix-matched reference materials is necessary. Given the lack of commercially available standard series, we have implemented a new method for generating matrix-matched standards using hydroxyapatite with known fluoride concentrations. This innovative material replicates the chemical composition and optical characteristics of teeth, enabling the optimization of LIBS acquisition parameters for enhanced sensitivity and linearity, achieving a LOD of 18 $\mu g \cdot g^{-1}$. To validate the effectiveness of this approach, we conducted fluoride measurements in teeth from rats exposed to varying levels of fluoride, alongside a control group. Comparative analysis was performed by utilizing ion-selective electrode analysis as a reference. This was extended to demonstrate the potential of this method to reconstruct historical fluoride exposure in early-life through quantitative mapping of fluoride distribution in human teeth. In a similar way, we propose determinate fluoride distribution in soft tissue, as brain sections, using home-made gelatin standards with known fluoride concentration as matrix match standard. This study presents a promising avenue for accurately assessing fluoride exposure in teeth and soft tissue using LIBS, allowing for the reconstruction of historical exposure patterns. Finally, this approach holds significant potential for enhancing our understanding of the health effects associated with fluoride exposure.

(ATOM-06.3)**Synthesis and characterization of matrix-matched standard for laser-ablation-based analysis of hair**

Matthieu Baudelet, Kaitlyn Bonilla, Charlene Harris, Chloe Phillips, *University Of Central Florida*

Chemical analysis of hair by laser-ablation-based techniques poses challenges due to a lack of matric-matched standards. In comparison to bodily fluids, hair provides a minimally invasive sample collection and a better stability for storage. Thus, this makes hair useful to keep a biological temporal record of organic and inorganic components in the body.

Utilizing this LA-ICP-MS and/or LIBS for elemental analysis eliminates the need for sample modification prior to analysis. It is nonetheless imperative for a calibration material to show similarity to the chemical, physical, and optical properties of its matrix. However, there is a lack of certified reference materials that meet these criteria all at once for chemical hair analysis. As a result, strategies that are not chemically sound are being relied upon by current industry and research. In this study, the use of a matrix-matched standard reference material will allow for the correction of sample matrix effects. The reference materials presently utilized for quantitative hair analysis include hair powder, dogfish liver powder, and glass. While posing similar chemical species to hair, these standards do not

accurately emulate its physical and optical properties. Thus, the development of a hair reference standard suitable for laser ablation is crucial.

We have developed a method for a new calibration material using keratin film as a matrix-matched reference standard for laser ablation. Characterization of the physical properties including physical and chemical homogeneity, stability, and dimensions of the film were studied. The reference material demonstrates comparable dimensions to a single hair strand. These results pave the way for a new set of standards for internal medicine, forensic toxicology, and anthropology.

(ATOM-06.4)**Analysis of lithium-ion battery materials by Laser-Induced Breakdown Spectroscopy (LIBS) and Laser Ablation-Inductively Coupled Plasma Mass Spectrometry (LA-ICPMS)**

C. Derrick Quarles, **Ross Coenen,** *Elemental Scientific, Inc.*

The current drive to improve technology has led to an ever-increasing demand for electric energy in the form of batteries. For example, vehicles, phones, and computers are all reliant on reliable lithium-ion batteries (LIBs) to provide long lasting power. The push to become greener has also put an emphasis on more reliable LIBs with better performance. One area of interest when it comes to performance is to determine the purity of the starting materials used to prepare cathode and anode materials and to ensure the ratio of constituents is within specifications. This is typically done by digesting raw battery materials, such as lithium nickel manganese cobalt (LNMC) powders, followed by elemental analysis using an ICP or ICPMS. Here we provide advantages for using sold sampling techniques, such as LIBS or LA-ICPMS, to analyze the starting materials and prepared anode/cathode materials. Spatial information can be obtained when using laser-based techniques, which allows for elemental mapping or depth profiling to gain additional information about the sample. By combining these techniques, elemental analysis can be performed in addition to measuring elements such as H, O, and F by LIBS.

(ATOM-06.5)**Detection of uranium in complex matrices via laser-based sampling**

Benjamin Manard, Hunter Andrews, C. Derrick Quarles, Tyler Spano, Daniel Dunlap, Veronica Bradley, N. Alex Zirakparvar, Cole Hexel, *Oak Ridge National Laboratory, Elemental Scientific, Inc.,*

The detection of target analytes, in low abundance, within a complex matrix, is the typical “needle in a haystack” problem. Regarding elemental / isotopic analysis, a traditional bulk analysis approach will ultimately homogenize the results leading to possibly a misinterpretation of respective processes. Being able to directly (spatially) characterize the sample can provide a more comprehensive understating of such sample. The work presented here will demonstrate how laser ablation sampling coupled to spectroscopic (LIBS) and mass spectrometric (LA-ICP-MS) techniques can extract valuable information (needle) amongst complex matrices (haystack).

23BIM02: Translating Multimodal Imaging Technologies into Routine Clinical Practice: Where do we Stand?, Sierra 2
Chair: Juergen Popp

(BIM-02.1)**Realizing the utility of infrared spectroscopic imaging for cancer pathology**

Rohit Bhargava, Kevin Yeh, Kianoush Falahkheirkhah, *University of Illinois at Urbana-Champaign,*

Infrared spectroscopic imaging combines the ability to record the native chemical content of tissues with the ability to visualize chemistry in its spatial diversity. For practical sample sizes, there is a need to record more data than a typical microscopy image (MB vs. GB) to record a sufficient level of detail in chemistry to address pathology needs. Trade-offs often have to be made between the closely related considerations of signal to noise ratio, spatial-spectral coverage, resolution and optical arrangements. Here, we present an approach to realizing the advantages offered by new ideas on fundamentally changing these trade-offs. We first describe a new microscope design for increased speed and rapid coverage that is useful for biomedical and clinical tissue imaging. Next, we describe integration of AI

methods that directly relate to pathology. Finally, we present examples of use cases to show the potential of this emerging technology.

(BIM-02.2)**Challenges and opportunity in translating in vivo Raman spectroscopy for clinical applications**

Anita Mahadevan-Jansen, *Vanderbilt University*

Our lab has been focused on developing Raman spectroscopy for in vivo detection of various clinical conditions including preterm birth, hydration status, otitis media, inflammatory bowel disease, oropharyngeal cancer etc. Regardless of the application, various challenges need to be addressed when developing Raman spectroscopy for in vivo use. These include study design, system calibration, probe design, system design, data processing and data analysis. In this presentation, I will review a few of our own as well as other groups' progress in translating Raman spectroscopy for clinical use and discuss future prospects of this technology.

(BIM-02.3)**Intraoperative tumor characterization via multimodal imaging**

Juergen Popp, *Leibniz Institute of Photonic Technology*

Reliably diagnosing and characterizing cancer, especially for surgical interventions, is a complex process involving a variety of imaging methods and, ultimately, time-consuming histopathology. To improve patient outcomes new techniques to provide diagnostically relevant information, quickly and reliably is required. Molecular spectroscopy could play a crucial role in addressing these unmet medical needs, as it offers the potential to provide besides morphological information also molecular fingerprint information in a non-invasive and labelfree manner.
We will report on the investigation of spectroscopic approaches with focus on Raman spectroscopy for intraoperative tumor diagnostics in terms of spectral histopathology. We will present novel multimodal labelfree spectroscopic instrumentation (like e.g. innovative Raman fiber probes, clinically usable non-linear multimodal microscopes or endospectroscopic probes etc.) for precise surgical guidance and intraoperative histopathological examination of tissue (staging and grading) under in-vivo or near in-vivo conditions in order to initiate an individualized therapy plan tailored to the patient as quickly as possible.
In order to take full advantage of these spectroscopic approaches a major step forward would be the implementation of spectroscopic-guided femtosecond ablation in a "seek and treat manner". In this way, it would be possible to have real-time monitoring of ablated features and enable 'seek and treat' applications. We will introduce a multimodal nonlinear microendoscope, which also allows for the ablation of biological tissue with femtosecond lasers.
Equally important as the development of clinically usable spectroscopic devices is the development of tailored sophisticated artificial intelligence based spectral analysis routines. Thus, the presentation will also introduce innovative spectroscopic dataset evaluation algorithms for the translation of spectroscopic data into quantitative diagnostic markers.
In summary the interplay between multicontrast spectroscopy either in a microscopic or endoscopic setting in combination with innovative artificial intelligence approaches for real-time spectral analysis and fs-laser ablation opens exciting new ways for an intraoperative histopathological tumor analysis and selective removal.

Acknowledgements
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(BIM-02.4)**In vivo bond-selective imaging by en-face detected mid-infrared photothermal microscopy**

Mingsheng Li, Hongli Ni, Jiaze Yin, Hongjian He, Yuhao Yuan, Guo Chen, Ji-Xin Cheng, *Boston University*

In vivo optical imaging with chemical bond selectivity plays a crucial role in both biological study and clinical applications. Compared with magnetic resonance imaging, optical imaging stands out due to its exceptional spatial resolution and heighten sensitivity. Mid-infrared photothermal microscopy (MIP) enables high-spatial resolution molecular imaging with micromolar-level sensitivity, thanks to its significantly larger cross-section area compared with coherent Raman scattering microscopy -by eight orders of magnitude. However, the challenges associated with in vivo MIP imaging arises from the low collection efficiency of scattered photons from deep tissue and the absence of an appropriate laser scanning technique. To overcome these obstacles, we developed an en-face detected MIP microscope that markedly improved the collection efficiency of scattered photons by 100 times. Consequently, our method facilitates in vivo bond-selective imaging on mice and the human body. It can visualize volumetric profiles of endogenous molecules in skin, such as lipid, protein, and water. In addition, this technique enables to monitor of rapid drug diffusion pathways in layers of skin.

(BIM-02.5)**Single-cell mid-IR Spectroscopy of the Disease Affected Neurons in Brain Tissue using Fluorescence-Detected Photothermal Imaging**

Aleksandr Razumtcev, Aris Polyzos, Hans Bechtel, Garth Simpson, *Purdue University, Lawrence Berkeley National Laboratory,*

Fluorescence-detected photothermal mid-IR (F-PTIR) microscopy is applied for chemically-specific imaging of brain tissue sections of mice affected by the neurodegenerative Huntington's disease (HD). Vibrational spectroscopy is a powerful tool that has the potential to reveal important conformational and structural changes in biological macromolecules that accompany disease progression. However, the widespread application of commercial vibrational imaging methods for biological research has been hindered by difficulties in cell registration within biological media due to the high chemical complexity of real-world samples. This issue can be alleviated by using instruments that support simultaneous fluorescence microscopy, owing to great successes in designing highly-specific fluorescence labels. The Simpson group recently demonstrated that the temperature sensitivity of fluorescence quantum yield can be exploited to locally probe the photothermal effect induced by the absorption of mid-IR radiation, leading to the development of F-PTIR microscopy as a novel method that combines the chemical specificity of mid-IR spectroscopy with the high spatial resolution and signal-to-noise characteristics of fluorescence microscopy. In this work, we demonstrate the application of F-PTIR microscopy for the characterization of freeze-dried fluorescently-labeled brain sections of HD-affected mice. Due to the fact that photothermal contrast in F-PTIR imaging is extracted from the modulated fluctuations in fluorescence intensity itself, neuron-specific labeling enabled studying only the most biologically-relevant cell types in the tissue samples with little background from the unlabeled regions. Furthermore, at high modulation frequencies of the incident mid-IR laser, the probe volume in F-PTIR is tightly localized around the emitting fluorophores, providing an increase in spatial resolution over complementary methods such as fluorescently-guided refractive-index based optical PTIR.

23FORENS04: International Mail Security, Southern Pacific A/G
Chair: Adam Lanzarotta
Co-Chair: Bhavik Vyas

(FORENS-04.1)**Analysis of FDA-Regulated Products for Drugs at International Mail Facilities**

Sara Kern, Adam Lanzarotta, JaCinta Batson, Michael Thatcher, Martin Kimani, Lisa Lorenz, Brian Boyd, Melissa Collins, Anvi Patel, Julio Arrecis, Kelsey Griffin, Fernando Gonzalez, Gregory Howe, Morgan Hudson-Davis, Mark Loh, Flavia Morales, Megan Sterling, Allison Reimer, Anthony Wetherby, Muhammad Altaf, David Laguerre, Donna LaGarde, Valerie Toomey, *Us Food And Drug Administration,*

In fiscal year 2022, over 354 million units of international mail were processed by the United States Postal Service (USPS), and many may have contained dangerous unknown, unapproved and misrepresented drug products under the purview of the US Food and Drug Administration (FDA). To increase the number of products inspected and protect consumers, the FDA's Forensic Chemistry Center (FCC) launched a satellite laboratory program outside of the Chicago International Mail Facility (IMF). Two analysts permanently staff this laboratory and analyze samples for the presence of active pharmaceutical ingredients (APIs) using an analytical toolkit that was extensively evaluated for ruggedness, ease of use, and speed during a pilot study. This toolkit consists of handheld Raman and portable FT-IR spectrometers, and a portable ambient ionization source coupled to a mass spectrometer that has detected over 289 unique APIs in drug products seized during the pilot and production program. This program was originally implemented to target opioids, particularly fentanyl and fentanyl analogs, but has evolved to include any type of FDA regulated product with an emphasis on complete unknown samples without labeling, which can be challenging even in a traditional brick-and-mortar lab with an arsenal of well-established techniques. Since production mode commenced in June, 2021, 985 samples were submitted for possible regulatory action. At least one API was detected by at least one device in, 76.9% of the samples. Of the APIs detected in these samples, several were either unapproved, controlled substances, and/or fall under the scope of section 801(u) of the Food, Drug and Cosmetic Act, which are drug products that have been determined to pose a significant public health concern.
Here we delve into the success and challenges associated with the satellite laboratory workflow and toolkit and discuss the evaluation of new technologies, including an atmospheric ionization mass spectrometer and a portable gas chromatograph with mass spectral detection (GC-MS). These instruments are expected to expand the capabilities of the toolkit and alleviate some of the issues encountered with the current equipment.

(FORENS-04.2)**Statistical Tools to Identify Reliable Discriminating Ions of Structurally Similar Fentanyl Analogs**

Isaac Willis, Victoria L. McGuffin, Ruth Smith, *Michigan State University*

A key challenge in seized drug analysis is the definitive identification of synthetic opioids, including fentanyl analogs. These analogs have flooded the market in recent years and, due to their structural similarity, the resulting electron-ionization mass spectra are highly similar. As such, identification of fentanyl analogs can be difficult based solely on mass spectral comparison.

In this presentation, a method to statistically compare two mass spectra will be discussed and the distinction of structurally similar fentanyl analogs will be demonstrated. The method uses the unequal variance t-test to test the null hypothesis (H_0) that the difference in intensity between corresponding ions is equal to zero. If H_0 is accepted for all ions in the scan range, the spectra are statistically indistinguishable. In contrast, if H_0 is not accepted for at least one m/z value, the spectra are statistically distinguishable, and such ions are identified as discriminating ions.

To demonstrate application of the method, this presentation will focus on two sets of positional isomers: ortho-, meta-, and para-fluoroisobutyryl fentanyl (o-, m-, and p-FIBF) and ortho-, meta-, and para-fluorobutyryl fentanyl (o-, m-, and p-FBF). Isomers within each set were analyzed by gas chromatography-electron ionization-mass spectrometry (GC-EI-MS) four times over an eight-month period.

Spectra within each of the four collections were statistically compared at the 99.9% confidence level and discriminating ions were identified ($t_{calc} > t_{crit}$, H_0 not accepted). However, due to inherent experimental and instrumental variation, the number and identity of discriminating ions varied across the collections. As such, ions were ranked in order of decreasing t_{calc} magnitude and averaged across the collections. The lowest ranked ions for a given comparison are those with the greatest difference in intensity and therefore, are more reliable for discrimination. In this manner, reliable ions to discriminate the FBF isomers and the FIBF isomers were ascertained. For example, for o-FBF and m-FBF, m/z 164 was identified as a reliable discriminating ion while, for m-FBF and p-FBF, m/z 234 was identified as a reliable discriminating ion. Each isomer comparison will be discussed in more detail and recommendations for accurate comparisons will be presented.

(FORENS-04.3)**Chemical Authentication and Determination of Composition of Counterfeit Drug Products**

Scott Huffman, *Bristol Myers Squibb*

Counterfeit medications continue to represent a global risk to patient safety and health. The authentication of medicines, and the detection of counterfeit products, is a priority for healthcare providers, regulatory agencies, law enforcement, and the biopharma industry in every country on earth. Additionally, the determination of the chemical composition of established counterfeits is important for building provenance around these illegitimate products, and for potentially linking cases to a common region or supplier. During this presentation we will highlight the analytical strategies and technologies used by Bristol Myers Squibb Forensics and Innovative technologies to determine the authenticity of our drug products, and the chemical composition of those that are found to be counterfeit.

(FORENS-04.4)**Integration of LC-MS and DART-MS in Routine Investigations of Suspected Counterfeit Pharmaceutical Drug Products and Product Quality Complaints**

Mark Wang, Scott Huffman, Ravi Kalyanaraman, *Bristol Myers Squibb*

Counterfeit pharmaceutical drug products endanger pharmaceutical supply chain integrity and patient safety. Product quality complaints negatively impact patient expectations of quality and personal perceptions of a company's performance. Therefore, the investigation of suspected counterfeit pharmaceutical drug products and product quality complaints is a vital component for pharmaceutical companies to ensure patient safety and confidence, to protect intellectual properties, and to meet regulatory requirements. Due to their short turn-around times and wide applicability, vibrational spectroscopic techniques and energy dispersive X-ray spectroscopy (EDS) have been routinely used in these investigations. Mass spectrometric techniques have been reserved for special or challenging cases. However, in recent years, we have increasingly utilized LC-MS and DART-MS in these investigations and achieved results which feature the enhanced detectability and specificity inherent in mass spectrometry. Presented here are some cases which demonstrate the feasibility of integrating LC-MS and DART-MS in routine investigations of suspected counterfeit pharmaceutical drug products and product quality complaints.

23IR05: Nanoscale IR Spectroscopy Theory and Applications, Sierra 3
Chair: Andrea Centrone

(IR-05.1)**NULL-DEFLECTION SCANNING PROBE INFRARED (NDIR) SPECTROSCOPIC IMAGING: FROM ANALYTICAL MODELS TO APPLICATIONS**

Seth Kenkel, Rohit Bhargava, *University of Illinois at Urbana-Champaign*

Nanoscale Infrared (IR) spectroscopic imaging techniques potentially offer super-resolved chemical information without labels but numerous technical challenges still limit reliable results. Confounding factors such as probe-sample mechanical coupling have already been identified; however, the extent of their influence has not been fully realized. Without a map to identify and navigate these technical challenges, further advances will likely not be possible. Here, we first review recent progress in analytical modeling of a cantilever in contact resonance and discuss complex effects such as probe-sample mechanical coupling, non-local forces, and multiplicative noise. Second, using the understanding obtained, we present a new Null-Deflection scanning probe Infrared (NDIR) spectroscopic imaging technique designed specifically to address these challenges and enable contact resonance IR imaging. Last, we demonstrate the potential applications enabled by the new approach.

(IR-05.2)**Understanding Cantilever Transduction Efficiency and Spatial Resolution in Nanoscale Infrared Microscopy**

JEFFREY SCHWARTZ, Georges Pavlidis, Andrea Centrone, *University of Maryland & NIST, University of Connecticut & NIST*

Photothermal induced resonance (PTIR), also known as AFM-IR, enables nanoscale infrared (IR) imaging and spectroscopy by using the tip of an atomic force microscope to transduce the local photothermal expansion and contraction of a sample. The signal transduction efficiency and spatial resolution of PTIR depend on a multitude of sample, cantilever, and illumination source parameters in ways that are not yet well understood. Here, we elucidate and separate the effects of laser pulse length, pulse shape, sample thermalization time (τ), interfacial thermal conductance, and cantilever detection frequency by devising analytical and numerical models that link a sample's photothermal excitations to the cantilever dynamics over a broad bandwidth (10 MHz). The models indicate that shorter laser pulses excite probe oscillations over broader bandwidths and should be preferred for measuring samples with shorter thermalization times. Furthermore, we show that the spatial resolution critically depends on the interfacial thermal conductance between dissimilar materials and improves monotonically, but not linearly, with increasing cantilever detection frequencies. The resolution can be enhanced for samples that do not fully thermalize between pulses (i.e., laser repetition rates $\gtrsim 1/3\tau$) as the probed depth becomes smaller than the film thickness. We believe that the insights presented here will accelerate the adoption and impact of PTIR analyses across a wide range of applications by informing experimental designs and measurement strategies as well as by guiding future technical advances.

(IR-05.3)**Spectroscopy and Imaging of Optical Near-fields on Individual Plasmonic Nanoparticles via Visible PiFM**

Sung Park, **Derek Nowak,** *Molecular Vista*

The ability to image the optical near-fields of nanoscale structures, map their morphology, and concurrently obtain spectroscopic information, all with high spatial resolution, is highly sought in nano-photonics. While photo-induced force microscopy (PiFM) with infrared light source is often used for nanoscale chemical studies, recent advances in tunable UV-vis-NIR light sources allow PiFM to characterize the optical near-fields of nanoscale photonic structures with equally impressive spatial resolution (~ 10 nm). PiFM measures the dipolar attractive force between the photo-induced dipole on the sample and the mirror dipole on the metal-coated tip, allowing it to measure the z-field associated with the photo-excited nano-structures without the strong background noise from parasitic scattering present on scattering scanning near-field microscopy (s-SNOM), currently the technique of choice for such studies. While background suppression techniques for s-SNOM provide adequate suppression in IR wavelengths, it is more difficult to do so in visible wavelengths without sacrificing the signal-to-noise significantly. In this paper, we present what we believe to be the first spectroscopic measurements of the plasmonic fields on individual nanorod and nanodisk samples with a spatial resolution of ~ 10 nm by coupling a light source that can tune the wavelengths continuously from 430 nm to 1450 nm. The spectroscopic data clearly shows that even with a single nano-particle, the resonance wavelength is determined by the local physical structure within the nano-particle. We believe that PiFM can effectively characterize heterogeneities of precisely manufactured nanostructures, SERS substrates, and heterogeneous (photo)catalytic samples.

(IR-05.4)**Photothermal Nanoscale Mid-IR Imaging - the Good, the Bad, the Ugly**

Georg Ramer, Ufuk Yilmaz, Lena Neubauer, Nikolaus Hondl, Elisabeth Holub, Yide Zhang, A. Catarina V. D. dos Santos, Bernhard Lendl, *TU Wien*

While direct interpretation of mid-infrared (MIR) spectra to identify the chemical composition of a sample is an important, though somewhat arcane, skill, ever more complex and larger IR datasets have made computer aided chemometric evaluation a widely used approach in qualitative and quantitative MIR analysis.

This same progression from direct interpretation of spectra to automated evaluiation is also now occuring in novel infrared imaging techniques, such as atomic force microscopy - induced resonance

(AFM-IR), a scanning probe based technique, that achieves infrared absorption imaging at < 20 nm spatial resolution, and far field MIR photothermal (MIP) spectroscopy.

In this work we focus on applying multivariate methods to photothermal super-resolution MIR spectroscopy techniques. Both techniques have in common that we like to pretend their signals are proportional to chemical concentrations - as we are used to from far field MIR imaging. However, both of them have additional contributions that need to be considered. For example, in contrast to bulk absorption spectra, AFM-IR and MIP spectra do not follow Beer's law and thus their signal does not depend linearly on the analyte concentration. Furthermore, at nanoscale spatial resolution lateral sample drift is noticeably affecting sample positioning. The optical non-linearity can be addressed by sample preparation and careful choice of sampling parameters. Thermal drift is a bit more challenging to solve in practice, but we can correct for it with software routines built using open source libraries.

In the case of MIP, sample geometry and sample refractive index contrast affect the signal and may cause false contrast. Care has to be taken not to misinterpret it as chemical information.

Here, we use examples from our own work to demonstrate how we've overcome these challenges.

23IR10: Instrumental Advances for Mid-IR Spectroscopy, Sierra 5
Chair: Young Jong Lee
Co-Chair: Bernhard Lendl

(IR-10.1)**Towards mid-IR photothermal lens spectroscopy for the analysis of liquids**

Gustavo Vinicius Bassi Lukasievicz, Elizandra Sehn, Alicja Dabrowska, Hongtu Cheng, *UTFPR / TU Wien*, *TU Wien, Technische Universität Wien*

Photothermal spectroscopy methods have been widely used to determine materials' thermal, optical, and mechanical properties. The mode-mismatched dual-beam photothermal lens (PTL) has been used due to its remote, sensitive, and non-destructive characteristics to detect a broad range of phenomena arising from the interaction of tightly focused laser beams and matter at different time scales.

In the PTL technique, the effect is probed by monitoring the probe beam phase shift caused by the surface expansion of the heated area, the photoelastic effects, and the spatial distribution of the refractive index within the sample and the surrounding medium. The transient signal is monitored at the far-field using a photodetector by analyzing the wavefront distortion of the probe beam. Thermal, optical, and mechanical properties can be quantitatively determined for solids, liquids, and gas. The applications involve material characterization of optical glasses, polymers, metals, alloys, semiconductors, fuels, and dyes.

Here we show the application of mid-infrared photothermal lens spectroscopy using a quantum cascade laser for the analysis of liquids. The PTL spectroscopy was used to obtain the mid-infrared absorption for different liquids without the use of expensive cryogenic detectors. The results show great potential for the method to be used in the characterization of different liquids and mixtures. Considerations about the theoretical model for the PTL technique in thin liquid samples are also presented.

(IR-10.2)**High-Sensitivity Infrared Absorption Spectroscopy for Aqueous Protein Solutions**

Seongmin Kim, Yow-Ren Chang, Young Jong Lee, *National Institute of Standards And Technology*

Infrared absorption spectroscopy can non-invasively quantify biomolecules and further elucidate their higher-order structures without separate sample preparation. However, in the conventional approaches, the strong IR absorption of water makes it challenging to measure proteins in aqueous solutions. Herein, we present an external-cavity quantum cascade laser (EC-QCL)-based IR spectroscopy equipped with a solvent absorption compensation (SAC) unit. The SAC method adjusts the incident

light spectrum to improve concentration sensitivity significantly. A series of measurements of aqueous protein solutions show a clear linear concentration dependence of Amide I and II bands from 100 mg/mL to 0.02 mg/mL without postprocessing. This new approach provides > 100 times greater sensitivity than conventional FT-IR spectroscopy. Rapid acquisition, wide frequency width, and no need for sample preparation will make this new IR spectroscopy technique a standard technique for characterizing the compositions and structures of protein solutions.

(IR-10.3)**Reading In-Between Spectra: Exploiting Laser-Based Mid-Infrared Spectroscopy with Chemometrics As A Tool to Study Continuous Unfolding of Proteins**

Shilpa Vijayakumar, Andreas Schwaighofer, Georg Ramer, Bernhard Lendl, *Technische Universität Vienna*

As protein function depends on in its correct folding i.e. its secondary structure, several industries including the pharmaceutical industry rely on analytical techniques for sensitive and dynamic detection of secondary structure changes (denaturation) for quality control. Fourier transform infrared spectroscopy (FTIR) by virtue of its sensitivity to different secondary structures and its capability to access a range of protein concentration makes it appropriate for the purpose. However, it struggles from its nemesis – water. To avoid total absorption, path lengths used in FTIR spectroscopy must hence be capped, consequently imposing challenges in protein denaturation studies that demand longer path lengths to avoid clogging. Tunable quantum cascade lasers (EC-QCLs) when used as light sources in mid-infrared spectroscopy, due to their high spectral power density, can offer this extended path length while retaining the benefits of FTIR spectroscopy, making it suitable for continuous, flow-through measurements.

This presentation demonstrates the use of an EC-QCL spectrometer covering the range of 1350 – 1750 cm^{-1} to study the continuous denaturation of proteins effected through two impulses, heat and chemical agents. These impulses themselves have spectral contributions, complicating the retrieval of protein secondary structure information. Chemometrics offers solutions to this in the form of algorithms, such as multivariate curve resolution alternating least squares (MCR-ALS), with the capacity to decompose complex matrices into component-relevant information. Applied to the thermal denaturation of bovine serum albumin (BSA) between 25-85°C, MCR-ALS indicates the formation of not one, but two intermediate structures. Furthermore, the effect of protein concentration on denaturation was investigated. The second example presents the challenge of highly interfering surfactant contributions overlapping with protein contributions. Two approaches were adopted to combat this; the use of a stand-alone MCR-ALS and the use of an automated baseline correction employing partial least squares regression (PLSR) to model the surfactant contributions, followed by MCR-ALS.

EC-QCL spectroscopy conjugated to chemometrics in both applications open promising avenues for automation and integrability to industrial processes as a consequence of the ruggedness and stability of such setups.

(IR-10.4)**Capillary Absorption Spectrometer (CAS) with Gas Chromatograph and Reactor for High Sensitivity Compound Specific Isotope Analysis**

Jason Kriesel, Emre Ozen, Kaori Emerson-Shurilla, Andrew Fahrland, *OKSI / Guiding Photonics, OKSI*

We describe a new approach for sensitive compound specific isotope analysis (CSIA) that utilizes a low-volume (~ 1 ml), compact gas cell, in a concept we refer to as a Capillary Absorption Spectrometer (CAS). In the CAS, an analyte is drawn into a hollow fiber, which has a reflective inner coating that guides a tunable laser beam to a detector. There is near unity overlap between the laser beam and the gas sample in the fiber, leading to a highly sensitive system with an ultra-low sample volume. The ultralow sample volume of the CAS opens up the ability to utilize the specificity and sensitivity of tunable laser absorption spectroscopy (TLAS) for sample limited applications.

For example, current CSIA systems characterize the stable isotope ratio of carbon (13C/12C) in a complex mixture by using a gas chromatograph (GC) to separate molecular species in time (elution peaks), followed by a combustion reactor to convert the carbon to CO2, which is then analyzed by an isotope ratio mass spectrometer (IRMS). The IRMS is an effective tool but has significant drawbacks in terms of relatively high cost, stringent vacuum requirements, and relatively large size, weight, and power. Alternatively, interfacing a GC to a cavity ring down or other multi-pass TLAS cell is not practical for sample limited applications due to the relatively large sample volume needed for these devices. In contrast, interfacing a GC to a CAS system is straight forward, and in fact, both the GC and the CAS utilize similar capillary tubing. The CAS can be directly coupled to the GC + Reactor enabling the elution peaks to flow directly into the CAS and analyzed in real-time with minimal sample requirements.
We will describe the overall spectroscopic concept and present results of isotopic analysis of biological samples, alkane mixtures, and food products. We will further describe how these measurements provide needed information to address important questions related to adulteration, degradation, provenance, and fidelity.

(IR-10.5)**High-Speed Infrared Spectroscopy and Analysis in Combustion Studies Using Swept-Wavelength External Cavity Quantum Cascade Lasers**

Mark Phillips, Austin Butler, Nick Glumac, Michael DeMagistris, Morgan Ruesch, Andrea Zambon, Neeraj Sinha, *University Of Arizona, University of Illinois at Urbana-Champaign, Combustion Research and Flow Technology, Inc. (CRAFT Tech)*

Absorption spectroscopy of gas-phase molecular species in high-temperature combustion conditions (flames, explosive fireballs, etc.) provides valuable information on chemical and physical properties in these challenging environments. The mid-wave infrared (MWIR) spectral region allows measurement of important primary combustion gases (CO, CO2, H2O, NOx), while the long-wave infrared (LWIR) spectral region gives access to a wide range of combustion and pyrolysis gases via characteristic "fingerprint" rotational-vibrational spectra. Absorption measurements through flame regions requires high speed operation to mitigate turbulence and track rapid chemical/physical dynamics, high source brightness to propagate through optically dense regions, and high spectral resolution to allow measurement of narrow spectral features from small molecular gases often of interest for quantification. Prior work has demonstrated the use of broadly-tunable swept-wavelength external cavity quantum cascade lasers (swept-ECQCLs) to measure multiple species and gas temperatures in high-explosive fireballs and biomass burning flames with temporal resolutions of 5-10 ms. Here we present new experimental results from broadband MWIR and LWIR swept-ECQCL measurements through propagating hydrogen/oxygen flames with up to 1 ms temporal resolution, to measure multiple chemical species and temperatures throughout the dynamic events.

For these experiments, two custom-built swept-ECQCL systems were used. The first operated in the MWIR region with a tuning range of 2010-2250 cm-1 (4.4-5.0 μm) and the second operated in the LWIR with a tuning range of 912-1170 cm-1 (8.5-11.0 μm). The swept-ECQCL systems were scanned continuously over ranges >150 cm-1 at rates up to 1 kHz to measure broadband absorption spectra during propagation of a hydrogen-oxygen flame in a combustion chamber. Spectral resolutions of 0.2-0.5 cm-1 combined with the large scan range enabled measurement of rovibrational lines of CO, CO2, and H2O over large band contours. Broadband absorption spectra were analyzed using automated quantitative spectral fitting algorithms based on HITEMP parameters to determine time-resolved temperatures and column densities of multiple reactants and products. Comparisons with computational fluid dynamics models were used to model path-averaging effects on measured spectra and analysis results, and to evaluate accuracy and precision of the methods.

23PAT04: In Situ Spectroscopy for industrial R&D, Southern Pacific E
Chair: Mark Rickard

(PAT-04.1)**Optimization-Based Strategies for Spectral Analysis and Kinetic Modeling**

Xiaoyun Chen, Thomas Krumpolc, Daniel Trahan, Lorenz Biegler, Michael Wang, *Dow, Carnegie Mellon University*

In-situ spectroscopy monitoring of reactions and processes are becoming increasingly popular due to multiple advantages such as the real-time feedback, non-intrusive and no-sampling required, and safe automated operations. To maximize information extraction from in-situ spectroscopy data set, many chemometric methods have been developed such as PLS, MCR, PCR, etc. Most of these methods do not take advantage of the kinetics information embedded in the spectroscopy data. A recent alternative method to these modeling approaches is to simultaneously obtain the reaction kinetic parameters with the curve resolution. Building on a nonlinear programming framework, the postulated reaction model is considered in the constraints of the optimization problem while also taking into account noise associated with instrument and model error. To further aid the practitioners with the rapid advancement of spectral analysis methods, an optimization modeling platform called KIPET was introduced (KInetic Parameter Estimation Tool). The KIPET approach is derived from maximum likelihood principles and uses nonlinear programming techniques and collocation methods to simultaneously solve a proposed reaction system, which has been shown to outperform traditional approaches like MCR-ALS. Several industrial examples will be presented to showcase the utility of this approach.

(PAT-04.2)**In Situ IR Study on Polyurethane Reactions**

William Wang, Lin Liu, Jake Grewe, *Lubrizol*

The application of in situ IR spectroscopy has been widely used in industry for decades. The method can provide more insightful information on the reaction, which includes the reaction kinetics and possible intermediate(s) involved. Such information drives innovation in process modification and optimization. Lubrizol Advanced Materials is one of the leading manufacturers of polyurethane dispersion (PUD) globally. PUDs are engineered polymers by dispersing polyurethane prepolymer in water. In this talk, applications of using in situ IR spectroscopy to monitor the PUD prepolymer reaction were discussed.

(PAT-04.3)**Mesoporous Materials and Mid-Infrared Spectroscopy for the Trace Monitoring of Contaminants in Process Analytical Technology**

Felix Frank, Bettina Baumgartner, Mattias Verstuyft, Nuria Teigell Beneitez, Dominik Wacht, Mauro David, Elsa Traxler, Borislav Hinkov, Dries Van Thourhout, Bernhard Lendl, *Tu Wien, Utrecht University, Ghent University-imec*

Mid-infrared (mid-IR) spectroscopy is a powerful analytical technique that has been widely used in process analytical technology (PAT) applications in the liquid and gas phase alike. It is a label-free and non-destructive technique that can provide real-time and continuous monitoring of chemical processes. This allows for rapid detection and correction of deviations from the desired process parameters, resulting in improved process efficiency and product quality. However, when performing measurements in the presence of water, mid-IR spectroscopy is limited by its strong absorption bands in the mid-IR region that overlap with many of the analyte bands. In the liquid phase, this significantly restricts the possible pathlength and, thus, the sensitivity. One way of working around these limitations is by employing preconcentration schemes, aiming at increasing the concentration in the volume probed by the IR beam while keeping the interaction length reasonably short.
In this work, we explored the combination of mesoporous films (e.g., silica, titania, zirconia) with evanescent field mid-IR spectroscopy and showed its capabilities for trace analysis of organic contaminants in gaseous and aqueous samples. For gas-phase detection, functionalized mesoporous materials were coated onto an integrated optics waveguide. Coupled with an external cavity quantum cascade laser, this approach enabled multi-analyte detection of volatile organic compounds with sub-ppmv limits of detection and enrichment factors of up to 10,000. The mesoporous films were found to be stable against high relative humidity.

Beyond demonstrating the raw analytical performance enabled by mesoporous enrichment layers, we also explored the synthesis and functionalization of mesoporous films, allowing to tailor the pores for analytical applications. For example, using titania and zirconia layers, both ionic species and organic contaminants could be detected in the low ppm range in aqueous samples with enrichment factors between 600 and 5000 by varying the sensor functionalization. Further, using these mesoporous layers, the phosphate absorption bands were accessible to IR detection, paving the way for high sensitivity phosphate sensing in water.

(PAT-04.4)**Measurement of Nitrogen-Containing Compounds and Oxyanions in Industrial Wastewater using High-Throughput Raman Spectroscopy**

Colin Couper, **Shaun Fraser**, Mark Kemper, *Tornado Spectral Systems*

Control of emissions from industrial wastewater is a regulatory requirement in all industries, and can also be valuable for minimizing waste. Common nitrogen containing compounds and oxyanions found in wastewater can be hazardous to human health and function as fertilizers causing environmental issues like algal blooms, but also represent opportunities for extraction or recycling.

Here we examine limits of detection for simultaneous, online measurement of important compounds in industrial wastewater, including ammonium, urea, nitrate, phosphate, and sulfate, in the context of a multipoint measurement system. Additionally, a comparison of univariate and multivariate model building methods, including peak area and PLS, is performed. These measurements can also be useful in a wide variety of other industries, such as production of fertilizers and chemicals, as well as bioprocess and biopharmaceutical applications.

By using High-Throughput Raman Spectroscopy, these measurements can be performed quickly and with an exceptionally low limit of detection generally below 100ppm, while measuring multiple points in the system.

23PMA06: Emerging Plasmonic Nanoparticles for Drugs and Pharmaceutical Analysis, Southern Pacific D
Chair: Malama Chisanga

(PMA-06.1)**Functionalized SERS sensors for the detection and quantitative analysis of narcotics**

Li-lin Tay, *National Research Council Canada*

Surface-enhanced Raman scattering (SERS) is an ultrasensitive analytical technique with molecular specificity, making it an ideal candidate for illicit drug monitoring. It is particularly valuable in the identification and quantitation of molecular species present at trace concentration levels such trace quantity of highly toxic synthetic analogues of fentanyl mixed with other drug matrix. Drug checking technologies provides a harm reduction measure by informing users of the composition of the drug. There are a number of potential drug checking technologies, such as Raman, FTIR, mass-spectrometry and colourimetric test strips. In this presentation, we will discuss a surface enhanced Raman spectroscopy (SERS) based sensor for detection and quantification of common narcotics such as fentanyl and heroin. We will discuss modification to the surface of gold nanostructure to improve the detection and spectral identification. We will present the use of a witness reference standards to aid the quantification process. Our results show that the optimized SERS sensors can generally out-perform the colorimetric test strip and can be a valuable tool in drug-checking and harm-reduction strategy.

(PMA-06.2)**SERS Biosensors for Early Diagnosis and Treatment Guidance in Plants**

Pietro Strobbia, *University Of Cincinnati*

Molecular analysis is essential for precise and data-driven agriculture, but its use in agricultural settings is limited due to the current burdensome analytical paradigm. Nanotechnology can

revolutionize molecular analysis by decentralizing it and making it easily accessible. This paradigm shift can result in more sustainable and efficient agricultural practices.

This work presents the development of a nanosensor technology for in-situ detection of infected plants. Our platform uses homogeneous surface-enhanced Raman scattering (SERS) sensing to detect viral infections in plants. The sensors are incorporated in gel networks, enabling sample preparation-free analysis in the field using a "touch-and-detect" method. The gels efficiently uptake genetic material, while the sensors give a specific and sensitive response to the target sequence. These sensors are versatile and can detect plant disease biomarkers (DNA), plant pathogens (viral RNA), and/or small molecule contaminants (pesticides). This presentation will focus on the use of these sensors to detect early viral infections in plants to guide treatment and mitigation strategies.

(PMA-06.3)**Tailoring The Nanoparticle Surface For The SERS Detection Of Drugs And Biological Analytes**

Chiara Deriu, Laura Fabris, *Politecnico di Torino*

In a SERS measurement, the intrinsically weak Raman scattering of an analyte that is adsorbed onto a suitable nanostructured surface is amplified by several orders of magnitude, enabling trace detection. Because the observed signal enhancement is the result of a plasmonic, near-field effect, conditions for signal observation, and consequent low limits of detection, are achieved when analyte adsorption on the substrate is thermodynamically favored.

When implementing SERS analytical protocols that are based on the use of colloidal nanoparticles as the enhancing substrate, it is fundamental to consider that these are never "chemically clean"; rather, they always bare a population of adsorbed species on their surface. As a result, the association constants that must be considered when performing SERS measurements in colloidal sols are not limited to those between the analyte and the plasmonic surface but should also include those between the surface and pre-adsorbed species, and between the analyte and pre-adsorbed species. The latter can be synthesis by-products, such as oxidized or unreacted reducing agent molecules, or other species that are intentionally added to improve the colloidal formulation, such as stabilizers. Because stabilizers typically represent most of the surface adsorbed species in a colloidally stable sol, failure to consider their energetics when implementing direct SERS protocols might result in failure to observe the desired SERS signal.

Despite its importance, the study of the interaction between colloidal sol stabilizers and plasmonic surfaces is an underexplored area of SERS research. In this presentation, different examples of such studies will be discussed, with a focus on how they can be leveraged for ad hoc SERS substrate and analytical protocol design. These include, but are not limited to, the stabilization of nanostars for tailored, class-specific analyte detection (i.e., a specific class of drugs or a family of biomolecules), the determination of experimental and theoretical thermodynamic quantities of stabilizer-metal systems, and their computational modeling.

23RAM11: Raman Standards, Cascade 3

Chair: Aaron Urbas

(RAM-11.1)**Semiconducting Nanowires for Metrological Calibration of Spatial Resolution in Raman Microscopy**

Sebastian Wood, *National Physical Laboratory*

Raman microspectroscopy is a widely used technique across multiple fields where chemical mapping of materials is required with sub-micrometre resolution. Whilst spatial resolution of Raman microscopy is a key performance parameter for such studies, there are currently no documentary standards and very limited reference samples available to address this need. Here we propose and evaluate a new reference sample for metrologically measuring the spatial resolution of a confocal

Raman microscope, which has been tested through an interlaboratory comparison involving institutions in 10 countries.

The proposed method of spatial resolution measurement is based on a line-spread function measurement using a nanowire feature that has a diameter much less than the expected point spread function of the instrument. In order to provide metrological traceability it is important that the nanowire features can be uniquely and repeatably identified, so that the nanowire dimensions can be measured using metrological atomic force microscopy (AFM). This is achieved using the dielectrophoresis concept, where inorganic semiconducting nanowires can be controllably deposited across gaps between pairs of metal electrodes. Other requirements of the sample are that it exhibits high Raman scattering contrast with the substrate, and is robust enough to endure repeated laser exposure. The structure and dimensions of the proposed sample are shown in Figure 1. A VAMAS (Versailles Project on Advanced Materials and Standards) international interlaboratory comparison study has been conducted to evaluate the proposed sample and associated measurement protocol. We will present the outcomes of this study.

As interest in super-resolution Raman spectroscopy methods grows, there is an increasing need for spatial resolution measurements that can be applied for resolutions < 100 nm. The techniques developed in this work are scalable and we have considered their feasibility for use as a reference sample for tip-enhanced Raman spectroscopy (TERS), including a demonstration using a nanowire with ~ 5 nm diameter.

(RAM-11.2)**Raman Data with CHARISMA**

Enrique Lozano, *ELODIZ Ltd*

The charisma project, an acronym for "Characterisation and HARmonisation for Industrial Standardisation of Advanced Materials" was established in 2021 under the EU-funded program HORIZON 2020 to harmonise Raman Spectroscopy for Raman data characterisation across the life cycle of materials, from the lab to production. We pay particular attention to how to address the consistency of the data along the research and industrialisation process and the use of a variety of Raman devices. We have prepared and tested a consolidated proposal for the calibration of the Raman units and the results of the first round-robin will be presented.

(RAM-11.3)**The Influence of Molecular Structure on the Raman Spectral Pattern and Reproducibility of Per- and Polyfluoroalkyl Substances in Liquid Extracts**

Seo Won Cho, Christina Remucal, Haoran Wei, *University of Wisconsin-Madison,*

As per- and polyfluoroalkyl substances (PFAS) pose a great threat to public health, the U.S. Environmental Protection Agency established the draft Method 1633 to detect PFAS in various environmental media based on solid-phase extraction and liquid chromatography-tandem mass spectrometry (LC-MS/MS). Though precise and sensitive, the operational complexity and high cost of the standard method hinder the regular monitoring of PFAS. Raman spectroscopy is a promising complementary tool for LC-MS/MS because of its fingerprinting ability for trace analysis, low operational cost, and fitness for field-deployable applications. For the practical application of Raman spectroscopy, a well-established Raman spectral library is a prerequisite due to the current lack of comprehensive and reproducible spectral data on PFAS. In this study, we propose a simple method to concentrate PFAS in organic solvents and to establish a Raman library for PFAS. We drop coated PFAS solutions onto aluminum foil and dried them to concentrate and secure PFAS in a solid form. The different functional groups and chain lengths affected the evaporation and crystallization behavior of PFAS. Raman maps were then collected from the sessile drops using a 532 nm laser and a confocal Raman spectrometer. Regardless of the chemical structure of PFAS, they shared common Raman peaks at around 300, 380, 524, 570, and 724 cm-1, while having varying peak-to-peak ratios. To differentiate PFAS congeners and alkyl acids, principal component analysis was performed on wavenumbers between 200 and 1,000 cm-1. This research created a novel reproducible Raman

spectral library of PFAS that will be a foundation for simple and facile PFAS screening using Raman spectroscopy.

(RAM-11.4)**Renewal, Revision and Modernization of the ASTM E13.08 Raman Spectroscopy Standards**

Li-lin Tay *National Research Council Canada*

The American Society for Testing and Materials (ASTM), is an international standards organization that develops and publishes voluntary consensus technical standards for a broad range of materials. ASTM technical committee E13 is focused on the advancement of molecular spectroscopy and separation science. It was established in 1950 and currently has over 125 members representing 12 countries globally. Its technical subcommittee E13.08 on Raman spectroscopy is responsible for a family of four Raman spectroscopy standards (E1683-02 performance of scanning Raman spectrometers; E1805-96 Raman shift standards for spectrometer calibration; E2529-06 Resolution test of a Raman spectrometer; E2011-13 Relative intensity correction). These four Raman standards are among the earlier documentary standards established for Raman spectroscopy and are broadly adopted by academics, instrument manufacturers and regulatory agencies. These standards were developed over two decades ago. There has been significant advancement in Raman instrumentation since their initial publication. In particular, the user community has grown to cover many advanced novel materials (e.g. 2D materials) and the rapid growth in portable Raman analyzer has in the last decade have raised new needs for documentary standards. Documentary standards should reflect the need of the broader Raman community. In this presentation, I will introduce the four existing Raman standards hosted by the E13.08 Raman spectroscopy technical subcommittee and discuss the plans for their renewal, revision and modernization. I will also briefly introduce other Raman spectroscopy standards in other ASTM technical committees which references to the existing E13.08 Raman standards and impact to these other standards. We welcome input from the broader Raman spectroscopy community on the discussion of future needs in Raman documentary standards.

23SPECIAL02: Spectrochimica Acta B - Award Session, Cascade 1
Chair: Alessandro De Giacomo

(SPEC-02.1)**Novel types of biomedical ICP-MS applications**

Frank Vanhaecke, Lana Abou-Zeid, Eduardo Bolea-Fernandez, Marta Costas-Rodriguez, Rinus Dejonghe, Rosa Grigoryan, Kasper Hobin, Tong Liu, Mina Nikolic, Kaj Sullivan, Ir. Thibaut Van Acker, Tom Van Helden, *Ghent University, University Of Zaragoza, University of Vigo,*

Since its introduction in the first half of the 1980s, ICP-mass spectrometry (ICP-MS) has been used for the bulk analysis of biofluids. Also in this early phase of development of the technique, its use in tracer experiments with stable isotopes was already explored. By now, the technique has secured its place in routine clinical labs, while the application range in a biomedical context is still being expanded. This presentation will focus on more novel types of biomedical applications explored within the A&MS unit of Ghent University.

A first application example that will be covered still addresses bulk elemental analysis of biofluids, but at < 1 µL sample consumption, as enabled by using a commercially available micro-flow injection unit coupled to a high-efficiency total sample consumption introduction system. Secondly, in tissue samples, not only the bulk concentration of elements provides relevant information, but also their spatial distribution. By using a state-of-the-art nanosecond ArF* excimer-based (193 nm) laser ablation (LA) set-up equipped with a low-dispersion ablation cell and aerosol transport system, elemental maps can be obtained at a pixel acquisition rate up to 1 kHz and with a spatial resolution down to ca. 1 µm. A third application example focuses on the determination of elemental contents in individual cells via ICP-MS operated in single-event mode. Proper cell fixation, the use of an adequate sample introduction system, and an ICP-MS unit with sub-millisecond detector dwell time enables elemental contents to be determined down to sub-femtogram per cell levels. A fourth and last application type to be discussed is high-precision isotopic analysis of essential mineral elements using

multi-collector ICP-MS. It has been demonstrated that the information embedded in the isotopic composition of such mineral element can either be complementary to that provided by its concentration or reflect alterations in the homeostasis due to physiological changes caused by disease or by any other process with higher sensitivity than the concentration does. As a result, isotopic analysis allows a more profound insight into biochemical processes and shows promise as a diagnostic and/or prognostic tool.

(SPEC-02.2)**A Compilation of Landmark Publications in Analytical Atomic Spectrometry**

George Chan, Gary Hieftje, Nicoló Omenetto, *Lawrence Berkeley National Laboratory, Indiana University, University of Florida*

The almost two-hundred years of development history in spectrochemical analysis accumulates a vast amount of literature. Many theories, physical foundations, and instrumentation of modern spectrochemical analysis methods date back to research performed several or even many decades ago. It can be difficult, especially for a young researcher, to become familiar with all the essential literature in the field as many of these seminal papers are now seldom cited directly. This long-standing limitation was acknowledged by Heinrich Kaiser, who asserted [1], "The main problem in spectrochemical analysis… is a problem of generations. … things which have been known for decades or half a century have been entirely forgotten or not taken up by the young people. … Apparently there is a lack of basic information, and the reason for this lack of basic information is that nobody is able to read the whole literature."

We started a project about four years ago in preparing a study-guide type of review in which we compiled a list of the most important landmark publications that have appeared since the origins of the field. Our approach has been unusual if not unique: we contacted a number of scientists, all highly prominent in the field of spectrochemical analysis, and asked each to name 12 to 48 publications that are deemed to be critical in shaping the field. Each suggested paper was accompanied by a brief discussion indicating what the key findings of the paper are and why it has been critical to the basic science and technology of spectrochemistry. We received responses from 48 scientists. A total of roughly 1,000 key papers covering more than 15 sub-disciplines of spectrochemical analysis are cited and discussed in the compilation.

In this presentation, the project will be overviewed, statistics of these key papers will be discussed, and a project update will be provided.

Reference:

1. R.C. Barras (ed), Transcript of first international conclave on unsolved problems in spectrochemical analysis (Ottawa, June 24-26, 1967), transcript available from Othmer Library, Science History Institute

(SPEC-02.3)**Advances On Micro Laser Induced Breakdown Spectroscopy And Micro X-Ray Fluorescence Mineralogical And Elemental Quantitative Imaging**

Cecile Fabre, Kimberly Trebus, Alexandre Tarantola, Jean Cauzid, Vincent Motto-Ros, Panagiotis Voudouris, *Georessources, Carleton University, ILM, University of Athens*

Mineralogical and petrographic studies require analytical methods capable to underline the repartition of major to trace elements within geological samples. The EMPA (Electron Microprobe Analysis) conventional method used for such investigation, but on restrictive zones, is on the verge to be reached by μLIBS (Laser Induced Breakdown Spectroscopy) and μXRF (X-Ray Fluorescence) techniques allowing the elemental cartography on thin rock sections or even larger samples. These spectroscopic methods with extremely fast acquisition speed (10 ms/pixel) are perfectly adapted to perform multi-elemental imaging of major to trace elements down to the ppm-level. Here, on a mica schist thin section that displays a wide paragenesis of minerals, μLIBS and μXRF quantitative elemental mappings are obtained using spot EMPA analyses as internal reference compositions. We exhibit the precision of these μLIBS and μXRF quantitative maps, varying slightly from EMPA calibrated data

for major and trace elements repartition in the sample. According to these oxide weight contents, a rapid mineral classification is obtained, with a very good discrimination between the minerals even for those with close compositions (alumino-silicates such as andalusite and kyanite) and within a complex material (Fe-oxides, quartz, micas, feldspars…).

(SPEC-02.4)**The Crucial Role of Molecular Emissions on LIBS Differentiation of Organic Compounds of Interest in Astrobiology under a Mars Simulated Atmosphere**

Javier Laserna, Laura Garcia-Gomez, Tomas Delgado, Francisco Javier Fortes, Luisa Cabalin, *Universidad de Malaga*

The influence of Martian atmosphere on the recombination mechanisms in laser-induced plasmas of organic compounds of interest in astrobiology has been closely examined. The proposed LIBS methodology reveals new insights concerning the effect of this factor on the molecular emission of organics. The presence of nitrogen, even in the short concentration found in the low-pressure Mars environment, impacts the plasma chemistry and the formation pathways leading to molecular species.

Characteristic atomic and molecular emitters (C, H, C2, CN, NH, OH and CH) were inspected in a set of selected organic compounds considered as meaningful biomarkers. For comparative purposes, LIBS analysis was performed in both low-pressure air and Martian atmospheres. Statistical analysis (linear discriminant analysis; DFA) suggested that satisfactory differentiation among materials was feasible under Martian conditions when molecular emissions are computed in the classification algorithm. Atomic lines (C, H) only contribute a mere 62% to the discriminating analysis, whereas the percentage of successful discrimination was considerably increased (up to 99%) by the progressive introduction of signals associated to molecular bands into the DFA analysis, thus confirming the major contribution of molecular information to the final sorting performance and the feasibility of discriminating closely related organic compounds by LIBS in the Martian atmosphere.

(SPEC-02.5)**Quantification of Major Elements in Rocks and Soils on Mars with SuperCam Laser-Induced Breakdown Spectroscopy (LIBS)**

Ryan Anderson, Paolo Pilleri, Travis Gabriel, Olivier Forni, Agnes Cousin, Roger Wiens, Sam Clegg, Jens Frydenvang, Ann Ollila, Susanne Schröder, Olivier Beyssac, Erin Gibbons, David Vogt, Elise Clavé, Jose-Antonio Manrique, Carey Legett IV, Raymond Newell, Joseph Sarrao, Sylvestre Maurice, Shiv Sharma, The SuperCam Team, *Usgs Astrogeology, Institut de Recherche en Astrophysique et Planetologie, Purdue University, Los Alamos National Laboratory, University of Copenhagen, Deutsches Zentrum für Luft- und Raumfahrt, Université Pierre et Marie Curie, McGill University, Universidad de Valladolid, University of Hawaii*

SuperCam is an instrument suite on the Perseverance Mars 2020 rover capable of collecting color telescopic images, visible to near-infrared spectra, Raman spectra, time-resolved luminescence spectra, and of conducting laser-induced breakdown spectroscopy (LIBS). LIBS spectra are used to quantify the major element oxides (SiO2, TiO2, Al2O3, FeOT, MgO, CaO, Na2O, K2O) in geologic targets on the surface of Mars at distances of up to 7 m. This quantification is accomplished using a suite of laboratory spectra of diverse geologic materials to train multivariate regression models. There are many different regression algorithms to choose from. We use cross-validation to optimize models for each algorithm and evaluate model accuracy using an independent test set of data that has been withheld from the cross-validation process. We also evaluate model results on data from Mars to ensure that the quantitative results are geochemically reasonable.

An initial major element calibration was published in early 2022, based on a database of 334 reference samples [1]. Since then, we have been working toward an updated calibration using an expanded database of laboratory data with >700 samples and an improved approach for cross-validation and model selection. We will present an overview of the current status of SuperCam chemical quantification efforts and discuss challenges and next steps.

[1] R.B. Anderson, et al. Post-landing major element quantification using SuperCam laser induced breakdown spectroscopy, Spectrochimica Acta Part B: Atomic Spectroscopy. (2022) 106347. https://doi.org/10.1016/j.sab.2021.106347.

23SPSJ04: NIR Spectroscopy (Applications), Cascade 4
Chair: Christian W. Huck
Co-Chair: Mika Ishigaki

(SPSJ-04.1)**Handheld NIR spectroscopy: a non-destructive, rapid and informative technique for quality control in the materials- and life-sciences**

Heinz Siesler, Marina De Gea Neves, Hui Yan, *Department of Physical Chemistry, University Duisburg-Essen, Jiangsu University of Science and Technology*

The presentation considers the rapid development of miniaturized handheld NIR spectrometers over the last decade and provides an overview of current instrumental developments and exemplary applications in the fields of material and food control as well as environmentally relevant investigations. Care is taken, however, not to fall into the exaggerated and sometimes unrealistic narrative of some direct-to-consumer companies, which has raised unrealistic expectations with full-bodied promises, but has harmed the very valuable technology of NIR spectroscopy, rather than promoting its further development. Special attention will also be paid to possible applications that will allow a user group that is not necessarily scientifically trained to solve quality control and authentication problems with this technology in everyday life.

(SPSJ-04.2)**Near-infrared spectral pattern classification of glycolytic reactions using oscillating reactions of yeast extracts**

Akifumi Ikehata, Miho Sesumi, *Food Research Institute*

To promote bio-applications of NIR spectroscopy, we need to understand the spectral patterns presented by multivariate analyses, such as PLS regression. These patterns can be inferred to originate not only from the objective compound but also from related metabolites, however this is still unclear. In this study, we will focus on the glycolytic reaction of yeast (Saccharomyces cerevisiae), an experimental microorganism that has been elucidated from the genetic level. Although the yeast extract is not alive, the autocatalytic action of enzymes sustains periodic reactions of carbohydrate metabolism for several hours. The oscillatory reaction with a cycle of 13 minutes can be monitored by the UV absorbance of NADH at 340 nm. UV and NIR absorption spectra of the same extractant in a cuvette were alternately measured at 90 second intervals for 2 hours. From the NIR spectra, we were able to construct a PLS regression to model the UV absorbance of NADH. In other words, the NIR spectra could capture the oscillating reactions of glycolytic metabolism. The problem is to understand the shape of the regression vectors and loadings of the latent variables. Since the PLS regression models only a periodic glycolytic reaction, background metabolisms are automatically excluded. 1H-NMR results performed separately clearly showed periodic time variability of individual metabolites. The results support the phase differences between metabolites predicted in the literature. The PLS regression model was interpreted by comparing it with metabolite kinetics obtained from the 1H-NMR spectra.

(SPSJ-04.3)**Hyperspectral image data analytics with deep learning fusion-nets**

Bosoon Park, Taesung Shin, *U.S. Department of Agriculture, Agricultural Research Service, USDA, ARS*

Foodborne pathogens cause a serious public health issue every year worldwide. Although various techniques have been used for assessment of bacteria viability, none of them was successful for implementing to identify live/dead bacteria rapidly without incubation process. Recently, hyperspectral microscope imaging (HMI) technology demonstrated the potential for a rapid label-free detection of foodborne bacteria. The HMI with deep learning methods accurately distinguished

between live and dead foodborne bacteria. In this study, three deep learning models called Fusion-Net I, II, and III were developed to classify pathogenic bacterial cells of several foodborne bacteria including E. coli, Listeria, Staphylococcus, and Salmonella using their morphological and spectral characteristics of live and dead bacterial cells. Three Fusion-Net models with inputs of spectra and morphological features from 546 nm band images of the cells performed to classify live/dead bacteria with over 93% accuracy, suggesting that live foodborne bacteria could be accurately identified by hyperspectral microscope imaging with machine learning algorithm effectively prior to causing foodborne outbreaks.

(SPSJ-04.4)**Unleashing the Potential: Overcoming Hurdles to Make Vibrational Spectroscopy a Routine Diagnostic Tool**

Bayden Wood, John Adegoke, Karin Jandeleit-Dahm, Diana Bedolla, Phil Heraud, Adele Kinces, Keith Dias, *Monash University,*

The potential of vibrational spectroscopy for disease diagnosis has yet to be realized as a routine diagnostic tool due to several reasons. First, the complexity of spectroscopic techniques, such as infrared and Raman spectroscopy, requires specialized expertise and instrumentation, limiting their use in routine clinical settings. Second, the lack of standardized protocols and large-scale validation studies across different diseases and populations has hindered their adoption. Additionally, biological sample variability and the presence of interfering substances pose challenges to accurate measurements. Technological advancements have improved the field, but equipment costs remain high. Finally, integrating vibrational spectroscopy into existing clinical workflows requires restructuring and optimization of healthcare systems. To overcome these challenges, efforts must focus on simplifying the techniques, establishing standardized protocols, accounting for sample variability, reducing costs, and streamlining integration with clinical workflows. The talk will focus on these challenges and how they are been met by the Monash Biospectroscopy Group that includes spectroscopists, clinicians, and industry stakeholders whose aims is to realize the potential of spectroscopy as a routine diagnostic tool.
In this context we have extensively investigated biofluids targeting diseases including malaria, HCV, HBV, SARS-CoV-2 and kidney disease. The talk will focus on the spectroscopic approaches to diagnosing these diseases using mid-IR, Near-IR, UV/Vis spectroscopy and multimodal spectroscopy highlighting the pros and cons of the different modalities.

(SPSJ-04.5)**Miniaturized NIR in Natural Products and Food Analysis - From Mechanistic Understanding to Framework Optimization**

Justyna Grabska, Krzysztof Bec, Christian Huck, *University of Innsbruck,*

Near-infrared (NIR) spectroscopy has revolutionized analytical chemistry by providing rapid, non-destructive, and cost-effective analysis. NIR spectroscopy is extensively used in various industries, such as agriculture, food analysis, forensics, security, and manufacturing, as a reliable quality control tool. Recent technological and methodological advancements have led to the development of miniaturized and portable instrumentation, as well as new methods for data analysis and interpretation, ushering in a new era in analytical spectroscopy.

This talk will explore the potential of the miniaturized NIR spectrometers in analytical applications, with a specific focus on natural products and food analysis. This talk will also address the challenges associated with the use of different miniaturized NIR spectrometers in analytical spectroscopy. These devices operate based on different technological principles, resulting in differences in their performance, sensitivity, and selectivity.

Furthermore, this presentation will delve into the mechanistic understanding of NIR spectroscopy and how this understanding can optimize framework development. Firstly, physical interpretation of NIR bands opens new possibilities of profiling the analytical potential of miniaturized sensors, which often can acquire only a specific fragment of the spectral (and thus compositional) information of the sample. Detailed dissection of chemometric models augmented by the interpreted information yields

the possibility to assess in detail the sensitivity and selectivity of a particular instrument against a specific compound or chemical moiety. Finally, interpreted information opens the pathway to the understanding of the impact of spectral noise on the analytical application. A "sample-specific" SNR values differ from the nominal SNR values, especially in the lower NIR wavenumber regions. Differences between instruments manifest themselves clearly, particularly in the case of strongly absorbing samples like water.

Plenary Sessions: NESAS and SAS Lester W. Strock Award; Maria Montes Bayon, Sierra 5

(PLEN-L3.1)**ICP-MS BASED STRATEGIES IN BIOMEDICINE: EXPANDING THE BOUNDARIES**

Maria Montes-Bayon, *University Of Oviedo*

The advantages in the use of inductively coupled plasma mass spectrometry (ICP-MS) in biomedicine have been widely acknowledged since its first introduction as elemental detection of ultratrace element in biosamples (e.g. serum, urine, etc). Namely, ICP-MS provides selective extremely sensitive and multi-elemental (and multi-isotope) detection capabilities even in complex samples that facilitates its use for quantitative purposes. But, although ICP-MS can be considered a purely elemental detector, the molecular specificity can be obtained by coupling it with separation techniques such as chromatography and electrophoresis. ICP-MS shows versatility and easy coupling to such techniques with the aim of monitoring the metals or metalloids associated to different biomolecules and, in this way, it becomes an attractive partner of ESI and MALDI for the study of those interactions in speciation and further, in metallomics studies. Nowadays, ICP-MS is completely immerse in the analysis “small individual objects” like nanoparticles and cells. The analysis of individual cells can only be achieved when the technology enables sufficient sensitivity to perform the determinations in the tiny amounts of matter contained in a single cell. The combination of a powerful strategy like single cell-ICP-MS (SC-ICP-MS) with techniques from the molecular biology like flow cytometry has yielded in the powerful mass cytometry (CyTOF) already in use in some laboratories related to biomedicine.
After almost 40 years of development, (ICP-MS) can hardly be considered as a novel technique anymore. Unless still in use in speciation and total elemental analysis, over the last decade, this technique has managed to uncover an entirely new application field, providing information in a variety of contexts related to the individual analysis of single entities (e.g., nanoparticles, cells, or micro/nanoplastics), thus addressing new societal challenges. In this presentation, the most remarkable applications of ICP-MS in biomedicine from speciation to single cell analysis will be highlighted with special emphasis on the need to combine multiple techniques to achieve deeper insights in the biomedical problems.

Plenary Sessions: AES Mid-Career Achievement Award; Robbyn Anand, Sierra 5

(PLEN-L3.2)**Electrokinetic Enrichment of Analytes Integrated with Label-Free Electrochemical Sensing - Scale-up and Scaling Laws**

Beatrise Berzina, Sungu Kim, Umesha Peramune, Kumar Saurabh, Sommer Osman, Echo Claus, Sanduni Devasinghe, Md Ruhul Amin, Madison Strait, Baskar Ganapathysubramanian, **Robbyn Anand,** *Iowa State University, Stanford University*

Sensors that leverage the influence of a binding event on charge transport, such as field-effect transistors and nanoporous membranes, are among the most sensitive because they translate localized binding into a change in a system-scale property. However, fabrication and custom functionalization of these sensors is not trivial, and their integration with protocols that pre-enrich target species and facilitate their transport to the binding site is an active area of research. In this presentation, we demonstrate that ion concentration polarization (ICP) in the presence of fluid flow drives stable focusing and efficient capture of charged analytes within a bed of probe-modified microbeads embedded in a microfluidic channel. This configuration combines several mechanisms known to stabilize ICP focusing for scale-up, thereby allowing for greater sample volumes to be swept without

compromising the efficiency of analyte separation and enrichment. A key finding is that ion conduction along the surface of the beads is a significant contributor to current through this channel segment under an applied voltage bias. Therefore, hybridization of a charged analyte to the bead surface leads to a shift in the slope of the current-voltage curve. This approach is versatile in that the analyte can be detected electrically, in the absence of a label. The resulting approach allows for a plug-and-play sensor using off-the-shelf microbeads and simple electronics, making it advantageous for point-of-need testing. Finally, we propose scaling laws for the enrichment and sensing of several classes of analytes including small molecules, nucleic acids, antibodies, and virus particles. Our results indicate that sensitivity is positively correlated with analyte size and charge.

23AES07: 50th Anniversary, Southern Pacific F
Chair: Tayloria Adams
Co-Chair: Erin Henslee

(AES-07.1)**Isotachophoresis theory and its application to sample preparation of nucleic acids**

Juan Santiago, Ashwin Ramachandran, Charles Blanluet, Diego Huyke, Alexandre Avaro, *Stanford University,*

Molecular diagnostics based on clustered regularly interspaced short palindromic repeats (CRISPR) enzyme systems have been the subject of intense, recent research and development. CRISPR-associated (Cas) enzyme assays are easily reconfigurable to different nucleic acid targets and highly specific. We are conducting studies of the basic CRISPR enzyme kinetics, and also studying on-chip CRISPR reactions controlled with electric fields. We discovered that the great majority of all CRISPR enzyme kinetics studies show data that grossly violate basic rules of mass conservation and rate laws. Following up on this, we quantified the kinetics of a range of CRISPR-Cas systems and demonstrated how these kinetics fundamental limit detection sensitivity. We performed a study of CRISPR specificity to small mutations, including single-nucleotide polymorphisms. We will also report on our ongoing development of microfluidics processes for rapid and automated CRISPR assays using on-chip isotachophoresis (ITP). ITP is an electrokinetic technique that can selectively and simultaneously purify, mix, and preconcentrate target species using shockwaves of ion concentration within microchannels. We use ITP for electric field control of CRISPR reactions to achieve low sample use and accelerated reaction rates.

(AES-07.2)**Contactless Dielectnrophoresis: History and Future Directions**

Rafael Davalos, Josie Ducan, *Virginia Tech*

Along with the benefits of traditional dielectrophoresis (DEP) modalities, Contactless Dielectrophoresis (cDEP) was invented to capitalize on attributes such as high-selectivity, sample sterility and viability, reduced effects due to joule heating, and inexpensive devices. A variation of insulator-based DEP, cDEP uses liquid electrodes separated from the sample channel with a thin membrane to generate a “contactless” field within the sample channel. The sample channel has cell-sized insulating structures for heightened sensitivity and device integrity. These posts allow for high-throughput batch collections of sorted populations of microfluidic samples. Further, the device is fabricated entirely from a polymer and does not require metal deposition.

Since it’s invention, contactless dielectrophoresis has been used in a myriad of applications from sterile sample clean-up to highly sensitive separations of cancer subpopulations. cDEP has been effective in separating glioblastoma cells from their stem-like counterparts with higher metastatic potential, explore the roles of cancer-associated macrophages and fibroblasts in the tumor microenvironment, and distinguish between stages of mouse ovarian cancer cell lines.

Along with its growing applications, the cDEP device design has also evolved. The original device design was a tapered channel where cells were characterized around the inlet and has been optimized in its newest generation as a triple-layer device with over 20,000 high-aspect ratio pillars designed

specifically for individual cell trapping. Design changes from over the past 15 has yielded tailored geometries and electric field distributions for selective batch separations.
The history of cDEP sits on its own precedence and the outlook for the future of this technology is ever-growing. In the laboratory, we are investigating the next generation of cDEP that allows for continuous separation and further high-sensitivity detection and separations for oncological precision medicine. Beyond the laboratory, technology has been licensed to a Blacksburg-based startup company to place this technology in the hands of those who will benefit the most.

(AES-07.3)**A History of Dielectrophoresis for Cell Characterization and Sorting**

Lisa Flanagan, *University of California, Irvine*

Dielectrophoresis (DEP) has a rich history, and its use in cell characterization and separation has improved our understanding of cell biology and expanded the application of electrokinetic technologies to complex particles. From the first article by Herbert Pohl describing DEP in 1951, and followed by his landmark book in 1978, DEP research, technology development, and implementation have grown exponentially. In particular, the utilization of DEP to study cells has flourished. A wide variety of cell types have been analyzed by DEP, including plant cells, bacteria, yeast, and a diverse array of mammalian cells. This retrospective will focus on mammalian cells, highlighting the biological and medical applications furthered by DEP and the evolution of DEP-based devices used to analyze and separate cells. Looking to the next 50 years, the data collected with DEP-based platforms will likely continue to transform biology and human health. For example, DEP could play a significant role in the analysis of sub-cellular particles, such as extracellular vesicles and exosomes, and begin to link individual cell electrophysiological properties to data obtained with exciting single cell analysis strategies such as single cell RNA sequencing. DEP has already revealed new insights in cell biology and the study of disease; it will be exciting to see what the future holds.

(AES-07.4)**Understanding Nonlinear Electrophoretic Effects in Microfluidic Devices**

Blanca H. Lapizco-Encinas, *University of California, Irvine*

Recently, there as a significant surge in the study of nonlinear electrophoresis and its application in microfluidic devices. As described by Khair [1], while the last century was marked by significant developments in the field of linear electrophoresis; the 21st century has witnessed major advances in the realm of nonlinear electrophoresis.

This presentation is focused on the recent developments and new understanding of nonlinear electrophoresis. As an electrokinetic phenomenon, nonlinear electrophoresis can be applied for the manipulation of a wide array of bioparticles, from nanoparticles (macromolecules, viruses) to microparticles (bacterial, yeast, mammalian cells). In particular, we are interested in understanding how particle characteristics (size, shape, electrical charge) affect particle mobility under nonlinear electrophoretic migration.

In contrast with its linear counterpart, the mobility of nonlinear electrophoresis is not independent of the electric field magnitude, which adds an extra layer of complexity to the understanding and application of this nonlinear phenomenon. Included in this presentation are our latest results on the characterization of the nonlinear electrophoretic mobility of polystyrene beads and cells. We will analyze the effect of particle/cell properties on their nonlinear electrophoretic mobility and demonstrate that good agreement is obtained between modeling and experimental results by including nonlinear electrophoresis effects.

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(AES-07.5)**Women in Electrokinetics**

Erin Henslee, Tayloria N.G. Adams, *Wake Forest University, University of California Irvine*

Since its discovery in the early 1800's, electrophoretic technologies have played a crucial role in scientific investigations across clinical, basic, and applied disciplines. The advancement in fabrication, computing, and microscopy has enabled a boom in research output over the last 50 years across fields from life sciences, chemistry, physics, to engineering. For example, a keyword search in Web of Science from 1970-2023 for "electrokinetics" resulted in over 2,000 research articles (filtered to remove review articles) with over 90% of those articles published since the year 2000. Since this boom in 2000, women have either been corresponding authors, lead author, or both and have contributed the top cited research articles on Web of Science with the key word "electrokinetics" (C.N. Mulligan, 2001; M. Gavrilescu, 2009). Women are leaders in the total number of research publications in areas such as "dielectrophoresis" (B.H. Lapizco-encinas, F.H. Labeed, A Ros), and have been recognized with numerous AES awards including lifetime achievement, mid-career, and service awards. In celebration of SciX's 50th anniversary and AES Electrophoresis Society's recent 40th anniversary (2021), we will focus this talk on the contributions of women in the fields of electrokinetics and look ahead to the next fifty years and the work to be done to support current and future women leaders in these fields of study.

23ATOM07: Early Career in Atomic Spectroscopy, Central Pacific A/B/C
Chair: Benjamin Manard

(ATOM-07.1)**Matrix-Matched Calibration Approaches for Quantitative Mapping of Various C-based Samples Using LA-ICP-MS**

Ana Lores Padin, Ir. Thibaut Van Acker, Beatriz Fernández, Ana Rua Ibarz, Rosario Pereiro, Frank Vanhaecke, *Ghent University, University of Oviedo*

Laser ablation in combination with inductively coupled plasma-mass spectrometry (LA-ICP-MS) has become an established micro-analytical technique for direct elemental mapping of a broad range of solid samples in different research fields, such as geology, environmental studies and life sciences. However, despite its promising features, e.g., multi-elemental analysis with a wide linear dynamic range up to 10 orders of magnitude, high spatial resolution down to the low μm level, minimal sample preparation and access to isotopic information, matrix effects and elemental fractionation pose a challenge to reliable quantification and in turn limit the robustness as a reference technique for imaging .
To cope with these challenges, external calibration based on matrix-matched certified reference materials (CRMs) is an adequate approach for LA-ICP-MS quantification. Nevertheless, the lack of CRMs for most sample types (especially those of environmental or biological origin) compromises its applicability. Thus, custom laboratory prepared standards using the same matrix as the sample (or as similar as possible) provide an alternative to overcome such limitations.
In this context, different LA-ICP-MS quantification strategies based on the use of matrix-matched calibration standards and their applicability for samples of diverse nature (biological tissue, carbonates and polymers) will be presented. Firstly, two approaches are introduced for analysis of biological matrices: the use of gelatin-based calibration standards and a more novel approach employing the same cell line as the sample to create cellular standards of which the composition completely matches that of the matrix . Both methods enable quantitative bio-imaging of proteins (after being tagged with metal-immunoprobes) along ocular tissues and human epithelial cells, respectively. Secondly, new homogeneous nano-particulate pressed pellets are currently being characterized and their use as an alternative to the NIST SRM 61X series glasses for analysis of biogenic carbonate matrices (e.g., otoliths and mussel shells) will be evaluated. Additionally, the challenges encountered in finding standards with a homogeneous elemental distribution to perform quantitative imaging of polymer samples will also be addressed in this presentation.

(ATOM-07.2)**An Early Career Perspective on Laser Ablation Plasma Spectroscopy for Nuclear Security Applications**

Kyle Hartig, Emily Kwapis, Kyle Latty, Justin Borrero, *Univeristy Of Florida*

Rapid, in-field, and standoff analysis of radiological materials is extremely important to nuclear nonproliferation monitoring and forensics applications. Rapid, in-field, and standoff analysis of solid and aerosol materials is possible with optical spectroscopy tools when combined with laser ablation; however, applying optical spectroscopy to the measurement of radiological materials presents numerous challenges.

Improvements in spectroscopic techniques have allowed for measurement of radiological materials and isotope ratios to be carried out at standoff distances under ambient atmospheric conditions, which has expanded the applicability of laser ablation-based optical spectroscopy techniques to a variety of scientific fields. These technological advances offer an in-situ measurement capability that was previously not available for radiological materials and isotope ratio analysis.

This talk will focus on radiological material detection and characterization through emission, absorption, and fluorescence spectroscopy of atoms and molecules in laser-produced plasmas as well as the use of laser plasmas as surrogates for detonation events. A careful review of the last decade of advancements in the analysis of nuclear materials using laser ablation-based techniques, which has seen an exponential increase in the number of articles appearing in the literature, will be presented. Perspectives on the technological gaps identified in the available literature and suggestions for future work will be provided in the context of a transitioning early career researcher to an established expert in this field.

This work is supported in part by the NNSA Defense Nuclear Nonproliferation Research and Development Monitoring Technology and Verification (MTV) Consortium and the DTRA Interaction of Ionizing Radiation with Matter (IIRM) University Research Alliance.

(ATOM-07.3)**Small Particles, Big Challenges: Comprehensive Multi-Elemental Analysis Of Discrete Samples Using ICP-TOFMS**

Lyndsey Hendriks, *TOFWERK*

Today single-particle ICP-MS (sp-ICP-MS) is a well-established method for the analysis of nanoparticles (NPs) in the scientific community. However, despite recent technological developments, the information provided by sequential mass analyzer is limited as they can only provide information for one (or maximum two) isotopes over the short transient signal produced by a single NP. This incomplete picture is disadvantageous for many applications. For example, when investigating the question whether the NPs detected are of natural or anthropogenic origin, only the determination of the intrinsic elemental "fingerprint" of NPs can answer the question. Hence, to overcome the limitations of sequential ICP-MS instruments for short transient analysis, a clear solution is to employ simultaneous full-spectrum mass analyzers such as time-of-flight mass spectrometers (TOFMS).

More specifically, I will describe how high-speed simultaneous multi-elemental detection with ICP-TOFMS can be employed for the detection and characterization of different discrete mass-limited samples, and address different challenges associated with quantification in complex matrices and accurate thresholding for robust NP identification. The potential of ICP-TOFMS for short lived transients will be highlighted through different case studies, involving nanoparticles, single cells and nanoplastics.

(ATOM-07.4)**Utilization of atomic spectroscopy at National Labs – an early career's perspective**

Benjamin Manard, *Oak Ridge National Laboratory*

Generally speaking, national laboratories approach scientific challenges across various arenas including national security, basic energy science, space exploration, and many more. These laboratories are meant to serve as institutes fostering personnel, technology, and infrastructure to tackle such scientific endeavors. Regarding atomic spectroscopy developments, one should consider the pioneers of such techniques including inductively coupled plasma (ICP) and laser induced breakdown spectroscopy (LIBS), and their respective national laboratory affiliations. Aside from instrumentation development, atomic spectroscopy is used routinely for various mission spaces including space exploration, national security, isotope production / discovery, etc. The talk presented here will deliver an insight to these arenas and describe how the national lab system has shaped my career as detail some of our recent research advancements in atomic spectroscopy at Oak Ridge National Laboratory

(ATOM-07.5)**Building your own fs/ns-LIBS system - from desperation to success and everything in between**

Cristina Méndez-lópez, Luis Javier Fernández-Menéndez, Cristina González-Gago, Jorge Pisonero, Nerea Bordel, *University Of Oviedo,*

Starting an academic career is never easy. If you also have to set up your experimental system from scratch, the difficulties multiply.

My enthusiasm for the LIBS technique goes back to my undergraduate days, when I had the opportunity to use it in my BSc thesis. At the time, I experienced first-hand one of its quintessential advantages: simplicity. A pulsed laser, a few lenses, a spectrometer and a detector, and you have a world of applications. But, what if instead of one laser you have two, one of them a femtosecond. And also (lucky you), a nearly empty optical table and the backing of a research group. Well, it turns out that setting up a dual-pulse LIBS system is more complicated than it sounds. You learn a lot in the process. You make mistakes. You fix them. Others pop up. And, of course, by the time you figure it out, the simplicity of the experiment is gone and you don't expect it back any time soon.

In this talk I will discuss what it is like to develop, on par and from scratch, a research career and a double-pulse LIBS system and, above all, highlight the work that we carry out as PhD students which is not published in papers but without which we could have not published any.

23AWD06: NESAS and SAS Lester W. Strock Award Symposium Honoring Maria Montes-Bayón, Cascade 1
Chair: Maria Montes-Bayón

(AWD-06.1)**A Personal Retrospective on the Analysis of Small Objects: Nanoparticles and Individual Cells**

Jörg Bettmer, *University Of Oviedo*

The early developments on the detection of single nanoparticles and individual cells using inductively coupled plasma-mass spectrometry (ICP-MS) [1, 2] have evolved the broad interest of the scientific community. Due to significant improvements of achievable instrumental sensitivity, a vast number of ICP-MS applications have represented a step forward to explore the elemental composition and characteristics of small objects like metallic nanomaterials and biological cells.
The journey of our laboratory working on the analysis of nanoparticles started in 2006 with the development of hyphenated techniques with ICP-MS [3]. This proof-of-concept study allowed the detection of gold nanoparticles much smaller than 10 nm, and later developments opened the applicability to other particle systems like silver, and iron oxide nanostructures. Its combination with single particle/cell ICP-MS also revealed the characterization of biogenic selenium nanoparticles synthesized by various organisms like yeast and fungi [4, 5]. Further examples of recent developments on the analysis of individual spores of bacteria will complete this presentation.

In summary, this work should highlight recent studies on ICP-MS detection of nanomaterials and single cells carried out in our laboratory in honour of Professor María Montes Bayón, recipient of the Lester W. Strock Award 2023.

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(AWD-06.2)**Exploration Of A Nitrogen Plasma For The Analysis Of Laser-Generated Aerosols**

Detlef Günther, Dylan Kaeser, Bodo Hattendorf, Joachim Koch, *Department Of Chemistry And Applied Biosciences*

Laser ablation-inductively coupled plasma mass spectrometry has become one of the most attractive techniques for direct solid analyses of trace elements and isotope ratios. Many successful applications have been reported. The major success of this technique is based on efficient aerosol transport and vaporization of the laser generated aerosols. Since many decades, helium mixed with argon prior to the introduction into the plasma, is the carrier gas of choice. Most recently, a prototype nitrogen plasma has been coupled to a mass spectrometer, which allows to use different carrier gasses for aerosol transport. We investigated a variety of gas combinations and measured the particle size distributions. Some of these results will be reported and their pros and cons for laser ablation-inductively coupled plasma mass spectrometry will be discussed.

(AWD-06.3)**Microwave-Enabled Ionization and Chemistries for Mass Spectrometry Analysis**

Steven Ray, *SUNY Buffalo Dept of Chemistry*

All mass spectrometry (MS) measurements require formation of a gas-phase ion in order for any subsequent analytical measurement, and as a consequence the ionization source is often termed ‘the bridge’ between the sample and the MS analysis. As a consequence, a large variety of ionization sources exist that exploit a number of chemical and physical mechanisms in order to form ions from the (mostly) neutral species found in samples. The mechanisms of ionization vary a great deal among the sources, however, in most instances ionization rate is optimized so as to increase the signal observed in MS. In recently studies, our laboratory has been researching the efficacy of microwave energy as a means of increasing these rates of ionization. Highly focused microwave fields have a number of potential mechanisms by which the critical mechanisms of ionization chemistries might be modulated. Microwave fields can be used to deposit thermal energy into ionization sources by dielectric heating, or by charged-ion interaction. In contrast to conventional convective heat transfer, microwave energy also influences different materials to much different extents, allowing energy to be deposited in a particular material type in preference to other materials. Microwave fields can also be focused into very low volumes using relatively uncomplicated electronics principles, allowing for very high power density that can be easily modulated. In this presentation, we will present several examples of the use of microwave fields to modulation ionization conditions for atomic and molecular ionization sources, and compare results to conventional ionization strategies such as electrospray ionization, atmospheric-pressure chemical ionization, and microwave induced plasma systems. We will also investigate the use of microwave energy to modulate and ‘speed up’ chemical reactions in the condensed phase, and explore the ways that microwave-modulated chemistry can be used to modify samples before ionization for mass spectrometry.

(AWD-06.4)**Analysis of unconventional elements via ICP-MS: Targeting Carbon and Fluorine**

Raquel Gonzalez De Vega, Jörg Feldmann, David Clases, *University Of Graz*

Since its commercial introduction 40 years ago, inductively coupled plasma-mass spectrometry (ICP-MS) has evolved to the most powerful detection technique for the analysis of trace and ultra-trace elements which relates to its high sensitivity paired with a vast linear dynamic range and methodic versatility. However, when targeting nonmetals by ICP-MS, some limitations arise due to the high ionization potential of these elements resulting in low ionization rates. Carbon and Fluorine are two examples for elements which are traditionally very difficult to target via ICP-MS and often omitted from analysis. However, recent advances have shifted the perspectives for the analysis of these elements.
In this work, we will showcase how Carbon and Fluorine can be targeted in ICP-MS. Specifically, we will present adapted and new methodologies for LA-ICP-MS, SP ICP-MS and HPLC-ICP-MS and applications for the determination of these two elements in different matrices.

(AWD-06.5)**The Role of Data Analysis in Analytical Chemistry**

Juris Meija, *NRC*

In 1786, Immanuel Kant denied chemistry the status of a proper science due to its experimental nature and lack of mathematical involvement. Contemporary analytical chemists now dedicate substantial time to interpret their data. An example of this shift is seen from the fact that one of the most cited articles of all time in the journal Analytical Chemistry focuses on data smoothing using least squares methods. The challenge for the analytical chemists is therefore to recognize data analysis practices as a natural part of the measurement and this talk will provide examples from modern analytical chemistry practice to illustrate the beneficial interactions between chemists, mathematicians, and statisticians that lead to more reliable measurements – from interpreting titration results in order to get precise concentration measurement, evaluating isotope patterns of a substance to obtain most likely molecular formula, or getting the most precise estimate for the Avogadro constant.

23AWD07: AES Mid-Career Achievement Award Symposium Honoring Robbyn Anand, Sierra 5

Chair: Robbyn Anand

(AWD-07.1)**Simplified Valve Control and Droplet Merging System for Sampling and Multiplexed Secretion Assays from Ex Vivo Adipose Tissue**

Christopher Easley, Andresa Bezerra, Md Moniruzzaman, Sabita Dangol, *Auburn University,*

The scale of microfluidic devices is well-matched to native flow conditions around cells and tissues in vivo. Several groups have used microfluidic sampling of ex vivo endocrine tissues for precise and sensitive analysis under continuous flow. However, most of these systems have been limited to temporal resolutions in the 2-10 minute range. Our group has used droplet-based microfluidics for rapid sampling of islet and adipose tissues with integrated on-chip assays to achieve sampling every few seconds, and the latest valve-controlled devices have been termed as microfluidic analog-to-digital converters (μADCs). Our μADC devices have demonstrated temporal sampling resolution (Δt) as low as 3.5 seconds, and they have recently revealed new biological information on the dynamics of lipolysis in adipose tissue.

Previous generations of these μADC systems were stationary instruments controlled by LabVIEW, with significant software and hardware expense, lack of portability, inflexibility, and complexity of programming. In this work, we discuss a new valve-based pump operated by an Arduino Mega 2560 board with open-source software. An new hardware controller includes 16 solenoid valves and can operate with both vacuum or pressurized air for normally-closed or normally-open valves. By analyzing normally-open valves in PDMS microfluidic devices with microscopy and video analysis (ImageJ), we confirmed the programmed valve timing during pumping and droplet formation. Droplet merging with a high frequency, AC voltage supply, was also updated with a hardware controller

(LM555 timer circuit), allowing a frequency range of 0.6 to 10.5 kHz and a voltage range from 100 to 1400 V for droplet merging. Merging was optimized to avoid excessive dielectrophoretic forces and droplet distortion, and a custom grounding electrode was used to shield the non-merging parts of the device.

Overall, we have developed a less expensive, portable, flexible, and simpler system to control droplet-based μADC devices, and we have applied this system to high resolution temporal sampling of adipose tissue during pharmacological modification of lipolytic process. This system should be suitable to control not only devices already well-established in our laboratory but also to control future devices for tissue-on-a-chip analysis, nucleic acid assays, on-chip assay calibrations, and various other applications.

(AWD-07.2)**Computationally Modeling Electrokinetic Phenomena: Complex Geometries and Small Electric Double Layers**

Baskar Ganapathysubramanian, *Iowa State University*

Electrokinetic phenomena are represented by the Poisson-Nernst-Planck (PNP) equations coupled with the Navier-Stokes (NS) equation. Direct numerical simulation (DNS) to accurately capture the spatio-temporal variation of ion concentration and current flux remains challenging due to the (a) small critical dimension of the electric double layer (EDL), (b) stiff coupling, large advective effects, and steep gradients close to boundaries, and (c) complex geometries exhibited by electrochemical devices. We address these challenges by developing a direct numerical simulation framework that incorporates: (a) a variational multiscale (VMS) treatment, (b) a block-iterative strategy in conjunction with semi-implicit (for NS) and implicit (for PNP) time integrators, and (c) octree based adaptive mesh refinement. The VMS formulation provides numerical stabilization critical for capturing the electro-convective instabilities often observed in engineered devices. The block-iterative strategy decouples the difficulty of non-linear coupling between the NS and PNP equations and allows using tailored numerical schemes separately for NS and PNP equations. The carefully designed second-order, hybrid implicit methods circumvent the harsh timestep requirements of explicit time steppers, thus enabling simulations over longer time horizons. Finally, the octree-based meshing allows efficient and targeted spatial resolution of the EDL. These features are incorporated into a massively parallel computational framework, enabling the simulation of realistic engineering electrochemical devices. The numerical framework is illustrated using several challenging canonical examples. This is joint work with Ali Mani (Stanford) and Robbyn Anand (Iowa State)

(AWD-07.3)**Increasing Sensitivity and Selectivity of miRNA Analyses with Thermal Gel Electrophoresis**

Thomas Linz, Mario Cornejo, *Wayne State University*

Gel electrophoresis is a common technique used for quality control validations of nucleic acid samples. However, miRNAs cannot be separated by gel electrophoresis because they all possess similar sequence lengths (~22 nts). The work presented here describes the development of a sensitive microfluidic platform to selectively analyze miRNAs using thermal gel electrophoresis (TGE). miRNAs and fluorescent probes were spiked into thermal gel and loaded into single-channel microfluidic devices. An injectionless analysis was developed that coupled inline analyte enrichment with an electrophoretic separation to quantify each target miRNA with high sensitivity. This innovative performance was then enhanced by incorporating a tapered design into the microfluidic channel. Separation resolution and limits of detection were further improved two-fold and ten-fold, respectively, using this elegantly simple approach. This method enabled the detection of miRNAs from cell lysates. To enhance selectivity of the analysis, a subsequent method was developed using high temperature (50 °C) to dissociate off-target miRNA-probe hybrids and only detect on-target species. This approach enabled multiple target miRNAs to be selectively quantified even in the presence of structurally similar interfering miRNAs. In summary, TGE provides a simple low-cost means of analyzing miRNAs with high sensitivity and selectivity to facilitate biomedical research.

(AWD-07.4)**Characterizing micro- and nanoplastics in human body fluids with dielectrophoresis**

Alexandra Ros, Timothy Long, Shulin Bu, *Arizona State University*

Microplastics have been recognized to pose a major environmental threat. They have been detected in aquatic and terrestrial systems in a large variety including polymers such as polyethylene terephthalate (PET), polystyrene, polycarbonate, polyethylene, polymethyl methacrylate (PMMA) and others. The size of microplastics ranges from mm-sized objects down to nm-sized particles and they are observed in various shapes including fibers. Recent studies indicate that animals, including aquatic sea life but also mammals ingest or otherwise are exposed to micro- and nanoplastics. It is thus not astonishing that micro- and nanoplastics have recently also been found in human body fluids such as blood. However, their impact on human health is little understood. To fill the knowledge gap on micro- and nanoplastic characterization within body fluids, we characterized PET and PMMA micro- and nanoparticles using dielectrophoresis at low frequency conditions and compare our findings with theory on double layer conductance. In addition, we established conditions mimicking blood constituent interactions with micro- and nano-plastics by treating the particles matching concentrations of high abundant proteins in blood, such as albumins. We observed unique behavior in dielectrophoretic trapping and will discuss how they could be used for future diagnostics of micro-and nanoplastics in body fluids.

23BIM07: Biomedical Spectroscopy and Imaging (CLIRSPEC), Sierra 2
Chair: Nike Stone
Co-Chair: Olga Eremina

(BIM-07.1) **A Resurgence in Nanoparticles: A Path Toward the Clinical Translation of SERS-based Imaging Contrast Agents**

Cristina Zavaleta, *University Of Southern California*

With the recent approval of the Moderna and Pfizer liposomal vaccines for COVID-19, we have witnessed a resurgence in the utility of nanoparticles in the clinical setting. Although some therapeutic-based nanoparticles have been clinically approved for decades, their clinical utility remained somewhat limited. Now that we have had the opportunity to appreciate the effectiveness of nano-based vaccines in a large population of humans, we should reevaluate how to exploit the many advantages that come from utilizing nanoparticles. Despite their use as therapeutic delivery agents in the clinic, nanoparticles have yet to see much progress in clinical translation as diagnostic imaging agents. Several clinical and preclinical studies support their use as imaging contrast agents, but their use in the clinical setting has been limited to off-label imaging procedures (e.g., Feraheme). In this talk, I will discuss the potential hindrances toward their clinical translation and alternative strategies we are taking to accelerate their utility in the clinic. I will also highlight the ongoing research in our lab that exploits the unsurpassed multiplexing capabilities of Raman imaging with SERS nanoparticles. With the recent success of nano-based vaccines, now is the ideal time to reimagine other clinical applications for nanoparticles to improve human health.

(BIM-07.2)**Cutting-Edge Biomarkers Discovery for Early Diagnosis of Central Sensitivity Syndromes Using Surface-Enhanced Raman Spectroscopy (SERS) and FTIR-Microscope**

Haona Bao, Siyu Yao, Silvia De Lamo Castellvi, Chelsea Goetzman, Zachary Schultz, Luis Rodriguez-saona, *The Ohio State University, Savannah River National Lab*

Fibromyalgia syndrome (FM) as one of the most prevalent central sensitivity syndromes which affects approximately 15 million individuals in the United States, continues to present significant diagnostic challenges. Additionally, the rheumatologic disorders like rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), and osteoarthritis (OA) further complicates the diagnosis and treatment process due to overlapping symptoms and psychosocial features with FM. This study aims to identify blood

serum-based biomarkers for FM and distinguish FM subjects (n=90) from individuals with other conditions (OA, n=30; RA, n=30; SLE, n=30) using vibrational spectroscopy technology, specifically Surface-enhanced Raman spectroscopy (SERS) and FTIR Microscope, coupled with supervised pattern recognition analysis. Low molecular fractions of blood samples were extracted by using a semi-permeable membrane (10 K) through centrifugal ultrafiltration. For SERS analysis, 10 μL of serum was mixed with 10 μL of 50 nm gold nanoparticles (AuNPs) and analyzed in a liquid form by a confocal Raman microscope with an excitation 633nm laser (acquisition time 10s, laser intensity 10%). On the other hand, 1 μL serum extract was placed on a microscope slide, future dried by a vacuum drying process, and analyzed by a FT-IR microscope (800-4000 cm-1, 64 coscans, ATR Ge crystal). Orthogonal Partial Least Squares Discriminant Analysis (OPLS-DA) was used to discriminate between FM and other rheumatologic disorders (RA, SLE & OA) through unique Raman and IR spectral fingerprinting signatures. Our OPLS-DA algorithms demonstrated clear clusters between FM subjects and other disease classes, with excellent sensitivity and specificity. Amide bands and aromatic ring structures, predominant in the regression vectors, may serve as candidate biomarkers for diagnosing FM syndromes. The novel aspect of this research is the discovery of reliable biomarkers for FM using SERS Raman and IR microscopy, which could lead towards early diagnosis of central sensitivity syndromes, ultimately reducing the burden on patients, their families, and society.

(BIM-07.3)**Characterization of Vaginal Fluid and Vaginal Lactobacillus using Raman Spectroscopy and Surface-Enhanced Raman Spectroscopy**

Anna Rourke-Funderburg, Andrea Locke, *Vanderbilt University*

Lactobacillus species are the main constituents of the vaginal microbiome and aid in inhibiting pathogenic growth. Decreases in Lactobacillus can lead to vaginal dysbiosis which can result in an ascending infection that can threaten the health of a pregnancy. Despite the importance of vaginal health during pregnancy, routine monitoring of this environment is not typically performed, and current methods are lacking. Raman spectroscopy (RS) is advantageous to fill this need due to the biochemical sensitivity this technique poses. Further, surface-enhanced Raman spectroscopy (SERS) can increase the usability of RS for dilute samples. In this study, we hypothesize that RS and SERS can detect distinct biochemical differences between Lactobacillus crispatus (L. crispatus) and Lactobacillus iners (L. iners), two of the dominant species in the vaginal microbiome, and further provide discrimination from vaginal fluid (VF). Liquid cultures of L. crispatus and L. iners were washed with deionized water and allowed to dry on tin foil for collection of RS spectra using the Renishaw inVia Raman microscope at 785 nm. VF was eluted from swabs by centrifugation and allowed to dry on tin foil for RS spectral collection. SERS spectra were collected by mixing each solution with gold nanoparticles and allowed to dry on tin foil. Principal component analysis (PCA) identified key RS differences in nucleic acid and protein content between L. crispatus and L. iners. Furthermore, sparse multinomial logistic regression (SMLR) discriminated between the two species with 100% accuracy and also highlighted variations in nucleic acid content between the species. Raman spectra of VF were analyzed against the two Lactobacillus spectra. PCA identified nucleic acid and protein content variations between the two bacteria and VF, and key markers only seen in the VF relating to acetic acid, lactic acid, and urea. SMLR discriminated the three groups with 100% accuracy. Finally, SERS of L. crispatus and L. iners revealed distinct signatures of each bacterium, including a reduction in the 732 cm-1 peak, representing the bacterial cell wall, in the L. iners spectra. In summary, RS and SERS results have demonstrated distinct variations in the biochemical makeup of VF, L. crispatus, and L. iners.

(BIM-07.4)**Deep UV Raman spectroscopy for probing active eukaryotic viruses**

Denis Rajnovic, Fatima Matroodi, Igor Lednev, Lamyaa Almehmadi, Barbara Rossi, Claudio Masciovecchio, Alessandro Marcello, *Elettra-sincrotrone And ICGEB, University at Albany, SUNY*

Deep Ultraviolet Resonance Raman Spectroscopy (DUVRR) is an emerging and powerful analytical tool used for detection and characterization of biological samples in a label-free and real time approach. The excitation in the Deep UV provides specific resonance enhancement of biological moieties, especially protein, DNA and RNA structures, allowing to efficiently detect biological Raman

markers in the spectra. It is well known that DUV radiation is a genotoxic agent. Prolonged UV light exposition induces damage to the genomes of viruses, breaking bonds and forming photodimeric lesions in RNA. These damages prevent both transcription and replication which leads to viral inactivation. Even though the effects of UV radiation on DNA of microorganisms have been well-recorded, its impact on RNA and RNA modifications is less known. A sample that is prone to such a damage are viruses, and with the SARS-COV-2 (RNA virus) outbreak, the need of fast tools for viral characterization and classification became even more necessary.
In this work, we will show how to obtain stable, high quality and information-rich DUVRR spectra of active Vesicular Stomatitis Virus (VSV) without affecting its viability, RNA and protein integrity. By opportunely tuning the excitation wavelength, we can detect and assign in the vibrational spectra specific markers of protein and RNA components useful to elucidate biochemical characteristics of active VSV virus. In addition, we can study the effect of DUV irradiation in the mechanism of inactivation of VSV viruses in order to find the most damaging wavelength for the active VSV virus. This information can further be exploited to build an effective countermeasure tool for virus deactivation.

(BIM-07.5)**Pharmacokinetic and Pharmacodynamic Tomography with Coherent Raman Imaging**

Dandan Tu, Conor Evans, *Massachusetts General Hospital, Wellman Center For Photomedicine, Mass. General Hospital, Harvard Medical School*

Coherent Raman scattering (CRS) imaging tools provide a label-free means to visualize specific molecules within tissue, making CRS methods well suited for solving challenges in drug development. We will present our efforts to leverage CRS tools to not only visualize the uptake of drugs within skin tissue, but also precisely quantify both pharmacokinetics and pharmacodynamics. We leverage deep learning methods, specifically the use of convoluted neural networks, for accurate feature extraction to measure pharmacokinetics across numerous different skin compartments. Using single and multicomponent compartment models, we can analyze the CRS-derived data to calculate traditional PK parameters such as Tmax and Cmax on both the micro- and macroscale. These approaches have been validated and now tested on multiple drugs in a range of formulations, demonstrating how CRS imaging tools can reveal new, previously-inaccessible PK and PD information. This research seeks to link the microscale information gained via CRS microscopy to the macroscale measurements made in traditional PK/PD labs to understand drug flow, the impact of structure and formulation on diffusion, and how these parameters relate to drug efficacy.

23CHEM05: Industrial/PAT Applications of Chemometrics, Southern Pacific E
Chair: Brandye Smith-Goettler

(CHEM-05.1)**Comparison Of 3-Way And 2-Way Multivariate Models For The Assessment Of Data Generated By Multidimensional Fluorescence A-TEEM Method**

Brad Swarbick, *KAX Group Pty Ltd*

Multivariate data analysis (MVDA) techniques are important tools for extracting important information from complex spectroscopic data. Many spectroscopic instruments generate data in a 2-way format, typically absorbance (or counts) vs. wavelength, generating a single array of data for each object measured. The acquistion of many objects leads to a matrix of objects vs. varaibles that can be analyzed and interpreted using methods such as principal component analysis (PCA) and partial least squares regression (PLSR).

Data generated by the A-TEEM (Excitation Emission Matrix (EEM)) system generates 3-way data per object in the form of an emission/excitation matrix of count data. The acquistion of a number of EEMs leads to the generation of a 'data cube' that cannot be readily analyzed using normal 2-way MVDA methods, but requires the use of advanced methods suited to 3-way data structures, such as parallel factor analysis (PARAFAC).

3-Way data can be matricized (sometimes incorrectly termed 'unfolded' into a matrix of data) in a manner that it is amenable to 2-Way analysis, however, the natural structure of the data may be lost during matricization, therefore, adding induced complexity and dependencies not present in the original 3-way structure.

This presentation describes the application of 3-way and 2-way data analytical methods to A-TEEM EEM data, first using a controlled data set and then a typical data set, showing how methods such as PARAFAC maintain the natural structure of the 3-way data after analysis. A comparison is then made to the application of 2-way analysis methods to the matricized 3-way data.

This, by no means, limits or precludes the application of 2-way analysis methods to matricized 3-way data, however, it highlights the importance of awareness on behalf of the data analyst to the generation of artefacts and dependencies, which may be introduced when such methods are applied to data sets that are more naturally suited to 3-way analysis.

(CHEM-05.2)**External Variable Augmented Iterative Optimization Technology (EVA-IOT): A Minimal Calibration Robust Modeling Approach to Monitor Continuous Pharmaceutical Powder Streams**

Natasha Velez-Silva, Adam Rish, Carl Anderson, James K. Drennen, III, *Duquesne University,*

Near-infrared (NIR) spectroscopy has been widely recognized as a powerful process analytical technology (PAT) for monitoring chemical composition of powder streams in continuous pharmaceutical processes. PAT methods using NIR employ multivariate models to translate spectroscopic signals into a response of interest. The implementation of pure component models, such as iterative optimization technology (IOT) algorithms, is gaining momentum within the industry, largely due to minimal calibration requirements. While IOT methods have recently demonstrated great potential for monitoring the quality of powder mixtures by NIR, the dynamic conditions of continuous manufacturing processes may limit the effectiveness of such approaches. The density variation introduced to NIR spectra that are collected from dynamic powder samples at different flow rates is detrimental to the prediction performance and robustness of IOT methods. This work presents a new approach, named external variable augmented iterative optimization technology (EVA-IOT), for enhancing the prediction accuracy and robustness of IOT methods based on augmenting the pure component matrix with the influential shape of non-chemical external sources of variability. This method derives the shape of non-chemical external variables from the latent structure of decomposition methods, such as unsupervised principal component analysis (PCA) and supervised partial least squares (PLS) regression, using NIR spectra from a single mixture collected at known levels of the external parameter.
Unsupervised and supervised EVA-IOT were applied to predict drug content from NIR measurements performed on dynamic powder streams of varying flow rates. NIR spectra collected at a constant chemical composition and variable flow rate/density were used to derive the spectral contributions of density in an unsupervised and supervised manner by PCA and PLS regression, respectively. The prediction performance of EVA-IOT on a test set with chemical content and flow rate variability was compared to base IOT and a global NIR-PLS model incorporating flow rate variability. The EVA-IOT methods accounting for density variation demonstrated superior prediction performance and flow rate robustness over base IOT. Supervised EVA-IOT achieved comparable prediction robustness to the global NIR-PLS model, while reducing the material burden by 86%, which makes the proposed method an attractive alternative over traditional extensive robust modeling approaches.

(CHEM-05.3)**Demonstration of Novel Model Diagnostic Based on Net Analyte Signal for Iterative Optimization Technology Algorithms**

Adam Rish, Natasha Velez-Silva, Samuel Henson, Md. Nahid Hasan, James Drennen, Carl Anderson, *Duquesne University*

The use of calibration-free or minimal calibration approaches has garnered increased interest as the application of spectroscopic process analytical technologies (PAT) has expanded in the pharmaceutical industry. Pure component modelling approaches such as iterative optimization technology (IOT), which require only pure component spectra and no mixture samples as model inputs, are desirable to achieve calibration-free or minimal calibration modeling. A key consideration for model deployment with PAT is appropriate model diagnostics which establish confidence in predictions without the use of reference data and identify unusual predictions. The industrial standard partial least squares (PLS) regression models use the well-established diagnostics Q-residual and Hotelling's T^2. However, the deployment of IOT is restricted by the lack of generally applicable model diagnostics that is specifically analogous to the Hotelling's T^2. A novel diagnostic for use with IOT based on comparing the shape of net analyte signals (NAS) between the pure components and a sample mixture utilizing the spectral angle (θ^NAS) is proposed. The θ^NAS indicates if an IOT prediction from a mixture spectrum is appropriate for a particular pure component matrix. To support diagnostic interpretation, the θ^NAS was decomposed into individual variable contributions that can be plotted. Since θ^NAS is a circular quantity, it was found to best interpreted using circular statistics and visualized on a compass diagram.
The novel θ^NAS diagnostic was successfully deployed to identify unusual near-infrared spectral samples with both chemical and non-chemical interferences from a set of pharmaceutical powder mixtures prior to an IOT prediction. When the unusual samples were accounted for using preprocessing techniques, the θ^NAS displayed a predicable response in parallel with a PLS model and its Hotelling's T^2. The θ^NAS was also sensitive enough to effectively demonstrate how accounting for chemical interferences within a pure component matrix directly enhanced prediction performance. These demonstrations of the θ^NAS diagnostic are intended to expand the application of IOT for PAT development.

(CHEM-05.4)**Calibration-Free Blend Uniformity Monitoring and Active Pharmaceutical Ingredient Potency Detection: The Role of Iterative Optimization Technology in Continuous Manufacturing Systems**

Samuel Henson, Adam Rish, James Drennen, Carl Anderson, *Duquesne University*

The pharmaceutical industry is taking active steps to achieve continuous manufacturing (CM) of drug products. Intermediate materials in CM processes are often inaccessible due to the interconnected nature of unit operations. The existing approaches to critical quality attribute monitoring via process analytical technology require multivariate models to facilitate data interpretation. Traditional calibration models such as partial least squares (PLS) regression are dependent on calibration data. Construction of a calibration set often requires access to intermediate materials for the collection of reference data. Limited material access in CM systems creates a challenge for calibrating PLS models, highlighting the need for modeling strategies which do not require sampling of intermediate materials or calibration data.
Calibration-free models are proposed as a solution to this challenge. Iterative optimization technology (IOT) is a calibration-free approach that requires only pure component spectra as model inputs. The current work demonstrates the applicability of IOT in a CM blender with physically inaccessible intermediate materials. IOT is used for both blend uniformity monitoring and active pharmaceutical ingredient (API) potency prediction. Calibration-free wavelength selection via a wavelength angle mapper (WAM) is demonstrated to improve the potency predictions, and the prediction reliability is assessed with a calibration-free diagnostic tool. The diagnostic is based on the spectral angle between the net analyte signals (θNAS) and provides an assessment of the prediction reliability. Blend uniformity is assessed with relative standard deviation for the potency predictions.
Overall, this calibration-free method is demonstrated to successfully monitor blend uniformity while providing accurate and reliable API potency predictions. The WAM method is shown to improve prediction performance for IOT, and the reliability of the predictions is confirmed with θNAS values. The proposed method is advantageous as material-sparing approaches are sought throughout the industry. Additionally, calibration-free approaches circumnavigate the challenge of inaccessible intermediate materials in CM systems. Minimizing the material burden associated with model

development while maintaining reliable, accurate model predictions is essential as the application of CM increases throughout the pharmaceutical industry.

(CHEM-05.5)**Integrating digital capabilities and data analytics for rapid resolution of manufacturing inefficiencies**

Brandye Smith-Goettler, *Merck & Co., Inc*

Advanced data modeling is routinely used to understand and remediate inefficiencies at different stages of pharmaceutical manufacturing. However, data scientists can spend several weeks immersed with subject matter experts to understand the given manufacturing process and scientific hypotheses for the inefficiency. The learning curve is reversed when a data science solution is found. This presentation will highlight a support system that integrates digital capabilities, machine learning, and guided data analytics to enable collaborative and rapid resolution of manufacturing inefficiencies.

23IR12: Mid-IR Sensing Schemes Beyond Absorbance Spectroscopy, Sierra 3
Chair: Bernhard Lendl

(IR-12.1)**Mid-Infrared Dispersion Spectroscopy – A New Avenue for Liquid Phase Analysis**

Alicja Dabrowska, Andreas Schwaighofer, Bernhard Lendl, *Technische Universität Wien,*

Mid-infrared (mid-IR) dispersion spectroscopy is an attractive, novel approach to liquid phase analysis that overcomes the limitations encountered in conventional mid-IR absorption spectroscopy. The technique detects phase shifts inherent with IR absorption, rather than measuring changes in intensity. It delivers quantitative and qualitative information about the sample equivalent to absorption spectroscopy while offering numerous advantages: immunity to source intensity fluctuations, constant sensitivity, baseline-free detection, extension of optimum pathlengths, and high dynamic range beyond the capabilities of the Beer-Lambert's law. [1]

In this work, we discuss the theoretical principles of the technique and experimentally demonstrate the advantages of dispersion spectroscopy over conventional absorption spectroscopy. Moreover, we present the latest configuration of the developed spectroscopic instrument for dispersion sensing in liquids. In brief, it consists of a Mach-Zehnder interferometer illuminated by a tunable quantum cascade laser. The sample is introduced to an instrument via a dual-channel transmission flow cell, placed between the interferometric arms, which is filled with a reference solution (solvent) and a sample solution (solvent + analyte) for the measurement. IR absorption in the sample solution introduces phase shifts between the interferometric arms proportional to the sample's refractive index allowing the dispersion spectrum to be recorded and analyzed. Our example applications demonstrate the power of our technique and the developed setup for analysis of various analytes (i.e., proteins, carbohydrates), complex mixtures, and chemical reaction monitoring. [2-3]

In summary, the presented work illuminates the potential of dispersion spectroscopy as an upcoming robust and sensitive way of recording IR spectra of liquid samples that can be harnessed to construct miniaturized, more reliable, and accessible sensors.

(IR-12.2)**IR Refraction Spectroscopy**

Thomas Mayerhoefer, Vladimir Ivanovski, Juergen Popp, *Leibniz Institute Of Photonic Technology, Ss. Cyril and Methodius University*

ABSTRACT
Infrared refraction spectroscopy [1] is a useful complement to absorption spectroscopy. Its advantages are its simplicity and the fact that it fills an important gap: it enables the quantitative interpretation of reflectance spectra with minimal effort. The refractive index spectrum is calculated from reflectance by disregarding absorption. The change in the refractive index is proportional to the concentration, and spectra with features similar to those of second derivative absorbance spectra can be obtained by numerically deriving the refractive index spectra. The peak values of the derived spectra indicate

oscillator positions and are approximately proportional to the concentration in a manner similar to absorbance. Unlike absorbance spectra, there are no baseline ambiguities for first derivative refractive index spectra, and in refractive index spectra, instead of integrating over a band area, a simple difference of two refractive index values before and after an absorption yields a quantity that is linearly correlated with concentration in the absence of local field effects.
By using the Kramers-Kronig relations, infrared refraction spectroscopy can be linked further to absorption spectroscopy. However, the need to extrapolate measured data into unknown regions can lead to errors and deviations from the true values in the known region. Recently, a fast and reliable method to determine the complex index of refraction function from reflectance spectra at normal or near normal incidence has been developed.[2] This method does not require extrapolation of the measured data for higher wavenumbers and provides simple and reliable material-independent ways to extrapolate the measured data for lower wavenumbers. As a result, it is possible to quickly and accurately extract spectral information from reflectance measurements at near normal incidence. A quantitative analysis of the obtained optical constants functions is possible with dispersion analysis, the much more advanced progenitor of band fitting.

Acknowledgement

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(IR-12.3)**Comparison of Attenuated Total Reflectance (ATR) to Reflectance Fourier Transform Infrared (FTIR) Spectra of Explosives for Real-Time FTIR - Differential Scanning Calorimetry (DSC) Measurements**

Gregory Klunder, Batikan Koroglu, Keith Coffee, Ana Racoveanu, Alan Burnham, John Reynolds, *Lawrence Livermore National Laboratory*

Differential scanning calorimetry (DSC) is an important technique used to evaluate the thermal stability of explosive materials and develop kinetic models for thermal degradation. Understanding the chemical changes that occur during the DSC analysis can also provide valuable information for the degradation mechanism. One method to evaluate the chemical changes in real-time is to measure FTIR reflectance spectra through a pinhole in the lid of the DSC crucible. In many cases, the FTIR spectra of the explosives are well characterized by either ATR or transmission spectroscopy. When the spectra of the material in powder form are measured in near normal reflectance, as is required for the real time analysis in the DSC, the reflectance spectra may not match the ATR spectra. This can be due to the strong absorption bands, restrahlen bands, and penetration depth resulting in spectral artifacts which can complicate the interpretation. Although sample preparation by diluting the sample in KBr can improve the spectra, this would in turn complicate the DSC measurements. The near normal reflectance spectra result from a combination of Fresnel (specular) and Kubelka-Munk (diffuse) reflectance. Isotopically labeled materials were also evaluated in order to help evaluate the thermal degradation mechanisms. Shifts in the ATR spectral peaks for isotopically labeled compounds were relatively small, however resulted in more significant changes in the reflectance spectra. The isotopic shifts were helpful in assigning the bands in the reflectance spectra. DSC analyses were performed with a 10°C/min temperature ramp to 330°C where it was held for selected times and then cooled. Real time reflectance spectra show changes in the chemical composition and the residue material was subsequently analyzed by HPLC. This presentation will cover the experimental considerations for DSC- FTIR, nuances of the spectral interpretations, chemical composition with supplemental LC-MS analysis including evaluations using isotopically labeled materials.

This work was performed under the auspices of the United States Department of Energy by Lawrence Livermore National Laboratory under Contract DE-AC52-07NA27344.

(IR-12.4)**High-Sensitivity IR Spectroscopy of Protein Drugs at Low Concentrations**

Young Jong Lee, Seongmin Kim, *National Institute Of Standards And Technology*

Infrared (IR) absorption spectroscopy is a powerful tool for characterizing pharmaceutical products and their structural conformations. However, conventional approaches to protein in aqueous solutions have been significantly challenged because the strong IR absorption of water overwhelms the limited dynamic range of the detection system and thus allows only a short path length and poor concentration sensitivity. Here, we demonstrate an adaptive solvent absorption compensation (SAC) approach can improve the concentration sensitivity and extend the available path length by distinguishing the analyte signal over the full dynamic range at each wavelength. We present QCL-based IR absorption spectra of hydrated proteins from >100 mg/mL to < 0.1 mg/mL protein concentration. The SAC results in the amide I band show two to three orders of magnitude enhanced signal-to-noise ratios compared to the non-SAC results. We also present recent advances in spectral range and speed of absorption measurement. A fully automated sample introduction system allows for measuring multiple protein drug products at their original formulations.

(IR-12.5)**Non-Invasive Glucose Monitoring Techniques using QCL-IR Lasers**

Edeline Fotheringham, Brock Koren, Taylor Stathopoulos, *DRS-Daylight Solutions,*

There are 422 million people worldwide living with diabetes1, and current methods for monitoring blood glucose levels can be painful, invasive, and potentially require surgical procedures. Determining a fast and reliable non-invasive method for monitoring glucose levels is a significant milestone on the path to better diabetes management. Multiple non-invasive monitoring approaches have been developed; however, mid-infrared (MIR) spectroscopy has the advantage of targeting the specific glucose excitation fingerprint to identify concentration with increasing accuracy.

Quantum cascade laser (QCL) sources are ideally suited for this application given their high spectral intensity across the fingerprint region and have enabled researchers to develop more advanced methods for non-invasive glucose monitoring. Using QCL-IR illumination sources, researchers are employing techniques such as photoacoustic and photothermal spectroscopy to detect glucose concentration levels in the interstitial fluid layer with a high degree of selectivity and specificity. The high quantities of data produced using QCL-IR sources have been passed into increasingly sophisticated machine learning algorithms to further improve the accuracy of glucose concentration results. The continued development of these techniques demonstrates the feasibility to achieve a fast, reliable non-invasive method for monitoring blood glucose levels.

23LIBS04: LIBS for Mining, Geology and Space, Southern Pacific B/C
Chair: Andressa Adame
Co-Chair: Aissa Harhira

(LIBS-04.1)**SuperCam Laser-Induced Breakdown Spectroscopy (LIBS) Results from Jezero Crater, Mars**

Ryan Anderson, Roger Wiens, Sylvestre Maurice, Arya Udry, Olivier Beyssac, Elise Clavé, Ann Ollila, Baptiste Chide, Benjamin Weiss, Shiv Sharma, The SuperCam Team, *USGS Astrogeology, Purdue University, IRAP, University of Nevada Las Vegas, Université Pierre et Marie Curie, DLR, Los Alamos National Laboratory, Massachusetts Institute of Technology, University of Hawaii*

SuperCam on the Perseverance Mars rover is the successor to the ChemCam laser-induced breakdown spectroscopy (LIBS) instrument on the Curiosity rover. SuperCam adds several additional techniques – visible to near-infrared passive spectroscopy, Raman spectroscopy, time-resolved luminescence

spectroscopy, a microphone, and color imaging – to ChemCam's combination of grayscale imaging and LIBS.

Perseverance landed in Jezero crater on Mars on February 18, 2021, and SuperCam has been collecting data almost daily since shortly after landing. SuperCam LIBS, in combination with the other SuperCam measurement techniques and data from the other instruments on the rover, has led to several notable results since landing. LIBS played an important role in the determination that the Jezero crater floor consists of stratified igneous deposits: the Máaz formation is a basaltic to basaltic-andesitic lava flow, and the Séítah formation is an olivine cumulate. SuperCam also has detected carbonate minerals along the rover traverse formed from the interaction of CO2-saturated water with olivine minerals. SuperCam has been used to analyze rocks with distinctive "purple" coatings and determined that the coatings are similar in composition to the pervasive martian dust. SuperCam's microphone can record the sound of the LIBS spark, and these recordings have been used to measure the speed of sound in the martian atmosphere, its attenuation with distance, and variations with temperature.

SuperCam plays an important role in the selection and characterization of samples being collected for return to Earth early in the next decade. As a rapid stand-off quantitative technique with small spot size, LIBS is very useful for tracking changes in composition as the rover traverses, and for surveying the rover workspace and selecting a sample representative of the desired geologic unit. SuperCam LIBS pits have also been used to mark the rock surface where drill-core samples will be extracted to preserve information about their rotational orientation.

As Perseverance continues to explore within and eventually outside of Jezero crater, SuperCam will continue to provide a rich set of combined spectroscopic, imaging, and acoustic measurements that can be used to improve our understanding of Mars.

(LIBS-04.2)**Analysis of Earthen-based Materials using LIBS**

Aissa Harhira, Josette El Haddad, Paul Bouchard, Francis Vanier, Elton Soares de Lima Filho, Christian Padioleau, André Beauchesne, Antoine Hamel, Francis Boismenu, Daniel Gagnon, Mohamad Sabsabi, *National Research Council Canada,*

The exploitation activities in the mining industry require tons of earthen-based material to be extracted and processed. For economical and environmental considerations, the industry is seeking a novel analyzer technology allowing a rapid measurement of the composition of raw materials prior to extraction and processing. Laboratory analyses are time-consuming and require an expensive preparation of samples due to their heterogeneity. Laser-Induced Breakdown Spectroscopy (LIBS) thus appears as a promising rapid and non-contact technique with little or no sample preparation. Combining this multielemental spectroscopic approach with multivariate analysis tools provides a powerful and enabling technique to estimate the composition of highly heterogeneous geological materials.
In this talk, we will illustrate examples of earthen-based applications of LIBS developed at the National Research Council of Canada (NRC). First, we present a method using LIBS to determine rapidly and without sample preparation the bitumen and mineral content in oil sands ores. Measurements in simulated mining conveyor conditions will also be discussed. Second, LIBS analysis of gold-bearing ores will be presented to illustrate the ability of the technique to quantify the gold content at the ppm range and to determine the abundance of mineral assemblages.

(LIBS-04.3)**Validation of Laser-Induced Breakdown Spectroscopy assisted by Laser-Induced Fluorescence (LIBS-LIF) for the measurements of platinum and palladium in solid ore**

Ismail Elhamdaoui, **Andressa Adame**, *Inrs*

I. Elhamdaoui1, S. Selmani1, F. Vidal1, M. Sabsabi2, M. Constantin3, P. Bouchard2

1Institut national de la recherche scientifique, Centre Énergie Matériaux Télécommunications, 1650 Lionel-Boulet Blvd., Varennes, QC J3X 1P7, Canada
2National Research Council Canada, 75 de Mortagne Blvd., Boucherville, QC J4B 6Y4 Canada
3Université Laval, Département de géologie et de génie géologique, 1065 Ave de la Médecine, Québec, QC G1V 0A6, Canada

1) Corresponding author: Ismail.elhamdaoui@inrs.ca

Determining the concentrations of precious metals in solid ore samples is a crucial undertaking within the mining industry. Laser-induced breakdown spectroscopy (LIBS) has been employed for this purpose; however, its detection limit often proves inadequate for measuring precious metals in ores. In this study, we examined the efficacy of a LIBS-LIF technique in enhancing the detection limit of platinum and palladium in solid ore samples. Calibration curves were established utilizing certified reference materials obtained from the Lac des Îles palladium mine. The LIBS-LIF technique achieved a platinum detection limit of 0.15 ppm, which is two orders of magnitude lower than that achieved by the LIBS technique alone. Consequently, the LIBS-LIF method was utilized to estimate platinum and palladium concentrations in six quarter core fragments sourced from the Lac des Îles palladium mine ore. The technique yielded concentrations comparable to those obtained through conventional chemical analysis for solid surface and pulverized rock assessments, with two exceptions where palladium exhibited significant heterogeneity within the ore. The findings of this study underscore the potential of the LIBS-LIF technique for determining precious metal concentrations in solid ore samples.

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(LIBS-04.4)**LIBS Analysis of Aerosolized Lithium Brines**

David Day, Morgan Jennings, Agustin Loureiro, *Sciaps*

With the steadily increasing demand for electric vehicles, there is a corresponding demand for lithium, the key metal component of rechargeable batteries. The majority of the world's lithium resources are subterranean lithium brines which range in lithium concentrations from sub-100ppm levels up to several percent after concentrating above ground. During the brine concentrating process there is an important need for quick analysis of lithium and other constituent elemental concentrations.
In this presentation we will discuss the development of a technique using Laser Induced Breakdown Spectroscopy for the analysis of lithium brine solutions. While our prior work was focused on the measurement of bulk aqueous samples, this work concentrates on the measurement of aerosolized lithium brine sprays. This new technique utilizes a handheld LIBS and has the benefit of eliminating splashing, allowing high frequency sampling, and no sample preparation. Data from both synthetically prepared samples as well as real brine samples will be presented showing that this method is capable of determining lithium concentrations from 10 ppm to greater than 1% lithium by weight. In addition to lithium, measurements of several other common brine constituents will be presented including sodium, potassium, magnesium, calcium, and boron.

(LIBS-04.5)**Recent ChemCam Observations on the Curiosity Mars Rover**

Sam Clegg, The ChemCam Science Team, *Los Alamos National Laboratory*

ChemCam is a remote sensing instrument that has been operating on the NASA Curiosity Mars Rover for over eleven years. ChemCam is the integration of remote Laser-Induced Breakdown Spectroscopy (LIBS) and a gray-scale Remote Micro-Imager (RMI). ChemCam uses a 1067 nm laser to generate the LIBS spark on rocks and soils up to seven meters away from the top of the rover mast. Multivariate and univariate models are used to extract elemental compositions from the LIBS spectra. The RMI is also used to collect high resolution context images of the samples before and after the target is analyzed with the ChemCam LIBS technique. This paper will highlight many of the recent observations along the traverse into the Sulfate Unit. Sulfur is ubiquitous on Mars and white calcium sulfate veins have been observed along the entire traverse. ChemCam has also recorded signatures of magnesium sulfates. Quantifying sulfur is a significant challenge in the LIBS spectra of geologic samples. Sulfur produces relatively few lines within the ChemCam spectral range and many of those lines overlap with many other prominent emission lines such as Fe. There are only a few weak interferences with the 564 nm sulfur line described by Dyar et al. 2011. A univariate model with this 564 nm line failed to produce a universal calibration independent of the sulfate molecular formula. A Partial Least Squares (PLS) model has been developed that successfully produced a universal model independent of the sulfate. This PLS model uses peak area spectra where the integrated peak areas are used rather than the full LIBS spectra. This increases the intensity of the relatively weak sulfur peaks. This model also uses the integrated sub-models developed by Anderson et al. 2017 for the ChemCam major element analysis. This paper will also summarize this sulfur multivariate model and the sulfur results observed along the traverse and into the Sulfate Unit.

23MASS04: Understanding Protein Structure with Mass Spectrometry, Southern Pacific A/G
Chair: Jared Shaw

(MASS-04.1)**Dissecting hierarchical organization of proteins and lipids organization in the membrane**

Kallol Gupta, *Yale University*

The cellular membrane plays an essential role in regulating the hierarchical organizations of membrane proteins (MP) and lipids that drive downstream signaling cascades. While nMS has been at the forefront of detecting these complexes, studying MPs with nMS demands prior dissolution of the cellular membrane through either chemical or mechanical means. Addressing this, we present lipid-vesicle nMS. This comprehensive tunable in-vitro platform enables us to study MPs directly from the lipid bilayer, where both lipid compositions, as well as specific bilayer properties such as membrane curvature, tension, and fluidity, can be customized to mimic a target physiological membrane. We demonstrate the feasibility of our approach by detecting a range of oligomeric MPs directly from in-vitro bilayers mimicking different eukaryotic organelles (e.g. mitochondria, endoplasmic reticulum, plasma membrane, synaptic vesicles, and Golgi). We further show our ability to detect MPs from membranes of different curvature and fluidity. Applying this to neuronal proteins we have discovered how specific binding of lipids to SNARE regulates the timescale of neurotransmitter release. In a separate example, leveraging on the tunability of our platform, we show how specific membrane lipids regulate the functional organization of membrane transporter. Finally, we expand this platform directly to native membranes. Taking different physiological membranes of prokaryotic and eukaryotic origin, we demonstrate our ability to detect MP complexes directly from intact lipid membranes, without requiring any prior dissolution of the bilayer. We further coupled this native top-down fragmentation to ID the membrane protein complexes. For top-down analysis, we make use of both collision cell HCD as well as ECD fragmentation, achieved by swapping the transfer multipole with an ExD cell (e-MSion). We demonstrate the broad applicability of our platform by taking membranes from different prokaryotic and eukaryotic cells, as well as specific intracellular organellar membranes, such as endoplasmic reticulum, plasma membrane, and mitochondrial membranes. We finally, apply this platform to show how we can capture a multi-protein bacterial complex, which is a key target for antibiotic development, and perform direct ligand screening from the native membrane.

(MASS-04.2)**Mass Spectrometric Characterization of Chemically and Natively Unfolded Proteins**

Ian Webb, *Iupui*

Ion mobility (IM) enables low-resolution protein structure determinations in milliseconds, yielding information about overall size and shape. IM/MS studies of cooperatively folded, well-structured proteins have revealed that when introduced under gentle conditions, these proteins retain their overall native-like structures. It is debatable whether this is true for intrinsically disordered proteins (IDPs) and regions (IDRs). Native mass spectrometry has been used to both support and refute claims that gas-phase ensembles are solution-phase ensembles of IDPs. In this talk, we will look at orthogonal methods such as machine learning, Monte Carlo and molecular dynamics (MD) simulations, CCS measurement and prediction, and solution and gaseous chemical crosslinking to provide a thorough examination of the structural fates of the IDP α-synuclein (SNCA). Specifically, we will present how phosphorylation induces ensemble-level conformational changes in the protein.

(MASS-04.3)**Protein Complex Heterogeneity and Structure Revealed by Native Mass Spectrometry with Electron Capture Charge Reduction and Surface Induced Dissociation**

Jared Shaw, Sophie Harvey, Chen Du, Vicki Wysocki, *University Of Nebraska Lincoln, The Ohio State University*

The assembly of proteins and other biomolecules into active cellular machinery is largely modulated by subunit interactions, protein posttranslational modifications (PTMs), and ligand binding. Advances in native mass spectrometry (nMS) have enabled rapid structural characterization of soluble and membrane protein complexes and very large assemblies. However, extreme heterogeneity in composition and PTMs can severely hinder mass determination and structural characterization. To address these challenges, we have developed a device combining ion-electron reactions with surface induced dissociation (SID) to expand nMS capabilities for characterization of large, heterogeneous proteins complexes. Electron capture charge reduction (ECCR) enables tunable gas phase charge reduction through the capture of low energy electrons to reduce charge without dissociation of protein complexes, as demonstrate from GroEL tetradecamer, ribosomal subunits, and heterogeneously glycosylated viral spike protein. The impact of gas phase charge reduction on fragmentation by SID was also investigated. Interestingly, gas phased charge reduction yielded fragmentation patterns nearly identical to solution charge reduction via addition of TEAA. Additionally, SID of the E. coli 50S ribosomal subunit yielded a variety of subcomplexes and ribosomal protein subunits enabling relative quantitation of modified forms.

(MASS-04.4)**Marriage Between Native Mass Spectrometry and Cryo-EM for Structural Analysis of Membrane Proteins**

Weijing Liu, Christopher Mullen, Donggyun Kim, Vadim Cherezov, Gregory Dodge, Barbara Imperiali, Hiruni Jayasekera, Michael Marty, Rosa Viner, *Thermo Fisher Scientific, University of Southern California, Massachusetts Institute of Technology, University of Arizona*

Membrane proteins (MPs) represents 60% clinical drug targets owing to their active involvement in cellular processes. The complexity of membrane mimetics for MPs solubilization pose the challenges for native mass spectrometry (nMS) analyses. Here, we systematically investigate the complexity of MPs in different membrane mimetics (detergent micelles, nanodiscs, and styrene maleic-acid lipid particles (SMALPs)) to establish mimetics-based nMS methods. Such nMS methods can be powerful and complementary to cryo-EM structure analysis. Firstly, rapid online buffer exchange-nMS (OBE-nMS) within minutes coupled to real-time data processing enables quick assessment of MPs prior to Cryo-EM analysis. Secondly, either charge detection mass spectrometry (CDMS) or proton transfer charge reduction (PTCR) is compelling to resolve heterogeneous MPs for benefiting further structural elucidation.
Firstly, detergent screening for OBE-nMS reveals LDAO is universal for characterizing both bacterial and mammalian MPs in detergent. Herein, we were able to detect 4 out of 5 different subunits plus

PTMs of GPCR-Gs complex (>170 kDa). Initial testing of MPs in nanodiscs using OBE-nMS employing mobile phase containing detergent resulted into nanodiscs dissociation into monomeric membrane scaffold protein and phospholipids. Removal of detergent from mobile phase intriguingly preserved the nanodisc integrity, and thus facilitate apply such method for characterizing MPs in nanodiscs. Rapid OBE-nMS plus real-time data processing empower nMS as a screening approach before sending biomolecules to structure determination by Cryo-EM.
OBE-nMS of MPs in detergent and nanodiscs cannot deny its limitation on resolving charge state of complex samples. The aforementioned GPCR-Gs complex in detergent and Wbap in SMALP remained unresolvable in ensemble measurement due to their heterogeneity. However, using CDMS revealed lot-to-lot variation of GPCR-Gs complex. The lot containing target MW identified by CDMS also showed evenly distributed particles in cryo-EM image. Additionally, the oligomerization is controversial of Wbap. The CDMS results not only unraveled its dimeric status but also confirmed its bulky environment composed of numerous lipids and SMA. Results of GPCR-Gs and Wbap using CDMS both align well with structures obtained by Cryo-EM.
We have proved the value of nMS for streamlining MPs structure analysis. Future work will evaluate PTCR performance on resolving MPs in complex mimetics.

23PMA08: Small Molecule Analysis in Biopharma, Southern Pacific D
Chair: Karl Burgess
Co-Chair: Katherine Hollywood

(PMA-08.1)**An Automated Workflow For Global Metabolomics Of Complex Biological Samples**

Martina Pičmanová, Tessa Moses, Joan Cortada-Garcia, Ms Georgina Barrett, Hannah Florance, Sufyan Pandor, **Karl Burgess,** *James Hutton Institute, Lonza, University of Edinburgh, Agilent Technologies*

Advances in high-throughput methodologies in synthetic biology require rapid and sensitive workflows in the metabolic phenotyping of complex biological samples. We developed a straightforward, easy to implement liquid chromatography - mass spectrometry (LC–MS) metabolomics method using a commercially available column that provides increased throughput. Since reducing run time impacts chromatography, we coupled the separation with ion mobility spectrometry to expand peak capacity. Additional confidence in identification was obtained using collision cross section measurements for the features. The rapid untargeted metabolomics workflow was developed with broad metabolome coverage, by combining zwitterionic-phase hydrophilic interaction chromatography (HILIC-Z) with drift tube ion mobility-quadrupole time-of-flight (DTIMqTOF) mass spectrometry. The method is acronymised RHIMMS for rapid HILIC-Z ion mobility mass spectrometry. RHIMMS demonstrates improved chromatographic separation for metabolites with wide physicochemical properties at 3.5 min per sample, while maintaining reproducibility over 200 injections. The combination of rapid chromatographic separation with ion mobility allows improved annotation and the ability to distinguish isobaric compounds. Our results demonstrate RHIMMS to be a rapid, reproducible, sensitive, and high-resolution analytical platform that is highly applicable to the untargeted metabolomics analysis of complex samples.

Reference: Rapid HILIC-Z ion mobility mass spectrometry (RHIMMS) method for untargeted metabolomics of complex biological samples. Metabolomics (2022) 18:16.

(PMA-08.2)**An Analytical Platform for Real-Time Monitoring of Biopharmaceutical Manufacturing Processes and Integration of a Raman Platform**

Maikel Gaitkoski, Noemi Dorival Garcia, Jonathan Bones, *NIBRT,*

Biopharmaceuticals comprise a wide range of products varying from nucleic acids, antibodies to viral particles. During manufacturing and storage, biotherapeutics are subject to various post-translational modifications (PTMs), which may affect bioactivity or stability and are classified as critical quality attributes (CQAs). The control of cell culture process parameters (CPPs) is crucial, as levels of PTMs can be induced or modified during production. Product characterisation during a bioreactor process is

highly complex and time-consuming. Furthermore, multiple analytical data are needed, which may not be available for several days, thereby slowing process development and preventing 'real-time' adjustment to the manufacturing process. To address these constraints, we have developed a fully automated, highly flexible sampling and analytical characterisation platform for direct monitoring of bioreactor processes, allowing the generation of high-frequency data, and requiring low-sample volumes. Aseptic sampling was performed using Numera® (Securecell), followed by different sample processing paths. One delivers the sample directly to BioProfile Flex® II (Nova Biomedical) for viable cell density information, and media/metabolite profiling. Numera® also generates cell-free samples, which are injected to a LC-Orbitrap MS system (Thermo Fisher Scientific) for titre and PTMs determination using native mass spectrometry. Lucullus® Process Information Management System is responsible for process automation and management of the platform, including integration of CPPs data generated by TruBioä software that controls the bioreactor process.

Sample process timing is critical to ensure that sampling cadence can be maintained operational at the highest sampling frequency across max. four vessels ultimately. Consequently, this platform was also integrated to a Raman platform (Kaiser Optical RXN) for in-situ real time monitoring. The novel combination of these platforms is incredibly valuable for the wider field of biopharmaceutical manufacturing, as for the first time, integrated and aligned data from both analytical strategies on the same process will be generated, and the associated information using the analytical platform, will enable the verification of Raman platform performance.

This automated platform will overcome the inherent complexity of the involved analytical methods and complicated data analysis, offering a feasible alternative for implementation in the regulated environment.

(PMA-08.3)**Raman Spectroscopy to Detect Lentivirus in Living Human Cells**

Rheta Elkhaira, Keita Iwasaki, Kosuke Hashimoto, Hidetoshi Sato, *Kwansei Gakuin University*

The purpose of the present research is to develop a method for detecting human infectious viruses in the absence of human patients. The previous study has demonstrated that Raman spectroscopy was able to detect adenovirus infection within 3 hours in living human cells due to DNA, and the spectral changes after 9 hours later can be attributed to changes of protein. Adenovirus is a nonenveloped double-stranded DNA virus that infects the host cell without integration into the host genome. The capability of Raman spectroscopy to detect adenovirus infection in living human cells has been demonstrated in the previous study, however, the infection mechanism of a virus in living cells vary between virus species. Therefore, it is important to investigate cell reactions against other viruses which have different infection mechanisms. In this study we used a lentivirus, an enveloped virus with a single-stranded RNA genome that integrates into the host genome during infection. A lentivirus vector that integrated with Green Fluorescent Protein (GFP) was infected into HEK293 cells. The chemical changes in the infected cells were observed using Raman spectroscopy. The Raman signals from the host cells before and after virus inoculation followed by chemometrics as advanced statistical methods enable us to discuss in detail concerning compositional changes in the culture cells due to cell reactions against the presence of the virus. The results showed spectral changes attributed to nucleic acid and protein after lentivirus inoculation at different time intervals which indicated cell reactions against the presence of lentivirus in the host cells. The results of this study revealed the potential of Raman spectroscopy to detect lentivirus infection in living human cells.

(PMA-08.4)**Application of real-time metabolomics to CHO cell optimisation**

Luke Johnston, Mark Rendall, Jeff Keen, Karl Burgess, *University Of Edinburgh, FUJIFILM Diosynth Biotechnologies*

Chinese hamster ovary (CHO) cells are the leading host cell chassis for biopharmaceutical bioproduction. CHO cell metabolism underpins recombinant protein production but is complex, with many facets of CHO biochemistry still poorly understood. Metabolomics is the study of small molecules within biological systems and can be applied to generate phenotypic cellular profiles. We

apply a combination of cutting-edge metabolomics methodologies to further elucidate CHO biochemistry, including development of an on-line metabolomics process analytical technology (PAT) platform for real-time CHO bioprocess control.

The off-line methods compromise a 3.5 minute, rapid HILIC-Z ion mobility mass spectrometry (RHIMMS) method for analysis of hundreds of longitudinal CHO bioreactor samples, and a 1H proton NMR metabolomics workflow. The findings from our off-line, untargeted methods inform our targeted real-time metabolomics platform (RTMet), which consists of a flow injection mass spectrometry system coupled directly to a bioreactor for extracellular metabolite analysis at 5-minute intervals during live bioprocesses. To the best of our knowledge, this is the first on-line mass spectrometry-based metabolomics method for analysis of CHO bioreactor systems. RTMet is now being translated to an on-line feedback control system based on live metabolomics information generated.

The RHIMMS dataset compromised hundreds of identified features in positive and negative ionisation modes. Metabolites with a wide range of physiochemical properties, including polar and non-polar metabolites, were annotated across hundreds of longitudinal samples. 1H NMR metabolomics facilitated identification and accurate quantitation of ca. 40 compounds, many of which are complementary to the IM-LC-MS analysis. RTMet permitted monitoring of circa 40 critical metabolites from central carbon and amino acid metabolism during live bioprocesses. Feed-back control of glucose and amino acid media addition was explored to improve bioprocess efficiency in response to the on-line metabolomics data generated by RTMet, enhancing cell growth and monoclonal antibody titre.

Taken together, these metabolomics platforms produce a veracious metabolic profile of an industrially relevant CHO cell line. The result shed insight into CHO biochemistry and tease novel avenues of investigation to further improve the CHO bioproduction chassis. Particularly, RTMet offers a new PAT platform by tracking and responding to metabolite levels in real-time as key process parameters.

23RAM05: IRDG, Cascade 3
Chair: Karen Faulds

(RAM-05.1)**Exploring Pharmaceutical Formulation Structure Using 3D Raman Chemical Images**

Liam Davison-Gates, Don Clark, Fiona Clarke, Andrew Ewing, *Pfizer*

Chemical imaging has allowed much greater understanding of the distribution of materials within pharmaceutical tablets and guiding the production of high-quality medicines. Currently 2D chemical imaging is the “go-to” method for assessing material distribution in a sample, however, due to the random nature of sample preparation this method can be limited in determining the true sizes of material domains within the sample. This issue has been addressed through producing 3D chemical images. This is achieved through 2D Raman chemical mapping and iterative sample milling, which allows a full 3D chemical chemical image to be constructed. These 3D chemical images can be used to assess the distribution of the active pharmaceutical ingredient (API) and excipients in a formulation and to accurately measure the interconnectivity of these domains which are obscured in 2D chemical images. From these data sets the surface area in contact between different materials can be calculated, along with the homogeneity of each material distribution, and the overall structure of the spatial networks within the formulation. This is important as not every component in a tablet formulation is present as discrete particles.

Limitations of this approach are the data acquisition time (2 hours) and instruments being idle during non-working hours due to the need for the sample to be milled manually. A high-throughput approach allows multiple tablets to be milled simultaneously, and the subsequent Raman map acquisitions to be performed in sequence with no human intervention. Although the time to a single 3D chemical image

does not change, the benefit is 3D images from multiple samples which improves the statistical ruggedness of a study data set.

These developments are being utilised to determine the relationships between the material distribution in a tablet formulation and its physical properties. A case study shows how the information from 3D Chemical Images provided a hypothesis that API stability within a tablet was linked to its particle size and contact with a specific excipient. In conclusion, integrating 3D Chemical Imaging into the pharmaceutical workflow can assist in troubleshooting tablet manufacturing processes, and assessing interactions between different components, to better understand tablet stability and performance.

(RAM-05.2)**Enzyme-Activated Biorthogonal Raman Probes For Targeted Tumor Imaging**

Swati Tanwar, Behnaz, Piyush Raj, Aruna Singh, Lintong Wu, Dian Respati Arifin, Michael T McMahon, Jeff W.M. Bulte, Ishan Barman, *Johns Hopkins University,*

Sensitive imaging of enzymatic activity within the complex biological environment has become increasingly important for aiding in the diagnosis and prognosis of cancer. Enzyme's ability to cleave a specific peptide bond with spatiotemporal control has inspired the development of synthetic peptide-based molecular imaging nanoprobes designed to self-assemble upon interacting with specific enzymes. The strategy of injecting small sensing molecules that self-assemble into larger nanoprobes in situ increases intracellular accumulation, reduces efflux, and permits durable signals for imaging. Among the proposed synthetic approaches, the biocompatible click condensation reaction between D-cysteine and cyanobenzothiazole (CBT) has gained attention for its advantage of intracellular self-assembly triggered with endogenous cellular molecules under physiological conditions, with a fast rate constant. Raman spectroscopy (RS) offers superior optical imaging capabilities with exquisite molecular specificity, making it a potentially important optical imaging technique for recording quantitative spatiomolecular maps from live and intact biological samples. Raman signals are also resistant to photobleaching and, hence, better suited for long time dynamic measurements. We envision that the integration of RS with enzyme-triggered intracellular self-assembling strategies will render a paradigm of bioorthogonal Raman nanoprobes for molecular imaging across different levels of bio-organization. Here, we report design of a fundamentally new class of Raman-active self-assembling bioorthogonal enzyme recognition (nanoSABER) probe for highly sensitive targeted tumor imaging. In nanoSABER, we introduce a tumor-penetrating peptide sequence, and vibrational tags having vibrational stretching frequency in the cell silent region, to collectively generate high optical contrast. Selecting legumain as a representative tumor-associated enzyme, we demonstrate its utility for targeted imaging of legumain activity across different levels of organization from molecules to cells and tissues. Our results demonstrate the potential of nanoSABER as a broadly applicable sensing platform for a range of targets for Raman-based tumor detection and evaluation of treatment efficacy.

(RAM-05.3)**Understanding Molecule-Metal Interactions through SERS**

Laura Fabris, Chiara Deriu, Shaila Thakur, Kaleigh M. R. Scher, *Politecnico di Torino, Rutgers University*

Over the past decades, surface enhanced Raman spectroscopy (SERS) and SERS sensing have made considerable strides toward the use of the technology in real-life applications, especially in biosensing. SERS-based biosensors have pushed detection limits down to femtomolar and even attomolar concentrations by utilizing diverse sensing modalities. SERS sensors can be implemented both in direct and indirect modality; in the first approach the SERS signal collected is that of the analyte under investigation, while in the second the SERS signal of a Raman reporter is leveraged as a proxy to the analyte recognition event. Regardless of the chosen approach, metal-molecule interactions are key to achieving an optimal sensor performance. Yet, these interactions are very complex to monitor, especially when the SERS substrate used is in form of a colloidal plasmonic nanoparticle. Furthermore, the results of a sensing experiment have shown to depend closely on the interaction of the analyte or the reporter molecule, and to vary substantially with the density of such molecules on the nanostructure metallic surface. Therefore, understanding metal-molecule interaction and the origin of their variability is of paramount importance, in particular when these experiments are carried out for

diagnostic purposed. In my talk, I will report on our work aiming at understanding, through SERS and other analytical techniques, the interplay between the molecular structure of the analyte or the Raman reporter molecule and the properties of the nanoparticles, including the surface chemistry, the crystal structure, and the shape. These results can inform us on the parameters we need to take into consideration when implementing a SERS sensor, in particular if it is aimed at answering medical or biological questions.

(RAM-05.4)**Design and Fabrication of Porous Silicon Nanoparticle Paper-based Sensor**
Huijin An, Rabeb Layouni, Sharon Weiss, Andrea Locke, *Vanderbilt University,*

Volatile organic compounds (VOCs) are chemicals that are excreted by various metabolic processes in different environments. These compounds are commonly found in environmental conditions such as soil, air, and marine environments, or in various biological conditions including body fluids, and exhalation, which are greatly influenced by disease states, or even genetics. However, the conditions for detecting and monitoring VOCs in the field are very limited because of the low sensitivity. Therefore, using light-based techniques to detect VOCs in various environments has the potential to provide an alternative approach for remote sensing due to its portability, rapid signal acquisition, and the need for minimum sample preparation. The porous silicon layer (PSi layer) can be fashioned into optical VOC sensors due to its reflectivity properties, large surface-to-volume ratio, and biocompatibility. However, the fabrication methods for designing optical sensors using silicon are mainly focused on PSi films which are brittle and have problems with mass transportation and hydrophobicity limiting their sensing application. Here, we investigate the design of an optically transparent paper-based substrate embedded with pore silicon nanoparticles for VOC detection. The optically transparent membrane allows for low background noise and provides structural stability for the PSiNPs to minimize the risk of breakage. Numerous PSiNPs, embedded throughout the transparent substrate, create a 3D structure to help improve the mass transportation issue observed by PSi films. The PSiNPs were synthesized via electrochemical etching and then added to the nitrocellulose membrane by vacuum filtration. Next, the paper was made optically transparent with the addition and evaporation of dimethyl sulfoxide. Finally, for use as a SERs substrate for VOC detection, the paper was plasma treatment to attach 24 nm gold nanoparticles (AuNPs) on the surface of the PSiNPs. Scanning electron microscopy was used to characterize the surface of the substrate. SERS measurements were taken with 4-mercaptobensoic acid to confirm that AuNPs were attached to PSiNPs and improved the SERS signals due to the large surface area of PSiNPs. This method can not only allow for simple and fast fabrication which provides cost-effectiveness, but also improve on the existing shortcomings of using silicon for small molecule detection.

(RAM-05.5)**Transforming Healthcare Pathways By Screening Multiple Biomarkers Via SERS-Lateral Flow Immunoassays**

Benjamin Clark, Sian Sloan-Dennison, Kathleen Scullion, Karen Faulds, Duncan Graham, James Dear, Paul Fineran, Joanne Mair, David Creasey, Cicely Rathmell, Dieter Bingemann, Jonathan Faircloth, *University Of Strathclyde, University of Edinburgh, Wasatch Photonics*

Acute liver failure (ALF) has a high mortality. The commonest cause is paracetamol/acetaminophen overdose. Paracetamol is a readily available drug for treatment of minor ailments; however, it is ingested in overdose by 40% of self-harm patients in the UK (resulting in approximately 100,000 hospital presentations per year).[1] The antidote to paracetamol overdose is n-acetylcysteine (NAC) which is only fully effective when delivered within around 8 h of taking the overdose. Therefore, treatment must be started as quickly as possible in those patients at risk of liver injury. The current gold standard for diagnosing liver injury is serum alanine aminotransaminase (ALT) activity. Unfortunately, ALT activity only increases in the later stages of the overdose resulting in the failure to correctly diagnose liver injury in the early stages of the disease. Cytokeratin-18 (cK-18) has been identified as a potential biomarker that will provide improved diagnosis of patients suffering from drug-induced liver injury (DILI) following paracetamol overdose.[2] cK-18 levels are significantly higher than ALT levels in the early stages of DILI onset. In order to fully utilise the benefits of cK-18 as an early DILI biomarker, the development of a rapid point of care test (POC) is required.

This work investigates the development of a rapid surface enhanced Raman scattering (SERS) based lateral flow immunoassay (LFIA) diagnostic test which can be used to quantify ck-18 levels associated with the early onset of DILI. Interrogation of a SERS-LFIA device with a purpose-built portable Raman reader will provide health-care professionals with an improved diagnostic test for efficient patient stratification. The SERS-LFIA device and portable Raman reader outlined in this research has been developed to detect clinically relevant ck-18 concentrations as low as 5 ng/mL in 30 minutes after application of a patient's fingerprick blood sample. The success of the device will be assessed during a retrospective clinical study involving 620 patients across 2 hospitals.

References:
1. W. M. Lee, Am.J.Med., 1994, 96, 36–45.
2. A. D. B.Vliegenthart, D. J. Antoine and J. W. Dear, Br.J.Clin.Pharmacol., 2015, 80, 351–362.

23SPR05: Plasmonics and Sensing, Cascade 4
Chair: Jennifer Shumaker-Parry

(SPR-05.1)**Probing SERS intensity fluctuations with high-speed acquisitions**

Alexandre Brolo, Nathan Lindquist, *University of Victoria, Bethel Univesity*

Surface-enhanced Raman scattering (SERS) is a promising technique for applications in analytical chemistry due to its high sensitivity and selectivity. However, the fully implementation of SERS as analytical tool can be challenging due to fluctuations in SERS intensities observed from diluted solutions, which complicate quantification. Those types of fluctuations have been assigned to evidence of single-molecule detection by SERS. Recently, we have developed new experimental methods capable of monitoring those fluctuations at high speed (sub-ms time scale). It is clear that, at those time scale, the SERS fluctuations are a more general phenomenon that can be observed even from single nanoparticles.

(SPR-05.2)**Detecting Fentanyl Analogs by Combining Surface-Enhanced Raman Spectroscopy (SERS) and Paper Spray Mass Spectrometry (PS-MS).**

Sevde Dogruer Erkok, Roxanne Gallois, Leon Leegwater, Pascal Camoiras Gonzalez, Arian van Asten, Bruce McCord, *Florida International University, ENS of Lyon, University of Amsterdam*

There is an on-going effort in the black market to make new drugs that are more potent and addictive. Due to these continual modifications, many fentanyl analogs are developed and mixed with other illicit drugs, such as cocaine and heroin. The presence of fentanyl analogs as mixtures in illicit drugs makes it hard to estimate their potencies. This makes the detection and differentiation of fentanyl analogs important. Most current screening methods have difficulty detecting the full range of opioid analogs due to a wide variety of structural variations. However, surface-enhanced Raman spectroscopy (SERS) can differentiate structurally similar fentanyl analogs due to its ability to yield spectroscopic fingerprints for the detected molecules. Once SERS has identified a fentanyl analog, an additional confirmatory technique is required to provide identification of the compound in a court of law. A second, more precise method, such as liquid chromatography-mass spectrometry, is necessary for typical mixtures, which include other drugs and diluents at very low concentrations. Most confirmatory procedures require time-consuming and expensive, highly sophisticated laboratory equipment and experimental procedures, which can delay critical information that might save a victim or identify a suspect. In this project, we propose to miniaturize and accelerate this process by combining SERS analysis and mass spectrometry on a single microfluidic device. This procedure, known as paper spray mass spectrometry (PS-MS), can isolate fentanyl analogs from even complex drug mixtures. Thus, we propose to develop a novel device capable of simultaneously screening and identifying unknown fentanyl analogs. Coupling portable SERS and PSI-MS methods provide strong confirmatory results for real-world samples and drug mixtures.

(SPR-05.3)**Towards Point-of-Care Methods for the Detection of Different Biomarkers in Blood Samples Using a Surface Plasmon Resonance (SPR) Sensor**

Caroline Dubois, Jean-Francois Masson, Danny Brouard, Jonathan Robidoux, *Université de Montréal, Héma-Québec*

Surface plasmon resonance (SPR) is fast, portable and reliable making it amenable for a point-of-care (POC) method for the detection of biomarkers in various matrixes. A step forward, in collaboration with Héma-Québec, will be presented with the quantification of IgA in plasma and ferritin in whole blood. The main focus of the Immunoglobulin A project is the protection of deficient individuals (under 500 ng/mL) who are at risk of having an anaphylactic shock when given a blood transfusion. IgA plays an important role in mucosal immunity and its deficiency is associated with auto-immune disorders like Crohn's disease. Qualification of IgA deficient blood permits to ensure reserves in blood banks and hospitals. For this protocol to be amenable to a point-of-care approach, a single use cartridge SPR permitted accurate readings and has brought us closer to a user-friendly SPR instrument. The main goal of the ferritin project is to develop a point-of-care method to quantify ferritin in human blood with a minimum volume of less than 200 uL. Ferritin is a good biomarker for iron, since its main functions are to store and detoxify it. It can be found in plasma with concentrations from 90 to 150 ng/mL and 90 to 200 ng/mL in women and men respectively. In the case of anemia, a concentration under 15 ng/mL is observed and is associated with iron absorption issues, certain infections, and loss of blood. Frequent blood donors can have a decrease of iron in their blood, which motivates our goal of using surface plasmon resonance (SPR) on site at blood drives. Taken together, these steps bring us closer to our final objective: an instrument that can be used on-site by a non-specialist.
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Poster Presentations

Wednesday Poster Sessions

(Wed-P1) **Investigating Recycled Battery Materials using ICP-MS and TG-IR Spectroscopic Methods**

Aniket, Kieran Evans, Chady Stephan, *PerkinElmer*

The process of recycling lithium-ion batteries has received some attention over the past few years due to the increase demand for raw materials in this area. The increased demand is largely as a result of an increased demand for electric vehicles and other e-mobility which may eventually outstrip the supply of raw materials from natural sources. As recycled materials begin to contribute a large share to the pool of raw materials available for battery recycling, having the tools to better understand and optimize the recycling process and final products will become more important.

This work aims to give a high-level overview of how already available analytical techniques, such as inductively coupled plasma-mass spectrometry (ICP-MS) and thermogravimetric analysis coupled to infrared spectroscopy (TG-IR) can be implemented to gain valuable insights about recycled materials.

We will first demonstrate the use of ICP-MS to determine impurities in two important potential final products of battery recycling, lithium carbonate and lithium hydroxide. As is the case when virgin raw materials are use, certain impurities must be below pre-defined limits to ensure good performance, safety, and longevity in the battery.

Secondly, we will look at the simultaneous identification and quantification of residual electrolyte in black mass produced from the battery recycling process. TGA allows for a sample to be heated and the weight loss to be measured as a function of temperature therefore providing quantitative information. IR spectroscopy may be used to identify the evolved gas and therefore elucidate the structure of the material that has decomposed or evaporated.

In summary, this work will demonstrate the sample preparation and analysis of different materials found throughout the battery recycling value chain and establish best practices for data collection and interpretation.

(Wed-P2) **Carbon Quantum Dots: A Novel Low-Dimensional Nano-Sized Organic-Based Fluorescent Sensor for Toxic Metal Ions**

PAPIA CHOWDHURY, *JIIT*

Carbon quantum dots (CQDs), is one of the youngest members of carbon-based nanomaterials which are rapidly gaining attention due to their simple synthetic accession, good bio-consonance, and several revelation applications including excellent tunable fluorescence properties, bleaching resistance, and biocompatibility. The lower toxicity of CQDs is opposing the disadvantages of quantum dots [1]. Low cost, eco-friendliness, presence of abundant functional groups (e.g., amino, hydroxyl, carboxyl), high stability and good water-soluble characteristics of CQDs help them be involved in medicinal applications like drug delivery, biosensors, medical imaging, LED, etc. Active optical signals due to fluorescence, photoluminescence, and phosphorescence are the main reasons that CQDs are actively used as optical sensors and bioimaging tools. The CQDs occupy a tiny spot that exhibits different optical properties on excitation due to variation of their complex surface states which contain the variable core structure of six-membered carbon rings and oxygen functional groups on the surfaces. The functional groups are usually carboxyl and hydroxyl in nature. On excitation, the anions and cations formed from functional groups used to recombine themselves. The variation of the electronic structure due to recombination creates many quantized energy levels which help the CQDs to produce different wavelengths adapting to different applications. However, many atomistic details of CQDs still remain unresolved.
The development of industries generates numerous heavy metal wastes that can cause direct or indirect harm to the environment and humans. Many hazardous heavy metals, such as copper (Cu), chromium (Cr), lead (Pb), zinc (Zn), nickel (Ni), iron (Fe), cadmium (Cd), mercury (Hg), tungsten (W) and silver (Ag) are toxic to living organism [2].
In the present work, we have synthesized fluorescent CQDs using hydrothermal treatment from the dried leaf as a carbon source and used the freshly prepared CQDs in view of their application to detect water-soluble hazardous metal ions. Prepared CQDs were characterized and tested with the help of several molecular spectroscopic techniques (UV-Vis absorption and fluorescence spectroscopy and FTIR spectroscopy). The present work opens a door to the study of new water-soluble and biocompatible CQDs by the use of their fluorescence sensing for the detection of hazardous metal ions.

(Wed-P3) **Investigating the bacterial protein quality control system with AFM-IR nanospectroscopy**

Wouter Duverger, Grigoria Tsaka, Katerina Konstantoulea, Ladan Khodaparast, Laleh Khodaparast, Nikolaos Louros, Joost Schymkowitz, Frederic Rousseau, *Catholic University Leuven / Flemish Institute of Biotechnology*

Protein aggregation is a universal phenomenon occurring, for example, as a response to cellular stress or a consequence of ageing. It is also causally linked to several human neurodegenerative diseases, such as Alzheimer's and Parkinson's.

FTIR spectroscopy has revealed that the secondary structure of aggregated proteins in bacteria depends strongly on the type of stress applied. However, these studies have always been performed in bulk and could not assess the variation within a sample. Therefore, we took advantage of the

exceptional resolution of AFM-IR and its sensitivity to mechanical stiffness to directly determine the structural and mechanical heterogeneity of protein aggregates inside bacteria.

We measured the differences between aggregates in situ using this emerging method for nanoscale infrared spectroscopy. We developed an image processing pipeline tailored to AFM-IR data to capture the concentration of misfolded protein (as measured by beta-sheet content) and their mechanical stiffness within the cells and automatically segment images into cells and aggregates. Our results show these parameters are not linked to an aggregate's size or location in the cell, but they do vary depending on the conditions in which the aggregates were formed: aggregates caused by a heat shock are larger, stiffer, and more numerous than spontaneously formed ones. Also, aggregates measured after a one-hour recovery period were less stiff yet had a higher concentration of misfolded protein than freshly formed aggregates. We offer evidence for a multi-stage aggregate disassembly process that is most likely caused by the consecutive actions of the bacterial chaperones DnaK and ClpB.

(Wed-P4) **Chemical Characterization of Single Seeds with Fourier Transform Near Infrared Spectroscopy**

Warren Edmunds, Justin Linehan, Ryan Smith, *Perkinelmer*

High-quality, high-throughput, and non-destructive chemical analysis of individual seeds would be of great utility for agricultural crop breeding and crop improvement programs. Infrared spectroscopy is a leading analytical technique for the non-destructive chemical characterization of organic materials. When considering measuring individual agricultural seeds, matching the infrared beam spot size to the small sample sizes required for measuring individual seeds is critical for collecting high signal-to-noise spectral data. This work describes the use of beam-condensing technology through simple optics as well as using a dedicated infrared microscope system to demonstrate "proof of concept" methodology for near-infrared spectra collection and chemical characterization of individual agricultural seeds. High-throughput data collection is demonstrated by using an automated sample stage on the IR microscope, with only a few seconds required for collecting an IR spectrum for each individual seed. The chemical variability observed amongst several batches of rapeseeds was analyzed using multivariate chemometric techniques PCA and PLS regression. The infrared spectra reflect the chemistries of crop quality indicators such as moisture, oils, fatty acids, and protein contents. Given adequate reference data, chemometric models can be developed for predicting such crop quality indicators.

(Wed-P5) **An Optimized Purification Protocol for Enzymatically Synthesized S-Adenosyl-L-Methionine (Sam) for Applications in Solution State Infrared Spectroscopic Studies**

ISAIAH ODEYEMI, Teri Douglass, Nosakhare Igie, James Hargrove, Grace Hamilton, Brianna Bradley, Cathy Lee, Brenden Le, Maitra Unjia, Dylan Wicherts, **Zackery Fernyhough**, Anjali Pillai, Shailendra Koirala, Laure Hagge, Raymond Trieval, Robert Fick, Allison Stelling, *UTD*

S-adenosyl-L-methionine (SAM) is an essential methyltransferase found in all living organisms. It is the second most common cofactor after adenosine triphosphate, donating a methyl group in cells. Methyltransferases are important drug targets in order to regulate important cellular processes, however these targets are difficult due to specificity issues that result in off-target effects in this large class of enzymes. This study aims to employ SAM vibrations as label-free probes to detect any druggable alternative conformers in each methyltransferase active site or as sensors of noncovalent interactions with SAM from active site residues. This information, along with inputs from crystal structures of the lead compounds in ternary complexes and computer modeling to explore potential conformers, can be incorporated into a drug design strategy aimed at improving drug specificity. To do this, we must first assign specific signals in SAM's Raman and IR spectra to motions of groups of atoms in SAM's structure, through incorporating isotopes into different positions of the molecule, and determining which spectral signals originate from which atomic groups in the molecule. Using SAM synthetase (metK), an enzyme that catalyzes SAM synthesis, commercially available isotopically labeled adenosine triphosphate and methionine precursors can be used to generate site-specific labeled SAM. However, the established purification protocols resulted in SAM

with excess salts, and counterion which produces large signals in the IR spectrum that interfere with detecting the signals from the SAM spectrum. We report a new purification protocol that removes the interfering counterion and reduces the salt concentration in SAM and we additionally present the first IR and Raman spectra of isotopically labeled CD3 and 13C-SAM. These findings will lay the groundwork for using SAM vibrations for label-free diagnosis of specific molecular conformations as well as sensors of noncovalent interactions to the cofactor from active site residues, substrates, and substrate inhibitors.

(Wed-P6) **Inhibition of vibrational energy flow within an aromatic scaffold via heavy atom effect**

Majid Hassani, *University Of Nevada Reno*

The regulation of intramolecular vibrational energy redistribution (IVR) to influence energy flow within molecular scaffolds provides a means to control fundamental chemical processes. Two-dimensional infrared (2D IR) spectroscopy is commonly employed to assess various energy transfer pathways by analyzing changes in the intensity of vibrational cross peaks. Previous studies using 2D IR spectroscopy have demonstrated the existence of multiple energy pathways between the azido and cyano vibrational oscillators in an aromatic scaffold, which are modulated by Fermi resonance. In this particular molecular scaffold, the heavy atom effect was utilized to impede specific energy transfer pathways. By eliminating these energy transfer pathways, through-space vibrational coupling is enhanced, allowing for the direct coupling between a selenocyano probe and an azido probe to be observed for the first time. Consequently, rectification of the molecular circuitry is achieved through the suppression of anharmonic coupling, leading to the emergence of distinct transfer pathways.

(Wed-P7) **Total Value Chain Testing Solutions for Biodiesel and Bioethanol Production – from Feedstock to Final Blend**

Nicholas Lancaster, **Chady Stephan**, Aaron Hineman, *PerkinElmer*

The demand for cleaner, greener renewable fuels is rising as the world strives for net zero carbon emissions. Biofuels, including biodiesel and bioethanol/gasoline blends, are established sustainable fuels with complex production processes. This poster provides an overview of the bioethanol and biodiesel refining processes and the analytical analyses conducted at each step.

Starting with feedstocks, we focus on quality control of key first-generation feedstocks like corn. Near Infrared Spectroscopy (NIR) is used to analyze moisture, protein, and starch content in corn germ, corn gluten meal, and gluten slurry. For biodiesel, we cover important vegetable oil feedstocks and their analysis using Gas Chromatography (GC) and Fourier Transform Infrared Spectroscopy (FTIR). Determining trace elements through Atomic Absorption Spectroscopy (AAS) or Inductively Coupled Plasma – Optical Emission Spectroscopy (ICP-OES) is crucial as they impact the final product.

Moving to the refining process, High-Performance Liquid Chromatography (HPLC) is used to monitor the fermentation process in bioethanol production. NIR is again employed to determine important properties of the by-product, dried distillers grains (DDGS). AAS and ICP-OES provide key nutritional information on mineral content in DDGS. For biodiesel refining, GC plays a crucial role in monitoring the esterification process. Determining methanol content, total and free glycerol content in B100 biodiesel, and trace elements ensure compliance with standard specifications.

Transport and blending processes are also covered. Ethanol denaturation is tested using GC to meet required specifications. Quality control testing of the blending process is vital, and FTIR and GC accurately determine biofuel content in final blends such as biodiesel (B5, B20, etc.) and bioethanol (E5, E10, etc.). Purity testing detects contaminants.

These analyses are conducted in-house by refiners/blenders or third-party contract labs and are crucial for various industries in the biofuel value chain.

(Wed-P8) **Structural Elucidation of Methylpyridine Derivatives Using Complimentary Analytical Techniques Coupled with Computational Modelling.**

Joshua Johnson, Ms Michael Bishop, David Archer, Brett Marsh, *Corteva Agriscience,* Ziyan Fan, University of California, Berkley, Hannah Zimmerman-Federle, *Indiana University - Purdue University, Indianapolis*

Methylpyridine derivatives are commonly used as active ingredients and intermediates in the agrochemical industry. These derivatives have seen a rise in utilization due in part to their scalability and affordability. The synthetic auxin herbicides have utilized methylpyridine derived compounds over the past 50 years in products such as picloram, clopyralid, and aminopyralid. Additionally, modern auxin herbicide products developed through structure activity-relationship (SAR) scoping such as halauxifen-methyl (ArylexTM active) and florpyauxifen-benzyl (RinskorTM active) continue to use these methylpyridine derivatives as key starting materials.
The presence of process impurities can have deleterious downstream effects and therefore, structural elucidation of small molecule impurities and metabolites is essential and required for registration of agrochemical products to understand the toxicological impact of impurities in both biological and environmental systems.
The continued development of various high resolution and tandem mass spectrometry (MSn) techniques has allowed for structural elucidation with higher levels of confidence; however, proposing definitive structures based solely on mass spectrometric data can still be challenging especially for isomeric compounds. In addition to the structure information derived from the GC/MS and GC/MS/MS techniques, gas chromatography – infrared spectroscopy (GC-IR) provides temporally resolved IR spectra which can be used for qualitative and quantitative analysis without the need for isolation of a specific impurity which is labor intensive and time consuming. IR spectra provide information about molecular vibrational and rotational states that can be used to determine the presence of different functional groups in unknown compounds. IR spectra can also be used to differentiate isomeric compounds; however, differences in the IR spectra can be subtle and difficult to intuit. To better utilize the IR data, IR spectra were calculated and compared to experimental data and ranked for decision making purposes. Density functional theory (DFT) calculations were used to calculate the energy minimized structures and their associated IR spectra.
Herein, we describe a workflow utilizing GC/MS, GC/MS/MS, and GC/IR for structural elucidation of methylpyridine derivatives used for the manufacturing of numerous agrochemicals. Additionally, we demonstrated how DFT calculations can be integrated into workflow to compare calculated spectra to experimental data to increase the confidence of the structure assignment.

(Wed-P9) **Vibrational energy flow in complex ligand scaffolds revealed via 2D IR spectroscopy**

Christopher Mallon, Mohammad Zafar Abbas, Matthew Tucker, Ana de Bettencourt-Dias, *University Of Nevada, Reno*

The ligands and other complexing agents of lanthanide coordination complexes act as point charges, producing an electric field, that induces interesting electronic properties on the metal or lanthanide core ion. There are few accounts of how the vibrational processes in the ligand influence the electronic properties of the lanthanide. Two-Dimensional Infrared Spectroscopy (2DIR) is performed on one of these ligands, TerPyridine-NO_2, which is a potential candidate for lanthanide coordination complexes. The complexes in question are expected to exhibit magnetic hysteresis, and thus has the potential to act as single-molecule magnets (SMMs). The magnetic states in SMMs are known to relax through the interactions of the vibrational states of the ligand and therefore can only keep its magnetic moment under certain blocking temperatures. 2DIR studies of TerPyridine-NO_2 reveal a network of coupled vibrational modes in the fingerprint region spanning 1300 cm-1 and 1600 cm-1. The resulting vibrational energy scaffold can provide key insights into the ways in which energy dissipation may occur through the vibrational modes of the ligand. The large number of vibrational couplings revealed in the 2D IR spectra suggests that excitations throughout the entire molecular structure can quickly redistribute and relax through these modes.

(Wed-P10) **Sophisticated ATR correction**

Thomas Mayerhoefer, Juergen Popp, *Leibniz Institute Of Photonic Technology,* William Costa, *Fiveash Data Management, Inc.*

Attenuated total reflection (ATR) absorbance spectra deviate from the Bouguer-Beer-Lambert (BBL) approximation, with high values of the absorption index of the sample [1] commonly associated with the nonlinear response of strong oscillator peaks. Therefore, for quantitative purposes, such spectra are only usable for weak absorptions with an absorption index smaller than 0.1, according to Hansen [2]. This limitation is counterintuitive, as ATR spectroscopy is usually used to investigate strong bands, which would require very thin layers to enable transmittance measurements. The reasons for this limitation is that ATR absorbance is not only a function of the absorption index, but also of the refractive index, which leads to redshifted band maxima and changed band shapes. Advanced ATR correction formalism can partially remove these shortcomings, but a reliable correction algorithm requires the calculation of the complex refractive index functions of the sample. We demonstrate such a correction procedure, which is much more sophisticated than the so-called advanced ATR correction formalisms. This sophisticated ATR correction formalism is also fully automated, and we used it to calculate the complex refractive index function for water based on 68 s- and p-polarized ATR reflection measurements in the effective angle of incidence range of 38.81 to 58.83° using a ZnSe ATR crystal on a fully automated ATR accessory and FTIR system. As a consequence of the relatively large number of independent measurements and resulting complex refractive index functions, we were able to determine the accuracy of the determined function. Because of the relatively small standard deviations across almost the entire range of angles, we conclude that our sophisticated ATR correction formalism is a reliable way to correct ATR absorbance spectra.

Acknowledgement

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(Wed-P11) **The Effect of Agglomeration of Colloidal Particles on Near-Infrared Light Scattering Properties: Studied by Molecular Dynamics Simulation and Electromagnetic Wave Theory**

Hyeonwoo Na, Hiroyuki Fujii, Kazumichi Kobayashi, Masao Watanabe, *Hokkaido University*

Colloidal solutions with high concentrations are gaining attention in several fields, including food science and chemical engineering. In these fields, non-destructive assessment of the agglomeration state is crucial. Near-infrared spectroscopy using scattered light has the potential for the assessment because light scattering is correlated to the agglomeration state. However, spectroscopy using scattered light is still under development because the correlation still needs to be clarified. Since light is widely scattered in colloidal solutions, a new spectroscopy is required to understand the connection between light scattering properties and agglomeration states. The dependent scattering theory, a type of electromagnetic wave theory, calculates the light scattering properties in concentrated suspensions. The theory requires the static structure factor, which characterizes the local structure at several times the particle diameter. In previous studies, the hard sphere model, which treats only repulsion between particles, has been widely used in the calculation of the structure factor. However, the hard sphere model cannot account for agglomeration. In this study, we utilized molecular dynamics simulations to compute the structure factor by treating the attraction between particles. The model can reproduce the agglomeration state. We modeled the colloidal particle interactions and did not include the interaction between the particle and solvent molecule because the particle interaction is dominant. As an interaction between particles, we employed the monodisperse Lennard-Jones (LJ) model with a particle diameter of 300 nm. We calculated the structure factors at different volume fractions from 1% to 20% and combined the dependent scattering theory to calculate the light scattering properties. Our results showed that the scattering properties with the LJ model are larger than those with the hard-

sphere model at more than 5% of volume fractions. This result means that the scattering properties reflect the change in the local structure due to the attraction between particles. Thus, it suggests a strong correlation between the scattering properties and agglomeration states.

(Wed-P12) **Light scattering properties for a coagulation process in soy milk**

Koyata Nishikawa, *Hokkaido University*

Products of soy milk, such as Tofu (soybean curd) have received great attention worldwide as a nutrient-rich and cholesterol-free healthy food. A technology for nondestructive monitoring for the coagulation process of tofu is important for its quality control. Tofu making is a coagulation process of soy milk induced by heating and addition of a coagulant. During the coagulation process, solid contents, complexes of oil bodies and proteins in soy milk' aggregate and form a network structure, so called curd. Since the complex size becomes larger and larger in the process, the near-infrared spectroscopy is expected to be a powerful nondestructive monitoring tool. However, the correlation between the scattering property and the coagulation state needs to be clarified. This study aims to clarify the correlation by a time-domain diffuse reflectance method. We prepared three concentration samples (5.5%, 8.3%, and 11%) by keeping the amount of coagulant, heating time, and temperature constant. We conducted a pointto-point scheme (point source and detector) measurement of the diffuse reflectance at the wavelength of 780 nm with the source-detector distance of 1.5 cm. We evaluated the reduced scattering coefficient as the light scattering properties. The results showed that the reduced scattering coefficient increased after the coagulation for all concentrations of soy milk, and the change was greater for higher concentrations. This result suggests the reduced scattering coefficient reflects the particle size increase in the coagulation process. In addition, different amount of solids makes a difference in the degree of coagulation' and therefore it causes a difference in the change of the reduced scattering coefficient higher concentration.

(Wed-P13) **Investigating the Stability of Biodegradable, Bio-Based, and Compostable Materials with Infrared Spectroscopy and Microscopy**

Lauren Ostopowicz, Victoria Benitez, Liang Zhao, Gilbert Vial, *Shimadzu Scientific Instruments*

Usable lifespans and decomposition products of plastics have been found to be detrimental to the environment. Plastic materials require years to decompose, eventually reaching the size classified as microplastics, which easily pass through water filtration systems. These potential environmental and health hazards lead to the discovery and implementation of biodegradable, compostable, and biobased materials. Though seemingly synonymous and environmentally friendly plastic options, they require specific conditions for complete deterioration that most consumers do not know or follow. This study investigates the chemical degradation rate of consumer plastic products labeled as biodegradable, compostable, or biobased plastic products under typical composting conditions. FTIR spectroscopy and micro-spectroscopy is used to track the chemical degradation changes over time and map the specific location of deterioration. The most stable plastic material was not an environmentally friendly plastic material and showed no chemical differences over the length of the study. Biodegradable, compostable, and biobased materials are composed of different starting materials, and all deteriorate at varying rates. Additionally, Wiley's KnowItAll™ spectra searching software and libraries are used to elucidate the various chemical information from the FTIR data to help understand the mechanism by which these environmentally friendly plastic options deteriorate into the earth.

(Wed-P16) **Chiral-Specific and Interface-Specific Vibrational and Electronic Spectroscopy of Ordered Assemblies**

Garth Simpson, *Purdue*

A theoretical framework and supporting experimental measurements are described for interface-specific spectroscopy of chiral molecular assemblies based on nonreciprocal absorbance circular dichroism. Specifically, criteria and approaches are described yielding interface-specificity typically reserved for even-ordered nonlinear optical spectroscopic measurements. Experimental measurements

of naproxen thin films yield UV-Vis circular dichroism results in excellent agreement with ab initio theoretical predictions with no adjustable parameters. Because these effects are fully electric dipole allowed, the chiral-specific response is distinct from that observed in isotropic assemblies and can yield dissymmetry parameters approaching unity. Analogous methods for achieving interface specificity in fluorescence and Raman spectroscopy of chiral assemblies is also considered theoretically, corroborated by prior results from others. All of these disparate chiral-specific and interface-specific phenomena can be interpreted within a relatively simple geometric model, providing a predictive framework for connecting broad classes of chiral-specific spectroscopies to molecular structure and orientation.

(Wed-P17) **Protein-Membrane Fiber Interactions in Hemodialyzers: A Multimodal Infrared Spectroscopy and Imaging Approach**

Suruthikha Vijay, William Querido, Azita HassanMazandarani, Rouzbeh Tehrani, Nancy Pleshko, *Temple University*

Visualizing and quantifying the interactions between proteins and the hollow fibers present in a high-flux hemodialyzer is essential to understand the efficacy of hemodialyzers at the molecular level. Two proteins of interest are albumin, an essential blood protein that needs to be retained, and beta 2 microglobulin (B2M), which in excessive quantities can lead to many issues, and must be removed during hemodialysis. Maintenance of levels of these proteins is of paramount importance, and techniques that can be used to quantify the interactions between the protein and the dialyzer fiber will help in determining the efficiency of the dialyzers. This research aims to establish methodologies to assess these interactions by different modalities of infrared spectroscopy and imaging. Samples of pure Optiflux dialyzer fibers, fibers that had come into contact with porcine blood through mock-hemodialysis, and pure bovine serum albumin (BSA) were collected and lyophilized. The used fiber samples were also embedded in an optimal cutting medium (OCT), and cross-sections were obtained via cryosectioning for imaging. Data collection was carried out by (1) attenuated total reflection (ATR) Fourier transform infrared (FTIR) spectroscopy using a Nicolet iS5 spectrometer (Thermo Scientific) with a resolution of 4 cm-1 and 32 scans, (2) FTIR imaging using a Spotlight 400 (PerkinElmer) microscope with a resolution of 8 cm-1, 2 scans, and pixel size of 6.25 micrometers, and (3) by optical photothermal infrared (OPTIR) imaging using a mIRage microscope (Photothermal Spectroscopy Corp.) with 2 cm-1, 4 scans, and a pixel size of 0.5 micrometers. Spectral data were processed using the Unscrambler X, Isys, and PTIR Studio software. Our results show that these techniques can identify and map the presence and distribution of proteins incorporated into the dialyzer fibers after contact with blood. In data from all spectroscopic modalities, along with polysulfone peaks from the fibers, we were able to identify clear Amide I and II peaks from protein, which had similarities to those of the pure BSA. This highlights the feasibility of these techniques to assess the interaction between blood-derived proteins and hollow fibers in a high-flux hemodialyzer.

(Wed-P20) **Overview of Additive Manufacturing and 3-D Printing Utilizing Numerous Analytical Techniques**

Thomas Dillon, **Chady Stephan,** *Perkinelmer*

Additive Manufacturing and 3-D Printing are two important industries that are very closely linked. Not only do innovations in either field drive the other but the way these materials are analysed in their respective laboratories is remarkable similar. In Additive Manufacturing and 3-D Printing laboratories the materials will be analysed for their chemical and mechanical properties. In this work I will give an overview into the many analysis performed in Additive Manufacturing and 3-D Printing laboratories. There are many analytical techniques that can be utilized to analyse 3-D printing materials and additives. Some instruments commonly used for chemical analysis are Gas Chromatographs (GC), Liquid Chromatographs (LC), and Fourier Transform Infrared (FTIR). These instruments provide a lot of valuable data at all stages of the supply chains for Additive Manufacturing and 3-D Printing. Mechanical and Thermal Characterization is a crucial role in any Additive or 3-D Printing Laboratory. Some examples of instruments that help mechanically and thermally characterize additives and 3-D

Printing material are Thermo-mechanical Analyzer (TMA) and Dynamic mechanical analysis (DMA). Instruments that perform Thermal and Mechanical measurements are essential to any Additive or 3-D Printing laboratory.
Additive Manufacturing and 3-D Printing laboratories are just like any other analytical laboratory where they must have the right instrumentation to get the answers you need. The instruments commonly used to provide these answers are found in both Additive and 3-D Printing laboratories. An effective analytical laboratory helps provide answers along the supply chain in any industry.

(Wed-P23) **Development of a Microwave-Enhanced Glow Discharge Atomic Emission Spectrophotometer**

Mitchell Stry, Steven Ray, *Department of Chemistry, Federal University of Piauí - UFPI*

The ability to detect metals in solid materials and thin-film samples at trace-level concentrations is a valuable analysis capability widely used in a myriad of applications. A glow discharge instrument coupled with a multichannel spectrophotometer detector allows for the detection of metals at low concentrations and for depth profiling of elemental composition. In this work, a conventional glow-discharge optical emission instrument is modified to employ microwave energy to increase the emission sensitivity of analytes. This alternative excitation source uses a microwave stripline architecture to introduce microwave energy into a conventional Grimm-style glow discharge lamp. When samples are sputtered by a glow discharge in an argon plasma environment, metals collide with free electrons and are excited to emission, allowing quantification by the spectrophotometer. The coupling of microwave energy into the glow discharge plasma increases the emission signal of free atoms, resulting in an increase in detection sensitivity. The design and construction of a specialized microwave-enhanced multichannel Roland-circle based spectrophotometer will be presented, and the benefits and feasibility of microwave coupled glow discharge spectrophotometry for the detection of metals in thin films will be presented.

(Wed-P24) **Real-time upstream process monitoring by Time-gated Raman spectroscopy**

Jacopo Zini, Amuthachelvi Daniel, *Janssen Pharmaceuticals,* Mari Tenhunen, *Timegate Instruments Ltd.*

The pharmaceutical and bioprocess industries have benefited from adopting Quality by Design and Process Analytical Technology (PAT) frameworks, enabling them to effectively optimize the process, real-time control, and ensure consistent product quality. As a label-free optical method and having only minimal interference from water, Raman spectroscopy has been explored as a PAT tool to complement or substitute time-consuming offline measuring methods. However, a major technological challenge in Raman spectral measurements is the sample-induced fluorescence background that obscures naturally weak Raman signals. This study addresses the fluorescence issue by using a Timegated® Raman spectrometer and reports inline, real-time monitoring of an antibody production process in an industrial bioprocess environment. The production of lactate, glucose, glutamate, glutamine, ammonia (NH3), lactate dehydrogenase (LDH), immunoglobulin G (IgG), and total cell density (TCD), viable cell density (VCD) at different runtime in a 50-litre bioreactor at Testa Center, Sweden was followed using a Timegated® PicoRaman M3 spectrometer (Timegate Instruments Oy, Finland) with a 532 nm pulsed laser.
In this study, we compared multivariate calibration models, hard calibration methods and deep learning based on time-gated Raman spectral data were built to predict the multiple key process parameters. In conclusion, the Timegated® spectrometer is shown to be an excellent candidate for real-time upstream process monitoring.

(Wed-P25) **Timegate Raman for continuous in-line monitoring of extracellular vesicles purification.**

Jacopo Zini, Heikki Saari, *Finnish Red Cross Blood Service, Helsinki, Finland*, Saara Laitinen, Amuthachelvi Daniel, Mari Mari Tenhunen, *Timegate Instruments Ltd.*

Extracellular vesicles (EVs) are nanoparticles released by cells in their environment. In mammals, EVs can be found in all biological fluids, and they are involved in cell-to-cell communication.
Although there are no approved clinical applications of EVs yet, numerous findings and clinical trials point out that, EVs can be used as novel drug delivery system or diagnostic tool. In this context, there is an unmet need to develop technology to monitor the downstream process of large-scale EV production. Among the process analytical technologies (PAT), Raman spectroscopy (RS) can be particularly suitable for this purpose. RS is a non-destructive, label-free technique which can resolve the chemical composition of a complex system, and it can be employed as a PAT tool for the downstream purification of other biological drugs such as monoclonal antibodies.
Here, we propose a Timegated® Raman spectrometer as an inline sensor to monitor an ionic exchange chromatography-based EV purification process. Chromatography processes are often monitored by UV–visible spectrophotometry. UV data reveal the presence of biological material in the elution buffer of the chromatography process; however, the data contain little information regarding the biochemical nature of the eluted material. Time-gated Raman data collected throughout the chromatography run disclose the biochemical composition of the eluted material. These data allow, not only the identification of the EV-containing chromatography fractions, but also effectively monitor the purification process pointing out eventual failure i.e., overload of the chromatography column or presence of contaminates in the EV fractions.
Our findings suggest that time-gated Raman spectra obtained by Timegated® PicoRaman M3 spectrometer (Timegate Instruments Oy, Finland) with a 532nm pulsed laser can be used to discriminate the EVs containing chromatography fractions from the fractions containing impurities.

(Wed-P26) **Electronic structure of lipid thin films using attenuated total reflectance spectroscopy in the far-ultraviolet region**

Yoshiki Hanjo, Yusuke Morisawa, *Kindai University*

We have developed a new spectroscopic technique using attenuated total reflection (ATR) in the far-ultraviolet (FUV) region; This technique allows us to observe electronic transitions from the σ orbital, which forms the backbone of organic molecules. Previous studies (Morisawa et al. 2015) have shown that the ATR-FUV spectra of low-temperature solid n-alkanes exhibit a decrease in excitation energy, suggesting changes in electronic state of the σ orbital due to interactions between alkyl chains. Inspired by these findings, our study aims membrane structural analysis through intermolecular interactions between alkyl chains in lipid bilayers.
We measured the ATR-FUV spectra of representative lipids, monostearin and monoolein, in cast films, lipid bilayers, and solutions. The obtained absorption band assignments were compared with quantum chemical calculations. We also investigated the differences in the spectra among cast films, lipid bilayers, and solutions. To prepared the cast films we cast monostearin and monoolein in 10 mmol/L chloroform. Lipid bilayers were prepared according to refence [1], and spectra were measured using our ATR-FUV spectrometer with the FUV optical path purged with Ar gas. Additionally, we varied the angle of incidence from 60 to 75 and measured the ATR-FUV spectra to observed changes in spectral response to the penetration depth.
In our presentation, we will discuss the band assignment of FUV absorption monostealin and monoolein, for cast film, lipid bilayers, and solutions. Furthermore, we will explore the dependence of the spectra on the penetration depth through measurements with varying incidence angles.

(Wed-P27) **Investigating the Solution Structure of Hydrate Melt (HM) using NearInfrared Spectroscopy**

Shoichi Higashi, Yusuke Morisawa, *Kindai University*

Conventional aqueous systems are not suitable for batteries with a wide potential window due to water electrolysis at 1.23V. However, they offer significant advantages such as fluidity and nonflammability. Hydrate melt (HM), a aqueous liquid electrolyte, is composed of two salts, bis[trifluoromethylsulfonyl]imide (TFSI) and bis[pentafluoroethanesulfonyl]imide

(BETI), along with a lithium ion in a ratio of LiTFSI:LiBETI:H_2O = 0.7:0.3:2 (Li-HM). Despite being an aqueous electrolyte, HM exhibits a wide electrochemical stability range, allowing for a potential window of over 3.0V. As the use of lithium-ion batteries continues to expand, sodium-ion batteries have also gained attention for their cost advantage and potential as large-scale storage batteries. This study focuses on investigating the properties of HM using near-infrared spectroscopy, specifically examining the differences in solution structure between lithium and sodium.
Infrared and Raman spectroscopy of the fundamental transition of OH stretching vibration region often overlook the signals of non-hydrogen-bonded environments due to the increased absorption intensity of hydrogen bonding component. This experiment aims to explore water-water hydrogen bonding and water-electrolyte intermolecular interactions in the $\nu_1+\nu_3$ transition region (OH overtone region) observed between 7000 and 6000 cm-1. Measurements were conducted using an FT-NIR spectrometer with a resolution of 4 cm-1.
For sodium HM, NaTFS and NaTFSI were used in this experiment. The NIR spectra in the $\nu_1+\nu_3$ region of water were measured for aqueous solutions of NaTFS at concentrations ranging from 1 to 5 mol/L, as well as a bisalt aqueous solution containing 5 mol NaTFS and 1 mol NaTFSI (Na-HM). The NIR spectrum of NaTFS mono-salt aqueous solutions and Na-HM, along with its second derivative, were compared with those of LiTFSI aqueous solutions and Li-HM. An absorption peak at 7180 cm-1 was observed in Li-HM, which was not observed in Na-HM. This band was not observed in the mono-salt LiTFSI solution, but only in Li-HM. In the NaTFS/TFSI solution observed in this study, the 7180 cm-1
component was not observed, suggesting that the solution structures of Li-HM and Na-HM are different.

(Wed-P28) **Development and Applications of Full-Vacuum FT-IR with Fully Automatic Switching Mechanism of Optical Elements for Seamless Analysis in Wide Wavenumber Range from Far-infrared to Near-infrared**

Kohei Tamura, Ken-ichi Akao, Yukihiro Ozaki, *JASCO Corporation*

The Fourier Transform-Infrared Spectrometer (FT-IR) has the ability to measure a wide range of wavelength from the visible to far infrared regions by changing optical elements such as the light source, beam splitter, window, and detector. In addition to the mid-infrared region which gives general molecular vibration information, the near-infrared region which provides information on overtones and combinations of molecular vibration, and the far-infrared region which yields information about crystal polymorphs and intermolecular interactions, can be obtained by one instrument.
However, changing these optical elements requires complicated operations. For example, nitrogen purge is often required to avoid overlapping of the absorption of water vapor and carbon dioxide on the optical path as noise in a spectrum. It is always necessary to break purge condition in the optical path and purge again whenever the optical elements are changed.

We have already developed the 'full-vacuum FT-IR' in which whole optical path can be evacuated for more efficient measurement than nitrogen purge method. This method is extremely effective in the far-infrared region, where the interference of water vapor is significant.
For more efficient analyses, we have developed the system with the automated changing mechanism of the optical elements, which can measure from 25,000 cm-1 to 20 cm-1 under vacuum condition without releasing vacuum condition.
In addition, we have recently advanced the beam splitter and detector for broadband measurement, and achieved to measure 6000 cm-1 through 30 cm-1 without changing any optical elements.
By combining this newly constructed FT-IR with the ATR accessory, which transmits light in wide wavenumber region, the system that can measure the wide range of spectrum from near- to far-infrared region without complicated operations or pretreatment has been completed.

In this presentation, the system configuration and features of this developed FT-IR for broadband measurement are described.
Furthermore, we will display the results of one-shot measurement of water spectra in the near- and far-infrared regions and the mid- and far-infrared spectra measured under vacuum conditions to

demonstrate the usefulness of this system and to discuss crystal forms of inorganic materials, which have been difficult to obtain in the past.

(Wed-P29) **Amyloidogenic and non-amyloidogenic molten globule conformation of β-lactoglobulin in self-crowded regime**

Sara Venturi, Barbara Rossi, Mariagrazia Tortora, *Elettra - Sincrotrone Trieste*, Renato Torre, European Laboratory for Non-Linear Spectroscopy, University of Florence, Andrea Lapini, Department of Chemical, Life and Environmental Sustainability Sciences, University of Parma, Paolo Foggi, University of Perugia, Marco Paolantoni, University of Perugia, **Sara Catalini,** *Department of Physics and Geology, University of Perugia*

Molecular insights on the β-lactoglobulin thermal unfolding and aggregation are derived from FTIR and UV Resonance Raman (UVRR) investigations. We propose an in situ and in real-time approach that thanks to the identification of specific spectroscopic markers can distinguish the two different unfolding pathways pursued by β-lactoglobulin during the conformational transition from the folded to the molten globule state, as triggered by the pH conditions. For both the investigated pH values (1.4 and 7.5) the greatest conformational variation of β-lactoglobulin occurs at 80°C and a high degree of structural reversibility after cooling is observed. In acidic condition β-lactoglobulin exposes to the solvent its hydrophobic moieties in a much higher extent than in neutral solution, resulting on a highly open conformation. Moving from the diluted to the self-crowded regime, the solution pH and consequently the different molten globule conformation select the amyloid or non-amyloid aggregation pathway. At acidic condition the amyloid aggregates form during the heating cycle leading to the formation of transparent hydrogel. On the contrary, in neutral condition the amyloid aggregates never form. Information on the secondary structure conformational change of β-lactoglobulin and the formation of amyloid aggregates are obtained by FTIR spectroscopy and are related to the information of the structural changes localized around the aromatic amino acid sites by UVRR technique. Our results highlight a strong involvement of the chain portions where tryptophan is located on the formation of amyloid aggregates.

(Wed-P30) **Optical and Structural Characterization of Plasmonic Nanocrescents as a Tunable Platform for Vibrational Circular Dichroism Spectroscopy**

Flore Elliott, *Westminster University*, Aria Ballance, Jennifer Shumaker-Parr, *University Of Utah*

Homochirality, where one enantiomer dominates over the other, plays a crucial role in biological and chemical processes. Understanding which enantiomer interacts with biological targets is vital for drug design. In the wake of the thalidomide incident, the Food and Drug Administration created a policy requiring the complete configuration of chiral compounds to be known. The issue with detecting chiral molecules is that their signals are too small to be interpreted when using optical techniques. Plasmonics offers a new method of identification by enhancing the optical responses of the molecules of interest. Localized surface plasmon resonance (LSPR) is the collective oscillations of conduction electrons on metallic nanostructured surfaces and can be measured in the ultraviolet-visible (UV-Vis) and infrared spectrum. Gold nanocrescents (NCs) with a range of sizes were fabricated as structures with sharp tips to enhance the electromagnetic field (EM) surrounding the crescents. To identify the most suitable NCs for experiments with chiral molecules, samples were analyzed based on their extinction when oriented at non-normal angles to the incident light. Atomic force microscopy (AFM) and scanning electron microscopy (SEM) were used to conduct three- and two-dimensional measurements of the size and height of the structures. AFM analysis shows NCs have a uniform tilt profile due to the deposition angle, along with varying heights of the structures. UV-Vis spectroscopy was utilized to determine the LSPR of NCs of varying sizes. Using linearly polarized light, each sample was rotated -30, 0, and +30 degrees with respect to the incident light. Of the NC samples analyzed, two of the samples showed promising LSPR spectra with strong plasmon bands and high extinction. We found that all samples exhibited the highest extinction at rotations of +/-30° and resulted in negligible differences between the two angles. The studies will be used to determine what

structural properties and optical behavior may be beneficial for developing NCs as a tunable platform for plasmon-enhanced chiroptical spectroscopy detection of chiral molecules.

(Wed-P31) **Effects of Crime Scene Contaminants on Surface-Enhanced Raman Analysis of Hair**

Isaac Juarez, Dmitry Kurouski, *Texas A&M*

Forensic analysis of hair is important as hair is one of the most commonly examined forms of trace evidence found at crime scenes. A growing body of evidence suggests that surface-enhanced Raman spectroscopy (SERS), a label-free and non-destructive analytical technique, can be used to detect and identify artificial colorants present on hair [2]. However, hair collected at crime scenes is often contaminated by substances of biological and non-biological origin present at such locations. In this project, we investigated the extent to which abiotic and biotic contaminants can alter the accuracy of SERS-based detection and identification of both permanent and semi-permanent colorants present on hair. In addition, we sought to understand the role that differing physical characteristics of contaminants played on influencing the acquired spectra.
Our findings show that some contaminants can reduce the intensity of the colorants' signals but do not obscure their detection and identification, while exposure to other contaminants, such as bleach or blood, eliminates SERS-based analysis of artificial dyes present on these samples. Regarding physical characteristics, we discovered that contaminant opacity generally does not affect the spectrum quality, but high contaminant viscosity does. We also found that contamination by acidic substances could destroy the colorant's spectral identity altogether, eliminating any future SERS-based colorant detection. Lastly, we found that washing the hair from the contaminated substance allowed for the underlying spectra to be identified around 95% of the time. These findings provide clearer insight to the potential and limitations of SERS in forensic investigation of colorants on trace hair evidence.

(Wed-P32) **Quantifying the ultrafast and steady-state molecular reduction potential of a plasmonic photocatalyst**

Chris Warkentin, Renee Frontiera, *University Of Minnesota*

Plasmonic materials are promising photocatalysts that interact strongly with light to produce energy-rich nanoscale environments. Hot charge carriers and heat produced in these environments can be harnessed to drive chemical reactions using visible and near infrared light. Though hot electron transfer has been suggested as the driving force in numerous plasmon-driven reactions, to date there have been no direct molecular assessments of the rate and efficiency of plasmon-to-molecule electron transfer, nor the energy of these electrons. Direct investigations of real-time dynamics in plasmon-induced chemical reactions are complicated by the rapid timescales involved and the fact that plasmonic optical cross sections are significantly larger than their molecular counterparts. In this work, we use ultrafast surface-enhanced Raman spectroscopy (SERS) to overcome these issues and quantify the transfer of electrons from a nanostructured gold plasmonic substrate to adsorbed methyl viologen molecules. Changes in the vibrational spectra of methyl viologen indicate a transient increase in reduced species after plasmon excitation, which we confirm with SER spectroelectochemistry on the same substrates. We report a reduction yield of 2.4 - 3.5% on the picosecond timescale, with plasmon-induced potentials ranging from -3.1 to -4.5 mV. Interestingly, a small fraction of these reduced molecules appear to be stabilized on the surface and remain for minutes. By quantifying plasmon-driven reduction in both the ultrafast and steady-state, we provide new tools and metrics toward optimizing these material-molecule interactions for selective and efficient photocatalysis.

(Wed-P33) **High sensitivity amplitude, phase, and polarization measurements for thin layer analysis with quantum cascade laser frequency combs**

Markus Mangold, Raphael Horvath, Jakob Hayden, Brianna Blevins, *IRsweep*
Nataraja S. Yadavalli, The *University of Georgia Athens*, Sergiy Minko, Andreas Furchner, *Helmholtz-Zentrum Berlin für Materialien und Energie*, Karsten Hinrichs, *Leibniz-Institut für Analytische Wissenschaften – ISAS*

The analysis of thin layered materials is challenging because of the inherently weak signals that are associated with thin layers. Ellipsometry relies on measurements of optical phase delays and polarization changes for studying composition and thickness of thin films. Recently, polarization dependent investigations with high spatial and temporal resolutions at high sensitivity and high spectral resolution became available by development of infrared laser based polarimetric methods. However, single wavelength laser techniques generally suffer from long measurement times to obtain a complete spectral analysis of a sample.
Here, we present quantum cascade laser dual-comb spectroscopy as a broadband laser technique for sensitive measurements of the reflectivity, dispersion, and anisotropy of thin layers. In a cryogenic surface reflectivity measurement concentrating on highest amplitude sensitivity, we showed that a reflectivity change associated with the formation of only 0.1% of a monolayer of methane is readily observed.
In a polarimetric study, we successfully demonstrated measurements of the polarization dependent complex transmission of a nanofiber scaffold.(1) Co-polarized (T_ss and T_pp) as well as cross-polarized (T_sp) transmission were studied. The phase sensitivity of asymmetric dual-comb spectroscopy allowed for simultaneous measurement of the corresponding phase delay, yielding the complete complex anisotropic transmission matrix. Measurements with an integration time as short as 65 µs covering more than 90 cm-1 centered at 1245 cm-1 with a spectral resolution of 0.3 cm-1 were demonstrated.
The demonstrated high amplitude, phase, and polarization sensitivity of quantum cascade laser dual-comb spectroscopy sets new standards for highest accuracy in thin film analysis. The short measurement time obtained in dual-comb polarimetry opens the door for studies of dynamic processes or rapid hyperspectral polarimetric imaging.

(1) Hinrichs, K.; Blevins, B.; Furchner, A.; Yadavalli, N. S.; Minko, S.; Horvath, R.; Mangold, M. Mid-Infrared Dual-Comb Polarimetry of Anisotropic Samples. Nat. Sci. 2023, 3 (2), e20220056. https://doi.org/10.1002/ntls.20220056.

(Wed-P34) **Quantitative evaluation of IR and corresponding VCD spectra**

Thomas Mayerhoefer, Singh, Jer-Shing Huang, Christoph Krafft, Juergen Popp, *Leibniz Institute Of Photonic Technology*

Vibrational circular dichroism (VCD) offers a way to determine the absolute configuration of a chiral molecule by comparing experimental and by quantum mechanical calculations computed spectra.1,2 In addition, classical treatments of IR and VCD spectra can provide valuable information. Such a classical treatment is dispersion analysis which is based on wave optics and dispersion theory.3 Dispersion theory was extended by Born and Kuhn between 1915 and 1930 to cover also chiral substances.3 This extension introduced coupling into systems of originally non-interacting damped harmonic oscillators. Accordingly, to quantitatively describe the dielectric function and the chiral admittance functions on which IR and VCD spectra are based, pairs of coupled oscillators are used. In addition to oscillator strength, damping and oscillator position, two more parameters are introduced per oscillator pair, which is the vertical distance between the coupled oscillators and the coupling constant. We determined the dielectric functions and chiral admittance functions of alpha-Pinene and Propylene oxide from their ATR and VCD spectra. These functions were modelled using pairs of coupled damped harmonic oscillators using one oscillator per peak. The coupled oscillators do not need to be directly adjacent concerning their oscillator positions, which complicates the assignment of conjugated pairs. On the other hand, conjugated oscillators cause bands with different signs the band areas of which are equal. As long as there is no band overlap, this greatly helps to identify the conjugated pairs. The coupling constants can also significantly affect the conventional IR spectra of chiral compounds, as coupling not only shifts peaks, but can also transfer oscillator strengths from one of the conjugated oscillators to the other.

Acknowledgement

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[3] Mayerhöfer, T. G.; Singh, A. K.; Krafft, C.; Popp, J.; in preparation.

(Wed-P35) **Cavity Ring-Down Spectroscopy: High Sensitivity in Littoral Environments**

Eric Languirand, Ian Pardoe, *DEVCOM CBC*

Cavity ring-down spectroscopy (CRDS) is a technique that can utilize the mid-infrared region of the electromagnetic spectrum to interrogate the vibrational modes of chemicals. CRDS is generally more sensitive than traditional gas-cell point detectors as the technique utilizes highly reflective mirrors within the cell making an effective optical path length on the order of kilometers. These type of systems therefore may provide more sensitive detection techniques than what is traditionally used.

This talk discusses some results from the Technology Experimentation and Characterization Field Trials (TECFT) at the Potomac River Test Range (July 2023), a test event in which chemical vapor and bioaerosol challenges are presented to developmental sensor technologies in an outdoor littoral, operationally relevant environment. A broad-band CRDS built from commercially-available components was evaluated at this event, and the results are compared directly to a representative, well-characterized commercially-available traditional FTIR point vapor detection system. This talk discusses some of the advantages and challenges associated with broad-band CRDS.

(Wed-P36) **Understanding Kink Formation In Cellulose Nanocrystals**

Sung Park, Yun Jing, Jia Hui Lim, Edgar Rauch, Yu Ogawa

Nanocelluloses, including cellulose nanocrystals (CNCs) and nanofibers (CNFs), are a class of fibrous crystalline nanomaterials extracted from land plants. They attract significant attention as an alternative to synthetic materials for their abundance, renewability, low toxicity, and excellent material properties. One under-explored feature of nanocelluloses, in the understanding of their structure-property relationship, is kink defects, i.e., sharp bends along the fiber axis of the nanoparticles. Kinks are common defects in fibrous materials, including carbon nanotubes, amyloid fibrils, and even macroscopic cellulose fibers. The nanoscale defects of soft materials, however, are less studied in comparison to those in hard solids, partly due to the lack of suitable analytical methods. In this study, we combine low dose scanning nanobeam electron diffraction (SNBED), infrared photo-induced force microscopy (IR PiFM), and molecular modeling to analyze the kinks observed in cellulose nanocrystals. The SNBED analysis provides spatially resolved crystallographic information on individual CNCs whereas IR PiFM provides local molecular information at and away from the kinks. This combination approach allows us to arrive at the structural details of soft matter defects at molecular and near-atomic resolutions. Such an approach is widely applicable to various semi- and polycrystalline soft materials. A greater understanding of the defect structure will lead to better exploiting the potential of soft materials, including biological and bio-based materials.

(Wed-P37) **Widefield Super-Resolution IR Imaging with Fluorescence Enhanced Photothermal Infrared Spectroscopy**

Mustafa Kansiz, Jay Anderson, *Photothermal Spectroscopy Corp.*

Optical Photothermal Infrared (O-PTIR) spectroscopy has established itself as a breakthrough vibrational microspectroscopy tool, offering significant advantages over the traditional FTIR/QCL & Raman spectroscopy, providing submicron simultaneous IR+Raman and fluorescence imaging, in non-contact mode with high sensitivity without any dispersive scattering artefacts.

O-PTIR has generated significant research interest and publications, however there still exists a demand for rapid, high sensitivity and high resolution widefield IR imaging. To this end, we have developed a novel widefield super-resolution IR imaging approach that utilizes the fluorescent signal directly for IR signal extraction. As the fluorescent signal is captured with a 2D fluorescence camera, this generates, simultaneously, widefield IR imaging as well as widefield fluorescence images. We have termed this - Fluorescence-Enhanced Photothermal Infrared (FE-PTIR) spectroscopy.
The key enabling factor here, is that when the wavelength of the IR pulses is tuned to a molecular vibration of fluorescently labeled molecules, the absorbed heat causes a modulation in the amount of fluorescent light emitted from the fluorophores and its surrounds. Coupled with the parallel data acquisition via the 2D (megapixel) visible fluorescence camera, using a standard glass objective of 50x, 0.8NA, single field of view for IR of 70x70um with 200nm pixels are possible. Compatibility with other standard visible glass objectives such like those with higher NA, or even immersion objectives opens up further possibilities for widefield super-resolution IR imaging.
FE-PTIR thus allows the IR spectroscopic analysis of specifically labeled regions (or autofluorescence) of biological cells and tissue, for example to study conformational stages of a specifically labeled class of target proteins.
FE-PTIR can enable the study protein misfolding associated with neurodegenerative diseases. Various examples from these applications will be provided.

(Wed-P38) **On-site quality screening of pisco distillates based on spectroscopy techniques**

Yalan Wu, Didem Aykas, Ahmed Menevseoglu, Siyu Yao, Luis Rodriguez-saona, *The Ohio State University*

Pisco is a Peruvian brandy obtained from the distillation of fermented Peruvian grape musts and juices. The distillation aims to recover the maximum amount of ethanol and positive characteristic aromas while minimizing the undesirable (methanol, copper, furfural and acetaldehyde) compounds in the distillate. Analytical methods for monitoring quality in spirit drinks are highly time-consuming and labor-intensive. We aimed to provide the Pisco industry with predictive algorithms interfaced with field-deployable portable spectroscopy (UV and FT-MIR) sensors for predicting multiple quality traits in their distillate products. In total 153 Pisco samples, 78 were obtained from the Peruvian market and 75 were donated by Bodega San Nicolas. The reference data of ethanol, methanol, acetaldehyde, ethyl carbamate and furfural content were analyzed by Gas Chromatography-Mass Spectrometry (GC-MS), and copper levels were assessed by Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES). Spectral data was acquired using a portable FT-MIR spectrometer (700 to 4000 cm-1) and UV-Vis system. Data analysis method by Partial least squares regression (PLSR) model was used to develop prediction models. Our reference results showed that in 11% of the samples ethanol level was below the legal limit (38%) indicating possible dilution with water. In addition, 53% of Pisco samples had higher levels of copper than the allowed legal maximum amount (5 mg/L). Nonetheless, ethyl carbamate and furfural concentrations in Pisco were not a safety concern. PLSR algorithms successfully predicted the ethanol (RP 1.0 and SEP 0.59 mg/100 mL), methanol (RP 0.92 and SEP 0.07 mg/100 mL) and acetaldehyde (RP 0.90 and SEP 0.23 mg/100 mL), copper (RP >0.95) contents in Pisco samples based on FT-MIR. Therefore, our results demonstrate that portable FT-MIR and UV-Vis spectroscopies are promising tools to predict quality parameters in Pisco samples providing reliable and rapid assessments for alcoholic beverage quality control purposes with less sample preparation and personnel training.

(Wed-P40) **Investigating local environment effects on intramolecular proton transfer dynamics in anthrarufin films**

Yingshi Feng, Christopher Jeffrey, Matthew Tucker, University of Nevada, Reno

Energy flow in small molecules is pivotal to the survival of many organisms. For example, UV screening compounds can dissipate large amounts of UV radiation to avoid apoptosis. The mechanisms for energy dissipation involve hydrogen bonding, proton transfer, conformational motions, and dumping into the vibrational manifold. Anthrarufin is a compound with a well-established pathway of energy flow. It undergoes either a single or dual intramolecular proton transfer.

The dependence of anthrarufin films on local environments is still far from understood. In this work, we found the time constant of anthrarufin photodegradation was decreased by a factor of 3 when enclosed in a polymer sheath. Ultrafast transient absorption will allow us to assess the excited state dynamics of proton transfer and vibrational relaxation to uncover the mechanisms within different environmental conditions.

(Wed-P41) **Standoff Infrared Detection of Thin-Layer Deposits Using Synthetic Reference Signatures from the Optical Constants**

Oliva M. Primera-Pedrozo, Charmayne Lonergan, Jeremy Erickson, Sarah Burton, Bruce Bernacki, Molly Rose Kelly-Gorham, Michael Wilhelm, Brenda Forland, Kendall Hughey, Tanya Myers, Timothy Johnson

Infrared spectroscopy has been used for many applications, but detection of surface deposits or stains remains challenging due to complex manner in which the liquid or solid (particle) deposits interact with light. In this study we report first efforts that use the laws of optical physics (scattering theory, Fresnel equations and the Beer-Lambert law) along with the complex indices of refraction to synthetically model or predict the spectra that are anticipated. The spectra are then added as endmembers to a spectral library used in the search algorithm to identify unknowns. To test the method, solid and liquid analytes were deposited as thin layers (e.g. ~2 to 100 μm) on painted and bare substrates using an ExactaCoat ultrasonic spray coater for use in standoff infrared measurements as well as laboratory hemispherical reflectance (HRF) spectra. The surface loading was controlled via a) analyte concentration (for solids) and b) programming the number of passes on the ultrasonic sprayer. To enable detection for different layer thicknesses and morphologies the use of physics-based synthetic infrared spectra to serve as endmembers in a spectral database is considered. The optical constants n and k were first used to generate a set of infrared reflectance spectra for different thicknesses of chemical layers (e.g. acetaminophen, methylphosphonic acid etc.) on various conducting and insulating substrates such as aluminum, wood, and glass. To gauge success, detection results using the synthetic spectra were compared to the results from laboratory HRF spectra collected for the same sample planchets used in the field standoff measurements. Preliminary results indicate good agreement between the synthetic reference data as compared to the lab-measured HRF data in terms of their ability to quantitatively predict longwave infrared data. The synthetic spectra were also compared with the standoff field reflectance data, and, using a first-order approximation, analysis found the thickness estimates in reasonable agreement.

(Wed-P42) **Flipping Out (Or In) About Vitamin E: What Nonlinear Optical Spectroscopy Can Teach Us About the Movement of Vitamin E Through Membranes**

Joshua Taylor, John Conboy, *University of Utah*

Vitamin E is a well-known lipid-soluble antioxidant that scavenges reactive oxygen species within the membrane to mitigate lipid peroxidation of the bilayer. Recently, molecular dynamic simulations have suggested vitamin E's scavenging capabilities are facilitated by nonenzymatic transmembrane translocation (flip-flop) of vitamin E between the two leaflets of the membrane. However, experimental evidence for vitamin E flip-flop is lacking, with most evidence pointing towards vitamin E acting as a membrane stabilizer embedded within the membrane. Here we show some of the first experimental evidence of vitamin E flip-flop in planar-supported lipid bilayers using the nonlinear optical technique of counter-propagating second-harmonic generation, which allows for direct measurement of vitamin E flip-flop without chemical or physical modification to the membrane. Studies on vitamin E at both biological and supraphysiological concentrations were conducted to determine its influence on vitamin E flip-flop and the phospholipid matrix. These results are some of the first to describe vitamin E translocation kinetics and thermodynamics through the membrane and opens the door to studying the kinetics of other minor constituents within membranes.

(Wed-P43) **Integrating AI-based Software RAMANMETRIX and Raman Spectroscopy for Real-time Analysis of Complex Bioprocesses**

Olivia Treuheit, Joerg Weber, Biophotonics Diagnostics Gmbh, Oleg Ryabchykov, Darina Storozhuk, Leibniz Institute of Photonic Technology (IPHT), Oliver Valet, MIBIC GmbH & Co KG

The accurate measurement of complex bioprocesses is crucial for the efficient production of pharmaceuticals and plays a significant role in food production. These intricate processes heavily rely on living organisms, necessitating the use of fast and high-performing process analytic instruments for precise control and timely intervention during perturbations.

In this study, we investigate the application of our AI-based software tool, RAMANMETRIX™, in conjunction with Raman spectroscopy for analyzing a complex multicomponent bioprocess. Specifically, a modified E. coli strain is cultivated in a fermenter and fed with glycerol. Samples are collected at regular intervals, and their spectra are recorded using a Wasatch Photonics Raman spectroscope. Additionally, these samples are subjected to analysis using high-pressure liquid chromatography (HPLC). RAMANMETRIX™ is employed to process and evaluate the collected data, involving steps such as data import, calibration, baseline correction, normalization, quality filters, and model building. Laboratory analysis is used as the reference for comparison. The resulting model, along with its associated parameters, can be saved and utilized for prompt evaluation of future samples, eliminating the need for time-consuming laboratory analysis. By monitoring key metabolites such as the carbon source and organic acids in real time, the process can be effectively controlled if required.

Furthermore, we explore the feasibility of online evaluation to enable live sample analysis without interfering with the ongoing bioprocess. To achieve this, a probe was employed in a viewport, enabling continuous spectrum recording. The spectra obtained from the above-mentioned method, along with HPLC evaluations conducted in the laboratory, serve as references for comparison.

This study demonstrates the potential of integrating AI-based software and Raman spectroscopy for real-time analysis of complex bioprocesses. The proposed approach offers a rapid and non-intrusive means of monitoring and controlling bioreactor synthesis, thus facilitating efficient pharmaceutical and food production.

(Wed-P44) **Precise Testing of Surface Modification/Functionalization Processes for Biotech Devices**

Sung Park, Derek Nowak, Padraic O'Reilly, Molecular Vista,

Surface modification or functionalization is used to manage the interaction of biomolecules, bacteria, viruses, and other molecules with the surface and is utilized in many industries including biotechnology, tissue engineering, biosensors, and semiconductor. While many methods are being developed for achieving the desired modification and functionalization, there remains a critical need for an analytical characterization tool for these processes since the existing techniques lack the nanoscale spatial resolution or sensitivity that many advanced applications require. The few surface-sensitive techniques such as ToF-SIMS and XPS are either destructive to the sample or cumbersome to use due to the requirement of vacuum in addition to falling short in spatial resolution. Out of practical necessity, water contact angle measurements are used to monitor the success of the functionalization step. This can lead to a false perception of success since it is blind to the actual identity of the molecules on the modified surface. In this talk, a nanoscale analytical technique called infrared photo-induced force microscopy (IR PiFM) is introduced. IR PiFM adds IR spectroscopy to atomic force microscopy (AFM) to enable sub-5 nm spatial resolution and sub-monolayer sensitivity for precise characterization of molecular identity and chemical state of organic and inorganic surfaces via their IR signatures. IR PiFM is robust enough and can be offered with precise motorized stages to monitor the surface of functionalized 8” wafers in biotech manufacturing processes. IR PiFM studies of various functionalized surfaces, both intentional and unintentional (i.e., contaminated), will be presented.

Thursday, October12, 2023
Oral Presentations

23AES04: Emerging Leaders, Southern Pacific F
Chair: Lisa Flanagan
Co-Chair: Alan Jiang

(AES-04.1)**Paper-based Microchip Electrophoresis for Point-of-Care Infectious Disease Testing**

Yi Yang, **Ran An,** *University of Houston*

Point of care (POC) infectious disease diagnostics and monitoring require rapid, accurate, and ideally quantitative tests to provide timely and proper treatment for patients. The current gold standard for diagnosing and monitoring infectious diseases are Reverse transcription-polymerase chain reaction (RT-PCR) and microarray hybridization due to their high detection accuracy of viral genome. However, these technologies require state-of-the-art laboratory infrastructure, including phlebotomists and skilled technicians, which are typically scarce or non-existent in low- and middle-income countries, where infectious diseases are most prevalent. Current POC solutions for detecting infectious diseases are POC PCR and antibody-based tests. However, POC PCR tests only provide qualitative disease detection, but not viral load quantification, which is essential to determine treatment for patients. Antibody based identification still limits timely virus detection due to the time-consuming process, including antibody incubation and target recognition. As a result, there is a dire need for a test at the point of need that is affordable, accurate, easy to use, with short turnaround times, that enables detection of infectious diseases in quantitative/semi-quantitative manners. Previously, we have developed a paper-based microchip electrophoresis system for quantitative hemoglobin level measurement and hemoglobin variant identification. Here, we have further developed this technology to conduct paper-based DNA electrophoresis as a detection method to quantify PCR-amplified DNA amplicons.
Our results demonstrated consistent separation and real-time tracking for these two electrophoresis markers, Xylene Cyanol and bromophenol blue, which are both widely used as agarose gel electrophoresis markers that migrate similarly to 3000 and 300 BP in 1% gel, respectively. In a customized benchtop system, we have demonstrated the feasibility of using Gazelle microcartridges to enable DNA electrophoresis on cellulose acetate paper pre-wetted with SYBR green in 1X TBE buffer. The total intensities of DNA bands were linearly associated with the DNA concentration. Additionally, within the 6 minutes tests, the electromigration speeds were inversely associated to the size of DNA fragments. Together, these preliminary results demonstrate the potential for paper-based microchip electrophoresis platform to provide rapid and consistent separation and (semi)-quantification of DNA for quantitative/semiquantitative infectious disease detection/monitoring.

(AES-04.3)**On the use of nonlinear electrokinetics for cell separations**

Alaleh Vaghef Koodehi, Curran Dillis, Adrian Lomeli Martin,, Blanca H. Lapizco-Encinas, Olivia D. Ernst, Rochester Institute of Technology

During the last decade, there has been a growing interest in the study of nonlinear electrophoresis (EP) of colloidal particles. The present work is focused on studying the influence of particle size and particle electrical charge on the nonlinear electrophoretic migration of particles. In order to design effective particle separations, it is necessary to understand how the mobility of the nonlinear electrophoretic migration of particles and cells depends on particle size and particle electrical charge. Presented here is the experimental assessment of the mobilities of colloidal polystyrene microparticles under the nonlinear electrophoretic regime considering two distinct electric field dependences (E^3 and

E^{3O2}). Nine different types of polystyrene microparticles were divided into two groups to study the effects of particle size and charge separately. The results showed that both mobilities had similar relationships with particle size and charge. The magnitude of both mobilities of the nonlinear electrophoretic velocities decreased with increasing magnitude of particle charge and increased with increasing particle size. Although the observed trends were not perfect, the results still provide valuable information. These findings will be useful in designing efficient charge-based and size-based separation of particles and cells.

Acknowledgments:
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(AES-04.4)**Integrated on-chip nucleic acid purification using isotachophoresis for sequencing applications**

Crystal Han, *San Jose State University*

The next-generation sequencing (NGS) has become an imperial tool in biomedical research, disease diagnosis, and predictive medicine since it provides a plethora of biological information. Regardless of application areas, all samples must be carefully prepared before being loaded for sequencing via a set of biochemical protocols including lysis, fragmentation, nucleic acid (NA) extraction, ligation, amplification, and size selection. In the most dominant NGS method, namely short-read sequencing by synthesis, a precise size selection of DNA is a critical step since the suboptimal size selection would cause inefficient cluster formation and poor performance. The conventional methods for DNA extraction and size selection mostly rely on the DNA-binding beads or columns, which are not amenable to automation, multiplexing, and integration due to high sample input requirements, a large volume of costly reagents, and lengthy protocols. In this presentation, on-chip isotachophoresis will be introduced as an emerging alternative to traditional nucleic acid purification. ITP is a type of electrokinetic method where heterogenous buffers are used to preconcentrate target molecules while rejecting others based on their electrophoretic mobility. It can be easily integrated with various electrophoretic separations to provide size selection and extraction simultaneously on-chip. The ITP-based integrated NA purification method is especially suitable for scarce samples because of its high yield for ultra-low input materials. It is also compatible with various downstream assays such as PCR, sequencing, and microarrays. For these advantages, the technique was applied to extract and size-select MNase-digested RNA from mouse embryos and human K562 cells to perform ribosome profiling from samples as low as single cells. The versatile applications of integrated ITP-based purification and future directions toward high throughput single-cell NA purification will also be discussed.

(AES-04.5)**Examining the biological properties of DEP-sorted mesenchymal stem cells (MSCs)**

Alonso Stephany, Zuri rashad, Sune Terbush, Kiara Lacy, Tayloria Adams, *UC Irvine*

Human mesenchymal stem cells (hMSCs) are used to treat a variety of diseases. One challenge in the field of stem cell therapies is the inherent heterogeneity that exists in patient samples leading to varied clinical outcomes. Cultures of hMSC contain stem cells, partially differentiated progenitor cells, and fully differentiated cells. Another limitation in hMSCs therapeutic potential is the inability to select specific cell subpopulations due to an insufficient number of biomarkers. Dielectrophoresis (DEP), is a cell characterization and sorting technique that offers the biophysical properties of cells as label-free biomarkers. In this work we have used a DEP-based microfluidic device, the cytochip, to generate trapped and untrapped subsets of hMSCs at a specific voltage/frequency combination. Two bioassays, cell differentiation and scratch, were implemented to assess the biological activity of the DEP sorted hMSCs. Our results showed differences in the a) adipogenic potential, b) osteogenic potential, and c) wound closure rates of the sorted hMSCs.

23ATOM09: Laser Ablation Based Atomic Spectroscopies: Fundamental and Applications, Central Pacific A/B/C
Chair: Jorge Pisonero

Co-Chair: Ana Lores Padin

(ATOM-09.1)**Advancing microplastics characterization with laser ablation-single particle-inductively coupled plasma-mass spectrometry**

Thibaut Van Acker, Ana Rua Ibarz, Frank Vanhaecke, Eduardo Bolea-Fernandez, *Ghent University*

Microplastics (MPs) are small plastic particles within a size range of 1 µm to 5 mm in diameter and originate from larger plastic waste that is broken down over time into smaller fragments and raise growing concerns. They tend to accumulate in water bodies, where they can be ingested by marine biota and end up in higher levels of the food chain. To date, there is no "universal" technique for the full characterization of MPs given their wide size range. Larger MPs (1-5 mm) can be recognized and sized visually and the smaller ones via microscopy, but no chemical information can be obtained in this way. Fourier-transform infrared spectroscopy and Raman spectroscopy are the most commonly used techniques for MNPs identification but these techniques are highly time-consuming, especially when addressing smaller MPs. There is an urgent need for novel analytical methodologies capable of detecting and characterizing sub-20 µm MPs (1-20 µm) to complement the existing techniques.[1] Mass spectrometry-based techniques demonstrate high potential in this context and in 2020, single particle-inductively coupled plasma-mass spectrometry (SP-ICP-MS) was added to this portfolio of analytical techniques, as it was demonstrated by pioneering work of our A&MS-research unit that MPs (1 and 2.5 µm polystyrene) in suspension could be detected one-by-one by monitoring the resulting transient $^{13}C+$ signal peaks using short integration times.[2] This methodology provides quantitative information about the particle size, size distribution, particle mass and number-based concentration. SP-ICP-MS can also provide valuable information regarding the trace elemental composition and this is particularly useful since MPs can adsorb and accumulate metals from their environment. The introduction of sub-20 µm MPs can be hampered by the limited transport efficiencies provided by liquid sample introduction systems and therefore, the potential of laser ablation as alternative sample introduction system for MPs characterization will be discussed. Specific attention will be paid to different polymer types within the sub-20 µm size range and the analytical figures-of-merit of this novel approach will be presented.

1. M. Velimirovic, et al., Anal. Bioanal. Chem., 2021, 413, 7–15.
2. E. Bolea-Fernandez, et al., J. Anal. At. Spectrom., 2020, 35, 455–460.

(ATOM-09.2)**Single spot Rb-Sr isochron dating of micas by LA-MC-ICP-MS/MS**

Alicia Cruz-Uribe, Cemil Arkula, Grant Craig, Claudia Bouman Joshua Garber, *University Of Maine, ThermoFisher Scientific Bremen GmbH™,The Pennsylvania State University, Bence Paul, Elemental Scientific Lasers*

Laser ablation coupled with tandem mass spectrometry is a burgeoning field for in situ Rb-Sr geochronology. The addition of Wien-style pre-cell mass filters on the Thermo Scientific™ Neoma MS/MS MC-ICP-MS enables simultaneous collection of on-mass and mass-shifted isotopes of Rb and Sr, with vastly improved precision over dynamic quadrupole-based instruments. Simultaneous isotope ratios of $^{87}Sr/^{86}Sr$ and $^{87}Rb/^{86}Sr$ in metamorphic biotite from western Maine were determined using an ESL™ imageGEO™193 excimer laser-ablation system coupled to the Neoma. Measurements were made on Faraday cups with 10^{11} or 10^{13} ohm resistors, with Rb+ at mass 87; Sr isotopes were reacted with SF_6 gas and measured as SrF+ at masses 103–107. Twenty-two laser spots from a single metamorphic biotite sample yield a "traditional" Rb-Sr isochron date of 289±6 Ma. Time-resolved signals reveal significant zoning in $^{87}Sr/^{86}Sr$ and $^{87}Rb/^{86}Sr$ within single spot analyses, which were used to construct single spot isochrons. Individual laser spots contain multiple isochronous subpopulations; some spots contain up to three distinct, statistically robust Rb-Sr isochrons that are decoupled from variations in Rb/Sr. Thirty-five isochron dates were determined using this "sub-spot" approach, with $^{87}Sr/^{86}Sr$ intercepts that systematically vary with Rb-Sr date; two-point isochrons were calculated for individual integrations (n=780) based on these variable intercepts. Both methods yield age peaks at 303, 270, and 240 Ma. These data suggest that the Rb-Sr system records multiple short-duration

heating, cooling, or fluid-alteration events spanning ~100 m.y. within small domains in single biotite crystals.

The punctuated nature of the events recorded is at odds with the long-lived cooling histories typically recorded in biotite by, for instance, $^{40}Ar/^{39}Ar$ geochronology. With their perfect cleavage and propensity to hold ions in interlayer sites, micas are not typically thought to record multiple geologic events, and have been considered particularly sensitive to resetting by thermal and fluid-mediated processes. The distinct isotope ratio and trace element zones demonstrated here in biotite mark a significant departure from the conventional view of Rb and Sr retention in mica minerals. The prospects for single spot isochron dating combined with isotope ratio and trace-element mapping by LA-MC-ICP-MS/MS open up a new frontier for mica geochronology.

(ATOM-09.3)**High Fidelity Imaging Of Earth Materials By Laser Ablation Inductively Coupled Plasma Time Of Flight Mass Spectrometry**

John Cottle, Andrew Kylander-Clark, Ciaran O'Connor, Robert Hutchinson, Lukas Schlatt, Phil Shaw *Nu Instruments, University Of California Santa Barbara, Elemental Scientific Lasers*

Recent advances in laser ablation systems and mass spectrometers have enabled the high-speed acquisition of high spatial resolution 2-D and 3-D 'images' of elemental and isotopic variations in a range of materials. With some notable exceptions, most applications of this potentially transformative technique have been in the field of biological imaging, and have not yet found widespread application in the field of earth science. Here we combine two recently developed technological advances to demonstrate the ability of this technique to resolve micron-scale elemental and isotopic zoning in a range of earth materials. We specifically target accessory minerals (e.g., zircon, monazite, titanite), rock-forming minerals (e.g., garnet, biotite), and carbonates (e.g., foraminifera, stalagmites) to assess the potential applications of this method. Analytical instrumentation consists of a newly developed "TwoVol3" laser ablation chamber installed on an NWR193 laser ablation device (Elemental Scientific Lasers) connected to a "Vitesse" time-of-flight inductively coupled plasma mass spectrometer (Nu Instruments). In Laser-based imaging applications, there is a key balance between the speed of acquisition and the required spatial resolution that places practical limits on the area that can be mapped and/or the spatial resolution with which differences in elemental concentrations can be detected. We present data demonstrating that the ultrafast washout (~1 ms), nm-scale stage precision, and the across-chamber <1% elemental reproducibility of the "TwoVol3" cell coupled with the ultrafast acquisition rate of the "Vitesse" increases the limits by which high-resolution, multi-element/isotopic maps can be generated in a given period of time. Together, these instrument attributes enable the routine production of multi-element images at a rate of up to 1000 pixels per second at detection limits in the ppm range.

(ATOM-09.4)**Fast full-elemental LA-ICP-MS: Using TOF-ICP-MS to easily gain a deep understanding of samples in a matter of minutes**

Lukas Schlatt, Phil Shaw, *Nu Instruments*

Laser ablation mapping is a well-established modern tool for the in-situ analysis of solid samples. The images obtained from these types of analysis can be used to understand the distributions of elements, which can have a great benefit for diagnostic purposes or to gain a deeper understanding of geological processes and many other uses. Getting to the perfect image is always a very difficult task though since many factors must be taken into consideration. For instance, a good middle ground must be found between the analysis time and resolution. When using a quadrupole, the dwell time also has to be matched with the laser frequency further complicating the setup. Additionally, the only a low number of elements can be examined, with the sensitivity for each being reduced by the addition of others.
Using a time-of-flight mass spectrometer eliminates almost all the previously mentioned issues. The speed of analysis when coupled to a state-of-the-art laser ablation system allows for the acquisition of

large maps in a very short time even at high resolutions. Furthermore, since all elements are acquired simultaneously at full sensitivity, there is no need to choose any isotopes or match the acquisition frequency to the laser.
Herein we will show data acquired using LA-ICP-TOF-MS showcasing the ease and speed at which data can be acquired. Moreover, the reduction of this data and interpretation of results will briefly be discussed.

(ATOM-09.5)**Dual-Comb Absorption Spectroscopy of Molecular Species in a Laser-Produced Plasma**

Ryan Rhoades,Ryland Wala, Jason Jones, John McCauley, Reagan Weeks, Sivanandan Harilal, Jeremy Yeak, Opicslah, Mark Phillips,*University of Arizona,Pacific Northwest National laboratory*

Dual-comb spectroscopy (DCS) has recently emerged as a promising technique for absorption spectroscopy of laser-produced plasmas (LPPs) due to its ability to measure spectra with simultaneous high spectral resolution, large spectral bandwidth, and high temporal resolution. DCS absorption provides a powerful complementary method to emission-based LIBS/LAMIS measurements, providing the ability to probe late-time, low-temperature LPP properties. We have previously used DCS to measure high-resolution broadband atomic absorption spectra in LPPs for species including rare earth elements (Nd, Gd) and metals (Fe, Rb), with analysis of spectra providing time-resolved atomic excitation temperatures and absolute column densities for each species. Here, we present experimental results using DCS to measure high-resolution absorption spectra of molecular oxide species CeO , SrO, and TiO formed in a LPP. Broadband absorption spectra of CeO are measured over a range of 781.1–791.5 nm with resolution as high as 2.4 pm, which is sufficient to resolve hundreds of closely-spaced individual rotational lines. Absorption spectra of SrO show the ability to resolve closely spaced rotational lines from three overlapping vibrational bands present between 784.6 –793.3 nm as well as 1005.5-1014.1nm. Comparison of high-resolution absorption spectra with lower-resolution LIBS spectra highlights the differences between rotationally resolved and unresolved measurements. Measurements of TiO are used to determine rotational/vibrational temperatures via comparison with spectral models. The ability to measure time-resolved high-resolution molecular absorption spectra in LPPs provides valuable information on chemical and thermal properties and dynamics of molecular formation. Furthermore, high-resolution absorption spectra are useful to developing and validating spectral models for diatomic molecules.

23BIM05: Nanotheranostics: Diagnosis and Treatment of Disease Using Nanomaterials, Sierra 2
Chair: Samuel Mabbott

(BIM-05.1)**Endoscopic Surface Enhanced Spatially Offset Raman Spectroscopy for the Detection of Colorectal Cancer**

Fay Nicolson, Dana-farber Bohdan Andreiuk, Eunah Lee, Andrew Whitley, Scott Rudder, Samuel Mabbott,Kevin Haigis, *HORIBA Scientific,Texas A&m University, Cancer Insitute / Harvard Medical School, Optosigma*

Here, we combine the use of "spatially offset Raman spectroscopy" (SORS) with that of Surface Enhanced Raman Scattering (SERS) nanoparticles in a technique known as "surface enhanced spatially offset Raman spectroscopy" (SESORS) to image deep-seated tumors. We will discuss the optimization of SORS instrumentation and imaging approaches, and subsequent application of SESORS to pre-clinical cancer imaging and delineation of tumor margins in Apcfl/+, Apcfl/+;KrasG12D/+, and GL261 mouse models of colorectal cancer and glioblastoma respectively. We demonstrate that our approach enables improvements in the non-invasive detection of these cancers due to improvements in SNR, spectral resolution, and depth acquisition, and can complement clinically approved radiographic techniques.

(BIM-05.2)**Novel Surface Modification and Time-Resolved Reading of Mn-Doped Nanocrystal Signal Reporter for Enhanced Bioassay Sensitivity**

Bryan Lee, Gita Kharal, Benjamin Sreenan, Claire Lin, Xiaoshan Zhu, *University Of Nevada, Reno*

Recently, Mn-doped semiconductor nanocrystals (NCs) with high brightness, long lifetimes, and low-energy excitation are emerging for time-resolved luminescence biosensing/imaging. Following our previous work on Mn-doped NCs, in this work we developed poly(styrene-co-maleic anhydride) (PSMA)-encapsulated Mn-doped AgZnInS/ZnS NCs as signal reporter for immunoassay on antigen capsular polysaccharide (CPS), a surface molecule and also a biomarker of Burkholderia pseudomallei which causes a fatal disease called melioidosis. To enhance the assay sensitivity, a surface treatment for PSMA-encapsulated NCs (NC-probes) was performed to promote the presence of carboxyl groups that increases conjugation efficiency between NC-probes and anti-CPS antibodies. Meanwhile, time-resolved reading on the luminescence of NC-probes was adopted to minimize the assay background autofluorescence. Both strategies essentially enhance the assay signal-to-background ratio (or equivalently the assay sensitivity) by increasing the signal and decreasing the background, respectively.
Through performing and comparing microplate-based immunoassays with different NC-probes (with and without surface treatment) and different signal reading methods (time-resolved reading and non-time-resolved reading), it was proven that the immunoassay adopting surface-treated NC-probes and time-resolved reading achieved a lower limit-of-detection (LOD) than the ones adopting non-surface-treated NC-probes or non-time-resolved reading. Moreover, the achieved LOD is comparable to the LOD of immunoassay using enzyme horseradish peroxidase as signal reporter. We will also perform and compare the surface-treated and non-surface-treated NCs in lateral flow immunoassay (LFI) platforms, applying the NCs to a point-of-care format for rapid detection of melioidosis.

(BIM-05.4)**Automating the Design of a Catalytic SERS Sensor for the Detection of Disease Biomarkers**

Steven Quarin, Amanda Macke, Ruxandra Dima, Pietro Strobbia, *University Of Cincinnati*

Viral outbreaks, such as the COVID-19 pandemic, have shown the need for point-of-need testing technologies that are both deployable and sensitive to better control the spread of these diseases. Reagentless surface-enhanced Raman scattering (SERS) sensors are a great candidate to develop these new diagnostic tests because they offer highly multiplexing capabilities, among other advantages. Their ability to function as a one-pot assay makes them ideal for point-of-care testing but currently these sensors lack the sensitivity necessary to detect viral biomarkers at clinically relevant concentrations. DNA catalysis can be used to improve the sensitivity of DNA-based assays; however, there is currently no design for reagentless SERS sensors that include a DNA catalysis process. Herein, we combine DNA catalysis with reagentless SERS sensors to develop a new sensor design with improved sensitivity. We systematically alter the composition of domains in the fuel strand to elucidate the thermodynamic framework that drives this catalytic sensing mechanism. This knowledge was then used in an algorithm for automated sensor design to target different sequences. Using this program, we created catalytic sensing mechanisms that can detect P. Falciparum (malaria parasite) and SARS-CoV-2 biomarkers. These sensors show a 20-fold increase in the sensitivity and limit of detection (LOD) compared to the non-catalytic sensors. This amplification can be further improved to a 36-fold increase with the addition of locked nucleic acids into the fuel strand. Additionally, we show single-base mutation selectivity between different variants of the SARS-CoV-2 virus. This development will allow for the rapid designing and fabrication of new sensors for rapid surveillance of emerging viral threats. Future advancements for reagentless SERS sensors and for the automated design program will be expanding the multiplexing capabilities and data analysis for different biomarkers.

Combing Nanoparticle Photothermal Therapy with Surface-Enhanced Raman Scattering (SERS) Temperature Feedback

(BIM-05.5)**William Skinner**, Renata L. Sala, Kamil Sokolowski, Oren A. Scherman, Jeremy J. Baumberg, Benjamin Gardner, Pavel Matousek, Nick Stone, *University of Exeter*

Nanoparticle-mediated photothermal therapy induces tumour cell death by elevating local tissue temperature via the absorbance of near-infrared (NIR) radiation. However, maintaining the right

temperature increase inside the tumour is crucial- too low, and cell death does not occur, too high, and necrotic cell death can occur.[1] Currently, there are few non-invasive techniques to monitor tissue temperature during photothermal therapy. In this talk, surface-enhanced Raman spectroscopy (SERS) is explored as means to optically measure the photothermal heating of colloidal clusters of gold nanoparticles (AuNPs). Raman spectra encode information about thermally excited states in the ratio of the Stokes and anti-Stokes vibrational modes. However, the intensity of anti-Stokes peaks in spontaneous Raman spectra are prohibitively low for in vivo sensing applications. AuNPs clusters with a surface plasmon resonance (SPR) in the NIR region can boost the spectral intensity of surface-bound molecules via SERS and have also been explored as potential photothermal therapy agents.[2] We leveraged the optical properties of AuNPs to create a dual nano-heater and SERS-temperature sensor to enable local temperature measurements during photothermal heating. Our photothermal therapy agent is fabricated by clustering AuNPs to shift their SPR towards the NIR region and optimise both heating and SERS response. In this way, AuNPs clusters can be simultaneously heated and report on local temperature via the ratio of the Stokes and anti-Stokes vibrational models of surface-bound biphenyl-4-thiol. This work demonstrates the use of a single photothermal therapy agent to heat and report on local temperature and in future will be combined with Surface-Enhanced Spatially Offset Raman Spectroscopy (SESORS) to simultaneously heat and measure temperature at depth in tissues.[3]

1. J. R. Melamed, R. S. Edelstein, E. S. Day, ACS Nano. 9 (1) (2015) 6-11.
2. T. A. Tabish, P. Dey, S. Mosca, M. Salimi, F. Palombo, P. Matousek, N. Stone, Adv. Sci. 7 (2020) 1903441.
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23CHEM06: Improvements in Field Sensing with Chemometrics, Southern Pacific B/C
Chair: Caelin Celani

(CHEM-06.1)**A Comparison of 3 Chemical Tools for Identifying the Geographic Region of Pinus ponderosa**

Erin McClure-Price, Pamela McClure, Edgard Espinoza, James A. Jordan, Ty Coplen, *US Fish and Wildlife Forensics Laboratory, US Geological Survey*

The need for identifying the geographic origin of timber in order to support law enforcement and protect endangered or protected tree species is a rapidly expanding global need. Previous studies using DART TOFMS have shown that it is capable of differentiating the origin of certain timber species, but more studies are needed before this new application is acceptable. For this study, five discrete populations of Pinus ponderosa were collected and analyzed using DART TOFMS, a LIBS analyzer, and ICP-MS. It was found that chemometrics applied to DART TOF mass spectra was able to resolve the P. ponderosa populations with an accuracy of 85%, and the results of the LIBS and ICP-MS analyses were greater than 95%. In conclusion, this research provides a case study for the continued expansion of DART TOFMS capabilities, as well as brings two affordable, relatively rapid, and novel approaches to the forefront of timber origin identification.

(CHEM-06.2)**Portable Instrumentation - Inside the Black Box**

Suzanne Schreyer, *Rigaku Analytical Devices*

Portable instruments are used in increasingly diverse applications, as use of portable instrumentation has become more mainstream. In many cases, it is the main type of instrumentation used to identify or classify materials.
This requires that the library and the method of analyses on the instruments are robust and relevant to use.
This presentation uses case studies of portable instruments in a variety of common use scenarios to illustrate the methods implemented in order to make these instruments fit for use in identification or quantitative analyses.

(CHEM-06.3)**Field studies of monumental paintings: exploring the trade-off between high spatial sampling at lower spectral resolution and range versus lower spatial sampling and higher spectral resolution and range for materials identification and chemical mapping**

Roxanne Radpour, John Delaney, *National Gallery Of Art, Ioanna Kakoulli, University of California, Los Angeles*

Reflectance imaging spectroscopy has emerged as a transformative tool in understanding a painting's chemical make-up, overall condition, and historical context by providing researchers the ability to produce labeled classification maps of constituent materials. Yet, within the realm of cultural heritage and conservation science, the majority of reflectance imaging spectroscopy applications are conducted within a controlled laboratory setting. In the case of in situ monumental art, including wall paintings, their study has primarily relied on portable single-spot spectroscopic techniques, multiband imaging, and supplementary use of chemical analysis of microsamples. Despite the largely non-invasive or minimally-invasive nature of these methods, their inability to provide spectral information across the entire composition constrains our ability to fully comprehend the overarching chemistry and condition of large-scale paintings. While field-deployable imaging spectroscopy systems developed for remote sensing applications in biology and agriculture can be applied to heritage studies, the high cost and power requirement necessary for the analysis of wall paintings renders the application of these systems less feasible. To address these challenges of technological application for in-field examinations of cultural heritage, this research introduces two reflectance spectroscopic imaging systems to better cater to the needs of monumental wall painting analysis in non-laboratory conditions. In one case a field-rugged point reflectance spectrometer having a large spectral range (350 to 2500 nm) and high resolution (1.4 and 2 nm) was repurposed into a raster scanning spectral imager; the second system is a compact, battery-powered hyperspectral camera operating in the visible-to-near-infrared (400-1000 nm) with comparatively higher spatial resolution, lower spectral resolution. To explore the capabilities and trade-off in spectral data quality of these systems, they were employed on both archaeological and Byzantine murals in Cyprus. The procedure for gathering information incorporated a range of data collection methodologies and processing procedures, such as, but not limited to, the use of multivariate statistical analyses in tandem with spectral angle mapping. The processed data resulted in chemical maps that highlighted artist materials, conservation treatments, and deteriorative agents. This information offered invaluable insights into the mural's composition, artistic techniques, and preservation status, thereby exposing artistic choices as well as the environmental impact.

(CHEM-06.4)**Surface enhanced Raman scattering (SERS) sensors: combining machine learning and nanosciences**

Jean-Francois Masson, *Universite de Montreal*

SERS and Raman spectroscopy yields large data sets with information-rich spectra. Classical linear methods are limited, especially for SERS spectra of single molecules, where the spectra are highly dependent on the orientation of molecules on surfaces and for large data sets. Methods from data sciences are increasingly used to classify spectra into categories and predict SERS spectra for new data based on trained algorithms. For example, we recently introduced the concept of SERS optophysiology, which combines a SERS nanosensor on the tip of a pulled fiber to provide spatially and temporally specific molecular information near or inside biological material. To accomplish this, a SERS nanofiber decorated with a dense and well dispersed array of Au NP has been developed for the measurement of neurotransmitters and other metabolites in proximity of cells. The nanosensors are thus highly compatible with current physiology experiments also relying on similar nanosensors based on electrochemistry and electrophysiology. Specifically, we will show that the SERS optophysiology nanosensor can measure a panel of metabolites near cells in a single experiment. The SERS spectra of these neurotransmitters were identified with a barcoding data processing method, processed with TensorFlow using a convolutional neural network architecture. This machine-learning driven data processing significantly improved the positive assignment rates for a series of metabolites and allows

for complex measurements of the cell's biochemistry. In addition, untargeted metabolite screening to classify COVID positive individuals from a healthy group will be shown.

(CHEM-06.5)**Using X-ray diffraction for the development of a reference methodology to validate chemometric models for quantifying respirable quartz by infrared spectroscopy**

Rachel Walker, Cody Wolfe, Milan Yekich, Emanuele Cauda, *National Institute For Occupational Safety And Health*

Infrared (IR) spectroscopy is advantageous for monitoring respirable mineral hazards in the field, such as α-quartz, because the instrumentation is portable, low-cost, and relatively easy to use by non-specialists. The deployment of IR field analysis methods would accelerate the ability to respond to exposures that threaten human health. For the promise of IR field analysis to be fully realized, the creation and training of multivariate or chemometric calibration models are required. The creation and training of the models require validation with reference values for the quartz mass concentration in designated reference samples. The objective of this study is to develop a methodology for measuring and evaluating reference values for validation of chemometric IR models. The methodology developed provides measurements of quartz abundance in reference samples for the development of computational models able to quantify respirable quartz while ensuring that the reference quartz values are of the highest reliability and accuracy. Sampling and preparation of the reference samples includes careful sourcing from the field to ensure wide geographic and mineralogical representation and size segregation to obtain particle sizes within the respirable regime. The reference method chosen to measure relative quartz composition in the samples includes powder X-ray diffraction (XRD) with Rietveld refinement and standardized quartz calibration techniques, such as internal standard or standard addition. Aliquots of the reference samples are then deposited onto pre-weighed filters for transmission IR analysis to provide the absorbance data which are correlated with the reference quartz values obtained through XRD and used to generate the chemometric models. Investigation of both field samples and synthetic samples are used to inform the evaluation of the reference method to provide a pathway for measurement of reference values of high reliability and accuracy. The performance of standard addition and internal standard calibration techniques for the synthetic samples are discussed and compared. Preliminary results indicate the calibration technique should be tailored according to sample characteristics such as quartz content, differences in X-ray absorption between quartz and the mineral matrix, and the extent of interfering reflections posed by minerals in the sample matrix.

23CTP/EARLY01: New Approaches in Instrumentation and Software Design, Sierra 5
Chair: Alexis Weber
Co-Chair: Francis Esmonde-White

(CTP-01.1)**Determination of Broadband-Light Atomic Absorption through Interferometric Spectrometry with a Spatial Heterodyne Spectrometer**

Yi You, Xunyu Li, Jens Riedel, *Federal Institute For Materials Research And Testing (bam)*

Spatial heterodyne spectrometry (SHS) combines the principles of interferometry and dispersion, harnessing the inherent strengths of both. The intrinsic optical Fourier transform within SHS reveals information embedded within its interferogram. This capability enables the discrimination of broadband and narrowband spectral information from one single image. In the context of atomic absorption, this means an absorbance spectrum can be derived from one exposure, eliminating the need for separate background collection. This feature is particularly advantageous for dynamic systems such as Fraunhofer-type absorption in laser-induced plasmas, as well as situations with an unstable background light source, like an argon arc lamp.
In this study, we integrated an SHS system with a flame atomic absorption (FAA) setup, highlighting the potential of SHS for high-resolution atomic absorption research. From just one image, unseeable interferometric components corresponding to narrow-band absorption lines were recognized. Specifically, an SHS absorption interferogram concomitantly houses both the illumination background and absorption ingredients, which can be differentiated via a set of computational procedures. We

demonstrate this capability with the construction of sodium (Na) absorbance spectra by employing this single-image technique.
Furthermore, the advancements in computational power, notably the parallel processing abilities of graphics processing units (GPUs), have ushered in real-time data analytics. This development has not only smoothed the dialing/tuning process of SHS-type Fourier transform spectrometers but has also reduced the cost of data interpretation in terms of time. Consequently, this fosters the discovery of novel analytical strategies.

(CTP-01.2)**Developing real-time solutions for quantification of protein in edible insect powders**

Silvia De Lamo Castellvi, Carmen Mendez, Celeste Aurora Matos Gonzalez, Yalan Wu, Luis Rodriguez-saona, *The Ohio State University, The Ohio State University-Universitat Rovira i Virgili*

Accurate and rapid determination of protein is of great economic importance to stakeholders in the insect supply chain. The most common approaches consist of Kjeldahl organic nitrogen estimation for proteins. This routine test is labor-intensive and require complex sample pretreatment, well-trained technicians to operate the instrumentation and are less amenable to be implemented for quality control at insect farms. The industry is therefore in need for an alternative method that can provide data in a timely and economic manner. Advances in NIR instrumentation coupled with multivariate statistical analysis techniques (chemometrics) have shown potential for analysis of complex multi-spectral information for the discrimination, classification, and identification of biological systems. The present study describes a new approach to predict the level of crude proteins present in commercial edible Tenebrio molitor, Alphitobius diaperinus, Locusta migratoria and Acheta domesticus powders mixed with among them and with different amounts of flours using near infrared combined with multivariate analysis. Edible insect powders were mixed with three types of flours (organic wheat, whole wheat, and chickpea) at concentration ratio ranging from 0 to 100% (n=180). Total nitrogen content was determined by Kjeldahl method, and the amino acids profile was obtained by HPLC. Spectral data was acquired using a handheld FT-NIR scanner (1350 to 2550 nm). Data analysis method by Partial least squares regression (PLSR) model was used to develop algorithms to easily predict insect protein with the proper nitrogen to protein conversion factors. PLSR results exhibited excellent signal-to-noise ratios and good linearity, predicting crude protein content with strong correlation ($Rcv \geq 0.96$) and low standard error of cross-validation (SECV=1.58-2.13) regardless the insect species tested.

(CTP-01.3)**Portable Shifted Excitation Raman Difference Spectroscopy -Capability for On-Site Analysis on Solids, Liquids and Gases**

Martin Maiwald, Kay Sowoidnich, André Müller, Bernd Sumpf, *Ferdinand-Braun-Institut*

Portable Raman spectroscopy is a powerful analytical technique for several in-situ application fields. However, laser-induced fluorescence and ambient light such as daylight are often a drawback for on-site analysis especially for weak Raman signals. To address these challenges, shifted excitation Raman difference spectroscopy (SERDS) has been successfully demonstrated which efficiently separates Raman signals from background signals, because of physical approach.
In this contribution, the capability of portable SERDS investigations are presented for real-world applications using an in-house realized portable SERDS sensor system. The key element of this device is a dual-wavelength diode laser emitting at 785 nm as the wavelength-stabilized excitation source for Raman spectroscopy and SERDS. This one-chip device provides two narrowband excitation lines with a flexible spectral distance up to 30 cm-1 at an optical output power up to 150 mW.
A rapid alternating operation between the two laser lines and a short measurement time down to 25 ms provided by the used spectrometer enables real-time SERDS investigations with the portable sensor system which are required for rapidly changing ambient light conditions, photobleaching and for applications with quick on-site decisions.
On-site soil analysis is performed and SERDS separates Raman signals of target soil substances e.g. calcite and dolomite from background signals and enables a discrimination of these soil carbonates even in mixtures.

Bovine milk is used as a strongly optically scattering liquid test sample. A qualitative analysis is carried out and show major components of milk, e.g., fatty acids, carbohydrates, and proteins. Beside the identification, a quantitative analysis is carried out using milk samples with different fat contents i.e. 0.1%, 1.5%, and 3.5%.
Ambient air gases, i.e., nitrogen, oxygen, and carbon dioxide are investigated under daylight conditions as test samples which generates weak Raman signals due to the low density of molecules within the excited volume. SERDS extracts the target signals and provides a 9-fold improvement of the signal-to-background noise compared to a background subtracted Raman spectrum using a daylight blank spectrum.
These results demonstrate portable SERDS as a powerful tool for on-site investigations and show the potential for further application fields, e.g., field geology and heritage science.

(CTP-01.4)**Single-particle analysis using time-of-flight-ICP-MS – What can we do with this new dimension?**

Lukas Schlatt, Phil Shaw, *Nu Instruments*

Single particle analysis has made great advances from its humble beginnings 30-40 years ago. Great improvements have been made in respect to the detection limits achievable and the quality of the data. These improvements are mostly down to the availability of ICP-MS instruments which are more sensitive and capable to measure transient signals faster. Most applications of SP-ICP-MS have used a quadrupole ICP-MS and have therefore been limited to the acquisition of just one isotope. In recent years, the advancement of technology has now made it possible to couple a time-of-flight mass spectrometer to an inductively coupled plasma source enabling the detection of multiple elements in a single particle event adding a new dimension to SP-ICP-MS.
TOF-SP-ICP-MS can allow for the examination of the elemental composition of individual nanoparticles, the quantification of endogenous elements in single cells and the identification of these cells based on labels, among various other insights. Additionally, new data analysis strategies have to be developed to understand the massive amounts of data which are acquired. Herein, data will be presented, showcasing the benefits that multi-elemental data can bring in various forms of single particle analysis ranging from analysis of individual cells to examination of contents of nanoparticles. Furthermore, various strategies will be shown which allow for in depth analysis of complicated datasets in a simple and easy to understand way.

23FORENS06: Pharma Forensics, Southern Pacific A/G
Chair: Ravi Kalyanaraman
Co-Chair: Scott Huffman

(FORENS-06.1)**Multirange Vibrational Spectroscopy for Pharmaceutical Forensics**

Mike Bradley, Robert Heintz, Stephan Woods, *Thermo Fisher Scientific*

Pharmaceutical forensics laboratories are confronted with a bewildering variety of samples for investigation. FTIR and FTIR Micro spectroscopy are workhorse tools in this setting. Most FTIR work concentrates in the mid-IR range (4000-400 wavenumbers) and involves the identification of components and contaminates, determining chemical distributions and concentrations, and building reports for various stakeholders. We will describe a single workstation that provides these mid/near bulk and micro analyses and also includes FT-Raman, near-IR (bulk and micro), far-IR (bulk), TGA-IR (deformulation), and even GC-IR, yielding a comprehensive pharmaceutical forensics toolbox. We will discuss how the versatility of this workstation gives maximum flexibility, especially while operating under one software umbrella.

(FORENS-06.2)**What's In The Bottle? Real Patient Complaints Submitted to FIT**

Brittany Handzo, Scott Huffman, Ravi Kalyanaraman, *Bristol Myers Squibb*

The Forensics & Innovative Technologies (FIT) team at Bristol-Myers Squibb conducts analytical testing associated with foreign/extraneous matter from manufacturing complaints, customer generated

product quality complaints (PQC), and suspected counterfeit drug products. A PQC investigation is defined as any customer generated, manufacturing, or packaging complaints related to the quality and safety of a product. Some PQC examples include possible failures of product specifications (empty bottles, broken seals, scuffs) or dissatisfaction with the appearance, package, labeling, or components of the product (staining, foreign materials). PQC complaints can be initiated from anyone in possession of a product, such as patients, doctors, or pharmacies, and are then sent to the FIT laboratory for evaluation. Typical analytical testing conducted for these investigations include characterization of foreign material, authentication of the drug product, or moisture testing for broken/disintegrated tablets.

PQC investigations are unique due to the non-conventional nature of the requests. Once a product is commercial and is assessable to patients, anything can happen since the product is outside BMS control. The objective of this work is to highlight real patient complaints submitted to FIT in 2023 including foreign objects, staining, and more, all of which were found inside real complaint bottles. These samples were tested using a variety of analytical techniques such as microscopy, spectroscopy, elemental analyses, and occasionally chromatography. The analytical workflow will be described, starting from sample receipt, sample analysis, and ending with investigation closure.

(FORENS-06.3)**Oh Pharmaceutical Forensic Microscopy, Where Art Thou Come From?**

Dale Purcell, *Chemical Microscopy, LLC*

Pharmaceutical forensic microscopy unites an eclectic mix of doctrines in scientific disciplines and principles governed by courts of justice. An interested attendee will be introduced to early founders and their foundational contributions. The works of Edmond Locard, Hans Gross, Paul Kirk, Walter McCrone, Charles Fulton, and others are fundamental in their applications to problem-solving using microscopy and microspectroscopy. The basic concepts of transfer of materials, the identification of the physiochemical nature of these materials, an attempt to determine the source of the materials, the inference of identity of source materials, an association of materials with a source or potential source, and an understanding of the events to assist with a reconstruction of the circumstances with the source.

Pharmaceutical forensic microscopy investigation may be summarized as the application of the microscope to perform microscopical examination of physical evidence by making detailed observations, measurements, and recordings of microscopic transfer (trace) evidence to assist in the resolution of contentious legal issues.

(FORENS-06.4)**Sourcing Foreign Or Extraneous Matter Using The Particle Approach**

Craig Schwandt, *McCrone Associates, Inc.*

“Novel Aspect:” Forensic analysis of pharmaceutical materials requires an alternate chemical paradigm.

One common objective of pharmaceutical forensic analysis is to determine the source of foreign or extraneous matter found in drug products. The first step is to identify the foreign material. Once the material is identified, the source of the material may be determined so that the production issue can be corrected. The question is how is this best done?

Conventional chemical provenance studies utilize increasingly sensitive analytical instruments to detect smaller concentrations of elements or compounds in a bulk material. The volume of material needed for analysis also continues to decrease. This ultrasensitive, bulk analysis approach is useful and benefits from analytical instrumentation advancements. However, it is ultimately limited because the key signals are diluted by the product matrix.

Selecting a different model, one that is not new but rarely practiced, is a better method. The key to the particle approach is isolation of the particulate of interest, whether they are foreign material or unexpected reaction products, from the product matrix. Isolation removes the dilution effect so that all analytical signals reflect the primary components of the particulate. This provides a clearer picture of the composition and form of the contaminant. Analysis results from isolated contaminants can be readily compared to possible source materials to determine the cause of the problem.

As an example, stainless steel wear particles are common in pharmaceutical products. Production equipment is typically constructed of common alloys such as 304 or 316 stainless steel. However, some drug products or production procedures may be incompatible with a particular alloy, causing wear or chemical degradation, and leading to contamination. With conventional analysis of the compromised drug product, there is an increase in iron content, but useful signature elements occur below detectable concentration. Particle analysis can distinguish the specific alloy producing contamination, which can help identify the source of the problem.

(FORENS-06.5)**Microscopy Tool Ideas In Parenteral Pharmaceutical Environment**

David R Martinez Wolcott, *Bristol Myers Squibb*

The parenteral environment in the pharmaceutical presents many challenges regarding identification, isolation, and further manipulation of foreign matter (FM) particles. Small particle isolation from a cylindrical vessel containing a liquid media presents a high-risk of sample loss operation.
The isolation and manipulation of (FM) particles suspended in an aqueous solution drives the need for modifying traditional tools for use in a denser media. As an example, there are known challenges such as the complications of maintaining the FM in focus when using tools such as tungsten needles or transfer pipettes. In addition, consideration of safety features during manipulation of potentially dangerous instruments such as syringes is also considered as design elements.
In this talk various innovative tools will be presented that have evolved over many years of practice needs in the parenteral pharmaceutical environment. These include direct contact tools such as tungsten needle modifications, microscope stage platforms and aids, and device manipulation tools. An interaction of audience experiences and brainstorming will be encouraged.

23IR06: Material Science: IR Nanospectroscopy Opens New Perspectives, Sierra 3
Chair: Ariane Deniset-Besseau
Co-Chair: Georg Ramer
Engrand,
(IR-06.1)**Nanoscale Insight into Paint Degradation Mechanisms**

Suzanne Morsch, Stuart Lyon, *University Of Manchester, Claudio Di Lullo, AkzoNobel*

Epoxy resins continue to be widely used in protective paints, particularly those employed as corrosion-resistant coatings in the packaging, marine and aerospace sectors. In this field, the long-term performance failure of nominally defect-free coatings commonly occurs in the form of localised under film corrosion spots in seemingly random locations. In the absence of leaching, hydrolysis or oxidative deterioration, coating degradation has instead been linked to water and ion transport through structural heterogeneities intrinsic to the epoxy matrix. However, nanostructural heterogeneities hypothesized to arise in network polymers, alongside any discrete transport pathways, have previously lain beyond the resolution of organic analysis techniques, and have thus remained experimentally and theoretically unresolved.

Over the past decade in Manchester, we have applied sub-diffraction limit infrared spectroscopy and mapping (the AFM-IR technique) to generate new insights in this area: for epoxy materials, we have confirmed the presence of historically disputed ‘nodule’ nanostructure and correlated this seemingly inherent feature of network polymers to inhomogeneous cross-linking, resulting in nanoscale variations in chemical functionality. Infrared mapping under humid conditions then demonstrated that this leads to inhomogeneous water uptake in the first instance and surface restructuring during prolonged immersion. Finally, the inter-relation between nanostructure, long-term water sorption and ultimately corrosion failure has been explored, and, surprisingly, nanostuctural heterogeneity is found to influence only the initial kinetics of water sorption into these materials. Water uptake, resistivity and ultimately performance failure is found to depend more on polymer flexibility.

(IR-06.2)**Travelling thru time with IR nano-spectroscopy**

Jérémie Mathurin, Laure Bejach, Emmanuel Dartois, Cecile Jean Duprat, Alexandre Dazzi, Ariane Deniset-besseau, Keyron Hickman-Lewis, *Institut De Chimie Physique, CNRS, Universite Paris-Saclay, National History Museum London*

AFM-IR is a well-established vibrational nano-spectroscopy technique, combining an atomic force microscope and a tunable infrared laser source to record the photo-thermal effect and access chemical information at the sub-micrometric scale[1]. This technique is already applied in a wide range of application fields but mainly to study pure organic samples such as polymers or biomaterials. Recent studies demonstrate a new capability of the technology: to analyse non-organic phases such as minerals and carbonates[2]. Those first results are not directly comparable to classic IR spectroscopy[3], it opens up new fields of investigation.

Based on those promising results, our group has started to develop and applied AFM-IR technique to ancient rocky samples. With its high sensitivity and resolution, AFM-IR can be used to probe in situ a small organic fraction on a mineral matrix without any further treatment, or extraction. This allows us to keep the mineral context and thus evaluate its interaction with the organic phase. We have successfully investigated i) organic matter inside meteoritic[4] and returned asteroid samples from Hayabusa 2 spatial mission[5], the most primitive form of complex organic matter, and ii) fossils embedded in rocks where the organic matter exhibits complex organisation. With these new applications, a window starts to open on the study of organic matter thru time from space to early life stage.

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3. Mathurin, J. et al. Photothermal AFM-IR spectroscopy and imaging: Status, challenges, and trends. J. Appl. Phys. 131, (2022).
4. Kebukawa, Y. et al. Complex mixture of organic matter in a xenolithic clast from the Zag meteorite revealed by coordinated analyses using AFM-IR, NanoSIMS and STXM/XANES. Icarus 400, 115582 (2023).
5. Yabuta, H. et al. Macromolecular organic matter in samples of the asteroid (162173) Ryugu. Science (80-.). 379, eabn9057 (2023).

(IR-06.3)**Probing Phonon Polaritons of Hexagonal Boron Nitride in the Aqueous Phase with Liquid-phase Peak Force Infrared Microscopy**

Xiaoji Xu, *Lehigh University*

Peak Force Infrared (PFIR) microscopy is an innovative atomic force microscopy technique that surpasses Abbe's diffraction limit, enabling chemical nanoimaging and spectroscopy in a wide range of samples, from soft materials to photovoltaic heterojunctions and polaritonic materials. In this study, we present the development and application of Liquid-phase Peak Force Infrared (LiPFIR) microscopy for soft matter and biological samples, enabling in situ tracking of polymer surface reorganization and detection of click chemical reaction products in the aqueous phase. The LiPFIR microscopy achieves ~10 nm spatial resolution in fluid phase infrared imaging and spectroscopy while providing complementary mechanical information. We demonstrate its application in revealing the chemical composition of budding sites in yeast cell wall particles in water, highlighting its label-free and non-destructive capabilities.
In addition, we explore the utilization of Phonon Polaritons (PhPs) of hexagonal boron nitride (h-BN) in the aqueous phase, because it offers potential applications in chemical sensing and polariton-enhanced nanospectroscopy. PhPs are collective phonon oscillations that hybridize with electromagnetic fields, allowing mid-infrared optical fields to match molecular vibrations. However, the strong infrared absorption from water has limited investigations and innovations in this field due to

the challenges in optical delivery and detection. Our solution involves the detection of photothermal responses induced by the excitation of PhPs using LiPFIR microscopy. We successfully measure the characteristic interference fringes of PhPs in isotope-enriched h-BN in the aqueous phase, extracting their dispersion relationship. The integration of LiPFIR microscopy in measuring mid-infrared PhPs in the fluid phase paves the way for novel research and development in mid-IR phonon polaritonics in water.
In conclusion, our research demonstrates the versatility and potential of LiPFIR microscopy in chemical nanoimaging, spectroscopy, and the investigation of soft matters and their transformations at solid/liquid interfaces. The application of LiPFIR microscopy in detecting phonon polaritons in the fluid phase further advances the field of mid-IR phonon polaritonics, opening new possibilities for chemical sensing and nanospectroscopy in aqueous environments.

(IR-06.4)**Poking into the nano-World: Infrared Nanospectroscopy for Chemical and Structural Analysis of Nanoplastics**

Clementina Vitali, , Hans-Gerd Janssen, Michel W. F. Nielen, Francesco Simone Ruggeri, *Wageningen University & Research*

In recent years, the emergence of microplastics (MPs) and nanoplastics (NPs) as ubiquitous contaminants has raised concerns about their potential implications for human health [1]. NPs, in particular, due to their sub-micrometre size, present an enhanced potential to cross biological membranes, and their translocation and accumulation in human tissues can lead to toxic effects on human body. The physical and chemical characterization of NPs is key for a better understanding of their exposure, fate, and impact on the environment and human health. However, bulk and micro-scale analytical methods lack the spatial resolution required to gather insights into the presence of NP contamination [2].

Infrared nanospectroscopy (AFM-IR) is currently the only analytical technique that can provide a comprehensive physico-chemical characterization of NPs. This is due to the unique combination of atomic force microscopy and infrared spectroscopy, which offers unequable enhanced spectroscopic sensitivity and nano-scale spatial resolution [3, 4]. Thereby, AFM-IR is a promising approach to advance our understanding of the behavior of NPs in complex systems and to address the growing concern of NP pollution.

In this presentation, we will illustrate the versatility of AFM-IR for NP analysis, emphasizing its ability to provide crucial information about the size, shape, chemical composition, and distribution of synthetic particles at the nano-scale. By discussing the analysis of a set of commercial bottled water samples, we then demonstrate how this method enables a thorough multidimensional characterization of NP contamination, by that concluding that the technique has great potential to contribute to the development of effective strategies for assessing the impact of NPs on human health and the environment.

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[3] F. S. Ruggeri, B. Mannini, R. Schmid, M. Vendruscolo, T. P. J. Knowles, Nat. Commun. 11 (2020).
[4] F. S. Ruggeri et al., Nat. Commun. 12 (2021).

(IR-06.5)**Infrared nanospectroscopy of local electric-field dependent effects probed with atomic-force-microscopy in contact mode**

Maria Eleonora Temperini, Raffaella Polito, Antonia Intze, Michele Ortolani, Valeria Giliberti, Tommaso Venanzi, *Italian Institute of Technology, Sapienza University Of Rome*

We have customized an atomic force microscope (AFM), equipped with metal-coated conductive tips and an infrared laser beam focused on the probe [1]. The setup allows to simultaneously apply a DC voltage to nanometric samples by the AFM probe and to perform tip-enhanced infrared nano-spectroscopy. First, we proved the operation of the new setup on graphene stripes connected to gold electrodes. We then moved to the study of thin films of the polymer PMMA (polymethyl methacrylate) and the piezoelectric polymer PVDF (polyvinylidene fluoride), on which we observed signatures of the vibrational Stark effect (VSE) and of other modification to the IR absorption spectrum as effect of the electric-field applied.
In the first experiment, we verified the operation as conductive-AFM of our customized setup by measuring the current-voltage characteristics of stripes of graphene and other 2D material flakes. We then illuminated the graphene stripes under the probe tip with the infrared quantum cascade laser and observed a clear voltage-dependent photothermoelectric effect at the nanoscale [2].
Then we studied the local IR absorption spectrum of spin-cast films of PMMA and PVDF with a thickness in the range of 20-100 nm. In the case of PVDF, we observed the presence of three different crystalline phases and we selected a quasi-monofasic domain to perform voltage-dependent spectroscopy. Voltage-dependent features are observed in both polymers, in agreement with FTIR measurements of homogenous films and attributed to the VSE [3].
This new advance in the infrared nano-spectroscopy approach paves the way to the study of electric-field dependent phenomena that can be probed with infrared radiation at the nanoscale. The setup can be useful in the study of inhomogeneous materials where a local response to the electric field is of interest, such as for the characterization of nanostructured devices and nanometer-thick samples.

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23PAT01: Looking 50 Years into the Future of PAT, Southern Pacific E
Chair: Marissa Dobulis
Co-Chair: Zoe Whalley

(PAT-01.1)**50 Years (and More) of PAT in Nuclear Materials Processing**

Robert Lascola, *Savannah River National Laboratory*

The application of PAT to nuclear materials production and cleanup is an interesting story with many parallels to commercial manufacturing. In some cases, the specific challenges of these materials have driven advancements in the field. In other cases, novel adaptations have been needed to implement state of the art methods in uniquely challenging environments. Examples of both types of work from the past 50+ years will be presented, drawing from technical reports and patents as well as peer-reviewed literature. In additional to this historical review, the current work of several DOE National Laboratories and collaborators in this area will be highlighted, showing that the dual tradition of innovation and targeted development remains strong.

(PAT-01.2)**Application of Structured Approaches to Implementation of Advanced Preprocessing Techniques**

Magdalene Chong, Alison Nordon, *University Of Strathclyde*

During development of process analysis methods, the measurement requirements can be overlooked whilst pursuing the best possible model. Therefore, a workflow approach has been proposed for implementation of an advanced preprocessing technique that places the measurement requirements at the forefront of any decision-making.
The workflow presented concerns application of an advanced preprocesing algorithm for temperature correction of spectra. Amongst the various methods available for temperature correction of spectra,

loading space standardisation (LSS) is relatively straightforward to implement with only a single tuning parameter to determine. The potential rewards in applying an advanced algorithm should be assessed against the monitoring requirements prior to expending the additional chemometric expertise in its implementation. Principally, this workflow uses a benchmark local isothermal model performance to establish the potential gains with removal of temperature effects before embarking on temperature correction. The workflow is applied to monitoring of ibuprofen concentration in ethanol/water by UV spectrometry. The gains in accuracy and precision of the predicted solute concentration in the absence of temperature were required to satisfy the monitoring requirements for population balance modelling. In this instance, the potential improvements in removing temperature effects were established before proceeding with an advanced algorithm and the advanced chemometric effort to implement LSS proved worthwhile.

Ensuring measurement requirements are fulfilled during implementation of advanced process analysis techniques

(PAT-01.3)**Towards Standardisation of Data Acquistion Software for PAT Applications**

Brad Swarbrick, Rajani Davuluri, Joonsup Lee, KAX Group Pty Ltd, Stephen Hammond, *Steve Hammond Consulting*

Instrumentation for Process Analytical Technologies (PAT) has experienced a technological revolution since the mid 2000's as a result of optical miniaturisation, faster computing power, wireless connectivity and a greater acceptance of technology, particularly in the regulated industries. Unfortunately, one of the drawbacks of having a wealth of instrumentation is the corresponding diversity of software applications that drive each instrument.

Software is as critical to instrumentation as the instrumentation itself. If users feel the software is too difficult to use, or they are burdened with having to learn 'another software' package, the overall result is less use of the instrumentation and therefore a potential loss of innovation. This too is coupled with the number of chemometrics software packages available to extract critical information out of the data.

It has become apparent that, just as communication standardisation protocols such as OPC have helped with instrument integration into processes and standardised data formats (GRAMS, JCAMP, etc.) have allowed easier data transfer between platforms, there is an overall need to have a standard, intuitive and easy to use base for spectroscopic data acquisition such that all hardware platforms can be used. Acquisition of data in a standard and secure format that can be used across a wide range of industries, while meeting the minimum data integrity requirements of each industry is highly desirable. Attempts to simplify this using the OPC-UA ADI specification have not resulted in any real progress.

This presentation outlines the environment required to introduce standardisation across all instrument platforms that will not only simplify the workload of analytical and process scientists/engineers but will also reduce lifecycle burdens on hardware manufacturers and industry practitioners alike.

Overall, successful PAT integration into any industrial application is a combination of correct choice of technology(ies), correct sampling and reliable model development. This can be better facilitated by the use of a standard software platform for data acquisition, visualisation and deployment that will ultimately allow instrument manufacturers to focus more time on developing improved hardware and provide end users with a single user interface.

(PAT-01.4)**Innovations in Online Analyzers: An End-User Perspective**

James Tate, *Ibird Consultants*

"Necessity is the mother of invention." Indeed, as needs have changed in the petrochemical manufacturing industry, so has the enabling technology used to operate these processes, including online sensors. Many of the innovations associated online sensor technology were spawned by

industrial users seeking to improve their processes. Today, the needs of our industry continue to evolve and likewise, there's a need to improve the technology necessary to meet these needs.

(PAT-01.5)**The use of simulation to optimize fiber optic probe performance for on-line applications**

Stephen Hammond, *Steve Hammond Consulting*

With the expansion in Continuous Manufacturing, probe based spectroscopy has become of growing importance with the capability to rapidly sample the powder bed of different processing steps, and continually characterize process performance during manufacturing.
Using a device that simulates the moving powder environment the sampling characteristics and capability of diffuse reflectance probes inserted into a moving powder stream to be thoroughly investigated and performance optimized. The device requires just 150g to complete multiple experiments to characterize the performance of multiple types of spectroscopy and probe designs. Key elements that can be characterized for individual types of spectroscopic probe (NIR , Raman, Fluorescence) during the development of the measurement systems were: speed of sampling, relevant (unit dose) contributing mass of material, and the ability to detect and react to variation in material properties. Also the ability to deal with mechanical disturbances in data streams that may cause significant issues with GMP results.
This approach has been used to understand the optimum use of probe-based instruments and simplified analytical model development and enhanced the ability for successful integration of an optimized spectroscopic probe approach into commercial manufacturing equipment.
A software platform has been developed to capture in real-time the data from multiple spectroscopies, and other devices such as motors, that enables efficient data collection and processing. Data from multiple sources is swept into a data alignment module and then into an SQL data base. Then an associated full function chemometric platform can provide extensive data exploration and analysis.
An important facet of the practical implementation of on-line analytical systems is the ability to detect and eliminate real-life disturbances in the sensor response unrelated to the product quality but are related to processing equipment, stops and starts for example. This new platform has the capability to in real-time, filter out such disturbances allowing only “clean” spectra to be used for study of sample characteristics.
This presentation will describe the spectroscopy, engineering development and supporting software that has contributed to a highly capable simulation and measurement system that is enabling fast development of robust PAT measurements.

23PMA05: Measurement of Proteins and Modifications towards Precision Medicine, Southern Pacific D
Chair: John Marshall
Co-Chair: John Wasylyk

(PMA-05.1)**Development and Implementation of Clinical Proteomics for Bedside Applications: Precision Oncology and Cancer Detection**

Emanuel Petricoin, *George Mason University*

Nearly all FDA approved or experimental targeted therapeutics in oncology work by either binding to proteins and delivering a cytotoxic agent or modulate protein expression and/or modulate protein enzymatic activity: it is the proteins that are the drug targets for most of these precision therapies, not the genes. Yet, paradoxically patient selection and companion diagnostics are currently dominated by genomic based approaches which are in fact can only infer what is happening at the protein level. Clinical case studies will be presented showing how cutting edge clinical proteomics technologies such as the reverse phase protein array (RPPA), which can quantitatively measure the expression and activation (phosphorylation) of hundreds of protein drug targets and map the signaling protein

architecture in microscopic quantities of tumor specimens- can be used for patient selection and stratification going beyond a genomics-centered approach to companion diagnostics and predictive marker applications. Many of the current FDA approved blood-based cancer detection and monitoring biomarkers such as PSA, CA125, CA19-9, etc. are proteins, and with recent technological advances in mass spectrometry providing tremendously increased analytical sensitivity, throughput and mass accuracy, the field of proteomics is currently poised to finally deliver on the long-standing promise of generating a flood of new biomarkers. However, since biomarkers that will have the best clinical utility at specific and sensitive early detection are likely to be of ultra-low abundance in the blood or body fluid of interest, proteomic based discovery approaches require commensurate new advances in sample preparation. Case studies will be presented on a novel nanotechnology wherein engineered hydrogel nanoparticles can serve as a biomarker harvesting and concentration "vacuum" that when coupled to high resolution mass spectrometry, identification of blood based novel protein biomarkers that can detect the presence of cancer at its very earliest stages are now being discovered.

(PMA-05.2)**Use of longitudinal serum samples for early detection and risk assessment of cancer**

Karin Rodland, Tao Liu,Vladislav Petyuk, Craig Shriver, *Oregon Health & Science University, Pacific Northwest National Labortory, Murtha Cancer Center*

Early detection of solid tumors through a simple screening process, such as the proteomic analysis of biofluids, has the potential to significantly alter the management and outcomes of cancers. The application of advanced targeted proteomics measurements and data analysis strategies to uniformly collected serum or plasma samples from the same individuals over time would enable longitudinal studies of cancer risk, progression, and response to therapy that have the potential to significantly reduce cancer burden in general. Leveraging the unique characteristics of the Department of Defense Serum Repository (DODSR), we applied robust, multiplexed targeted proteomics measurements to longitudinal serum samples from 175 patients diagnosed with HNSCC and 175 matched healthy controls, representing a total of 978 serum samples drawn at the time of diagnosis, 2 and 4 years prior to diagnosis, and 2 years after diagnosis, and archived in the DODSR. Following immunoaffinity depletion, serum samples were analyzed by targeted proteomics assays for multiplexed quantification of a panel of 146 candidate protein biomarkers from the curated literature. By applying a Random Forest machine learning model to the resulting data, we derived a 13-protein signature that distinguishes cases versus controls based on longitudinal changes in serum protein concentration. The abundances of each of the 13 proteins remained constant over time in control subjects. The area under the curve for the derived Random Forest classifier was 0.90. The 13 protein signature was validated in an independent cohort of 24 patients and matched controls, with an AUC of 0.80, demonstrating that use of longitudinal samples to mitigate the effects of inter-person variability has significant potential to identify biomarkers for detection and risk stratification.

(PMA-05.3)**Giving Gold Wings with Bright and Stable Mass Spectrometry Tags**

Nathaniel Dominique, Isabel Jensen, David Jenkins,Gurkiran, Knoxville, Chandler Kotseos, William Boggess, Jon Camden, *University of Notre Dame, Kaur University of Tennessee*

Gold nanoparticles (AuNP) are a promising contrast agent for laser desorption/ionization mass spectrometry (LDI-MS) and enable applications ranging from biological imaging to anticounterfeiting. Although thiol ligands are the dominant route to tune the AuNP surface for LDI-MS, thiols fragment extensively and do not ionize readily. To address these limitations, we designed a modular N-Heterocyclic Carbene (NHC) mass tag with superior LDI-MS performance to conventional thiol ligands. A suite of NHC-tagged AuNPs were prepared and benchmarked to state-of-the-art thiol-AuNPs. This comparison illustrates that NHC mass tags surpass thiols as evidenced by orders of magnitude higher ion yield and dramatically reduced fragmentation. Then, these bright mass tags were harnessed for chemical reaction monitoring, multiplexed imaging, and information storage/recovery. These results demonstrate the remarkable performance of NHC ligands as mass tags and point to new analytical applications of well-established NHC chemistries.

(PMA-05.4)**COVID19 versus ICU Respiratory Distress Controls or Normal Human Plasma by Liquid** Chromatography Nano Electrospray Ionization and Tandem Mass Spectrometry

Jaimie Dufresne, John Marshall, *YYZ Pharmatech*

COVID19 versus ICU controls and normal human EDTA plasma were separated over quaternary amine resin and digested to tryptic peptides for liquid chromatography nano electrospray ionization and tandem mass spectrometry. The resulting MS/MS spectra were correlated to the peptides of the human proteome by the X!TANDEM algorithm to establish the p-value and FDR corrected q-value of protein identification. The observation frequency of the identified proteins was counted using SEQUEST and compared by Chi Square. The COVID and normal plasma was identified on both linear quadrupole ion trap and the orbital trap that showed excellent agreement. There were large differences in observation frequency for many proteins between ICU versus NHP and COVID vs ICU that included many known acute phase markers like ORM1. Analysis by STRING indicated that many proteins associated with cellular motion, the regulation of the immune response, RNA binding and regulation were observed to be increased in the plasma of COVID19 versus ICU and NHP patients. The results from PCR, LC-ESI-MS/MS of tryptic peptides, and cytochrome ECL assays confirmed that mitochondrial components were present in the plasma, in agreement with the established central role of the mitochondria in SARS-COV-2 biology.

(PMA-05.5)**The Tryptic Peptides and Proteins of Fetal Versus Adult Serum from MS/MS Spectra**

Jaimie Dufresne, **John Marshall,** *YYZ Pharmatech*

Fetal versus adult bovine serum were extracted with organic solvent versus chromatography over quaternary amine resin followed by digestion. The peptides were identified and quantified by random and independent sampling with LC-ESI-MS/MS. The best fit per MS/MS spectra (BFPS) was computed from X!TANDEM and SEQUEST. Observation frequency was computed alongside analytical and statistical controls of blank injections and random MS/MS spectra. Analyzing the extensively fractionated serum samples with the X!TANDEM algorithm yields more than 13,000 protein gene symbols, accessions or loci with at least three peptide matches and an FDR corrected q-value of less than 1% error ($q \leq 0.01$). The protein p-value and FDR q-value from X!TANDEM showed qualitative and quantitative agreement with the SEQUEST cross correlation algorithm corrected against random MS/MS spectra controls on ≥ 12,000 protein gene symbols from serum. The independent comparisons of fetal versus adult serum samples, extracted peptides versus digested proteins, X!TANDEM versus SEQUEST algorithms, and tryptic (TRYP) versus phosphotrytpic (STYP) peptides, all showed qualitative agreement by Venn diagram and quantitative agreement by linear regression. Proteins associated with cellular growth and development such as actin and extracellular matrix factors were enriched in, but IgG chains were apparently absent from, fetal serum. Growth factor ligands including LEDGF (PSIP1) and soluble receptors were observed in fetal serum and adding LEDGF to adult serum results in more rapid cell growth and avoided cell death.

23RAM14: Industrial Raman, Cascade 1

Chair: Ian Lewis

(RAM-14.1)**Process Analytical Utility of Raman Spectroscopy in Therapeutic T-Cell Manufacturing**

Shreyas Rangan,Katherine N. MacDonald, Smilla Colombini, Miles Huynh, Hans Georg Schulze, Martha Z. Vardaki, Michael W. Blades, Megan K. Levings, Robin F.B. Turner, James M. Piret, *University Of British Columbia*

T-cells are a rapidly emerging therapeutic cell type with diverse clinical applications ranging from organ transplantation and graft versus host disease to cancer therapies. In cell therapy manufacturing, T-cells are typically isolated from the patient or a matched donor, genetically modified if needed, expanded to large numbers in culture, and infused into the patient. Assessment of cell quality before,

during, and after the culture is essential to maximize therapeutic benefits. We tested two T-cell types relevant to cell therapy research with Raman spectroscopy – regulatory T-cells (Tregs) and conventional T-cells, to determine the suitability of Raman spectroscopy as a process analytical technology in T-cell manufacturing.

Tregs effectively suppress immune response, such that clinical trials are investigating Treg therapies to treat several autoimmune diseases. We worked with Tregs isolated from pediatric thymuses – a byproduct of open heart surgery. We investigated Raman spectroscopy as a tool to monitor thymic Treg populations expanded over a 21-day culture. Raman spectra were collected from dry-fixed samples and univariate analyses were used to establish profiles for expanding Tregs. This revealed consistent trends in Raman bands associated with cellular proteins (1003 cm^{-1}) and lipids (1450 cm^{-1}), that varied predictably with activation. Raman markers indicating poor culture outcomes were identified. Multivariate analyses revealed that Tregs were distinguishable from conventional T-cells isolated from the same donor, enabling monitoring of unwanted cell types.

Conventional T-cells are the starting material for CAR T-cell therapy, a last-resort therapy that has shown promise in treating a variety of cancers and has received FDA approval for the treatment of several blood cancers. We isolated mononuclear cells from human blood, purified T-cells, and assessed both mononuclear and purified T-cells using Raman spectroscopy. We discovered correlations that indicate the proportion of T-cells in the starting mononuclear cell preparation, as well as the distribution of T-cell subtypes in the purified material, a critical quality attribute of the CAR T-cell product.

These results demonstrate the potential of Raman spectroscopy as a process analytical technology for therapeutic T-cell manufacturing. The adaptation of existing real-time Raman probes to therapeutic manufacturing standards should enable effective and continuous process monitoring.

(RAM-14.2)**Automation and sampling technologies enabling new frontiers in Raman spectroscopy as a PAT for bioprocessing**

Karen Esmonde-White, Maryann Cuellar, Sean Gilliam, Justin Moretto, Ian Lewis, *Endress+Hauser*

Raman spectroscopy is the leading PAT in upstream bioprocessing due to its robust, scalable, and proven technology. Raman provides deep levels of real-time process understanding and feedback control without destructive sampling. Since 2011 there have been significant innovations in probe technologies, integration with automation platform, cross-scale transferability, calibrations, and ease of use. In this presentation, we review the evolution of Raman probe technologies, monitoring and control applications, and industry trends driving PAT requirements for calibrations. The review provides a historical context for new technologies supporting inline Raman for micro-bioreactors, single-use (SU) systems, and downstream bioprocessing. As we have observed throughout the evolution of Raman in bioprocessing, new applications are enabled with new technologies. Recent examples demonstrated successes in CPP control beyond glucose and target protein critical quality attributes (CQA). Finally, calibration technologies ensure a well-characterized instrument performance necessary for model transferability. Integration of calibrations into lab-scale or manufacturing-scale bioprocessing probes were achieved using standard emission sources. Micro-, SU bioreactors, or flow cell formats require a different approach to calibration, and we will discuss how those requirements informed on development of new calibration tools.

(RAM-14.3)**Quantitative Analysis Of Common Components In A Chemical Mechanical Polishing Slurry Using Raman Spectroscopy**

Michelle Sestak, Timothy Holt, *HORIBA Scientific*

Chemical Mechanical Polishing (CMP) is a crucial step in semiconductor manufacturing. Quantification of the components of the slurry is critical to ensuring an effective slurry that will not damage the wafer. Current techniques for quantification, such as Ion Chromatography (IC) and High-

Pressure Liquid Chromatography (HPLC) have excellent limits of detection, but they are difficult, costly, and require experienced users. In this application note, Raman spectroscopy is explored as an easier and more flexible technique for quantifying common components in a CMP slurry, such as benzotriazole and glycine, without any sample preparation or costly consumables. Results show that Raman spectroscopy can reach estimated limits of detection and quantification of benzotriazole of less than 0.025% and 0.10% (both in mass percent), respectively, making Raman spectroscopy an ideal alternative to the more costly and time-consuming techniques like IC and HPLC.

(RAM-14.4)**Automated Quantitative Raman-based Analysis of (Microplastic) Particles and Fibers down to 1 µm**

Natalia Ivleva, Oliver Jacob, Alejandro Ramírez-Piñero, Martin Elsner, *Technical University of Munich*

Microplastics (MPs, synthetic polymer particles and fibers in the size range of 1 µm–1 mm, Hartmann et al., 2019 doi.org/10.1021/acs.est.8b05297) are detected in the environment all around the globe, as well as in drinking water and food, thus raising concerns about their impacts on the environment and human health. Since more hazardous effects are expected from smaller MPs, reliable quantitative analysis is required. Here, Raman microspectroscopy (RM) is suitable for the chemical identification and quantification of MPs in the entire size range (Ivleva, 2021 doi.org/10.1021/acs.chemrev.1c00178). Our open-source program TUM-ParticleTyper (von der Esch et al., 2020 doi.org/10.1371/journal.pone.02347) enables the automated detection, quantification, and morphological characterization of (plastic) fragments in dark-field images of optical microscopy, followed by the automated RM-based identification of MPs and non-plastic fragments of up to 7000 particles/fibers down to 10 µm, randomly selected on the entire filter. Since the number of particles/fibers increases with decreasing the particle size, it become nearly impossible to detect all particles down to 1 µm on the entire filter. Therefore, we developed novel alternative strategy – random window subsampling, where the automated acquisition of optical image and localization of (MP) fragments are followed by RM measurements from window to window. We also introduced a bootstrap method, to provide an error quantification with confidence intervals from the available window data. We implemented in TUM-ParticleTyper 2 new RM measurement algorithm that computes confidence intervals on-the-fly during the analysis and automatically stops, if an appropriate number of fragments is identified, thus improving time efficiency (Schwaferts et al., 2021 doi.org/10.1007/s00216-021-03326-3). Additionally, we implemented advanced image processing for better recognition and morphological characterization of (microplastic) particles and fibers, including automated adaptive de-agglomeration and improved shape classification.
In this presentation we will introduce our new open-source software program TUM-ParticleTyper 2, which enables automated analysis of (MP) particles and fibers down to 1 µm. Repeated measurements of "in-house" produced secondary reference MPs were evaluated, to assess the precision of the whole procedure. Finally, applicability of developed automated Raman-based method for the detection, identification, quantification and characterization of MP particles and fibers from different environmental samples (water, sediments) will be demonstrated.

(RAM-14.5)**In situ Raman-electrochemistry for Routinely Characterizing Electronic Properties of Dopped SWCNTs**

Joanne Yam, Adam Hopkins, *Metrohm*

Electrical conductivity of single wall nanotubes (SWCNTs) plays a critical role in high-performance flexible batteries. Doping SWCNT is a well-known technique to modify their electronic properties and improve electrical conductivity. In this presentation, we demonstrate in situ Raman-electrochemistry as a unique technique for both predicting electronic properties and understanding the charging mechanism of SWCNTs. We demonstrate this by electrochemically doping SWCNT with both chloride and tetraethylammonium tetrafluoroborate to study simultaneous changes in Raman intensity and/or shifts with respect to applied potential. Our study shows hysteresis of the cyclicvoltammogram and Raman spectra during doping cycles. We demonstrate the impact of doping on D, G, 2D and RBM bands of the nanotubes at different voltage states.

23RAM16: Nano Raman 2, Cascade 3
Chair: Andrew Whitely
Co-Chair: Abdrey Krayev

(RAM-16.1)**High Resolution Ambient Tip-Enhanced Optical Microscopy and Spectroscopy**

Patrick El-Khoury, *PNNL*

This talk will highlight our group's recent adventures in tip-enhanced optical microscopy and spectroscopy. Emphasis will be put on high spatial resolution Raman measurements performed under ambient laboratory conditions. Fundamental studies targeting molecules interacting with plasmonic nanostructures and nanoparticles will first be presented. These studies motivate multimodal (non)linear nano-spectroscopy and nanoimaging measurements that report, e.g., on the resonances and spatial distributions of optical fields confined to the nanoscale. Altogether, our studies reveal a complex interplay between photons and electrons on the nanoscale, particularly at plasmonic nanojunctions. The implications of our finding on studies employing SERS and TERS to track plasmon-induced chemistry are numerous. The talk will therefore end by highlighting some of the lessons learned in this exciting research area.

(RAM-16.2)**Unconventional Moiré Electrons and Phonons in the Magic Material Probed by Tip-Enhanced and Gate-Dependent Raman Spectroscopy**

Andreij Gadelha, Douglas Ohlberg, Cassiano Rabelo, Eliel Neto, Thiago Vasconcelos, Joao Campos, Jessica Lemos, Vinicius Ornelas, Daniel Miranda, Rafael Nadas, Fabiano Santana, Kenji Watanabe, Takashi Taniguchi, Benoit Troeye, Michael Lamparski, Vincent Meunier, Viet-Hung Nguyen, Dawid Paszko, Jean-Christophe Charlier, Leonardo Campos, Luiz Cancado, Gilberdo Medeiros-Ribeiro, Ado Jorio, *Federal University of Minas Gerais*

We unravel the nanoscale properties of electrons and phonons of twisted bilayer graphene (the magic material). Beyond expanding the optical spectroscopy limits, by revealing the twisted bilayer graphene crystallographic structure using visible light, our discoveries unravel exotic physics. We observe the localization of lattice dynamics, whose properties follow the moiré crystallographic structure. We also measure an astonishingly strong and persistent electron-phonon coupling on the microscale. Our combined results support the unconventional behavior of the magic material and pave the way for future discovery of strong-correlated interactions between electrons and phonons. Our findings were possible due to unique tip-enhanced and gate-dependent Raman techniques, which allow spectroscopic investigations at the nanoscale and with Fermi-level tuning on the microscale. In summary, our work uncovers magic material's unique properties, elucidating the role of local electrons and phonons and their possible influence on graphene's superconductivity.

(RAM-16.3)**Investigation of Transition Metal Dicalcogenides Flakes with TERS and Wide-field Raman Microscopy with Stochastic Optical Reconstruction.**

Joachim Jelken, Cedric Lambin, **Francois Lagugne,** *Western Ontario University*

Tungsten, Molybdenum and Vanadium disulfide flakes were investigated using tip-enhanced Raman spectroscopy, kelvin force microscopy and nanomechanical measurements revealing details that could not been seen in standard Raman microscopy. These complementary approaches are well adapted to understand how the surface of 2D materials develops and how their mechanical properties can reveal hidden structures leading to a better understanding of their formation.
Specifically, a flower-like structure was observed at the surface of WS2 flake and was revealed by Raman measurements conducted in confocal and in tip-enhanced Raman spectroscopy modes providing excellent optical contrast to differentiate the different domains of this secondary structure based on the change of intensity of the lattice vibrations.[1] Beyond topography measurements, nanomechanical measurements were used to yield the origin of the hidden flower. In the case of VS2,

spiral structures were observed at the surface of the flakes due to the orientation of platelets with different orientations. The local work function and TERS were investigated for single VS2 flakes.[2] In addition to TERS spectroscopy we have applied wide field microscopy with stochastic optical reconstruction on Raman- and PL maps of MoS2 flakes under mild and strong irradiation conditions.[3] Local transient phenomena can be revealed with this approach highlighting that the edges of the flakes are sensitive to oxidation. The results are correlated with atomic force microscopy (AFM), including friction force microscopy, and Kelvin probe force microscopy (KPFM) measurements to obtain information about the influence of the irradiation on the topography, friction force and work function of the flakes.

[1] "The Hidden Flower in WS2 Flakes: A Combined Nano-Mechanical and Tip-Enhanced Raman Exploration", J. Jelken, M. O. Avilés, F.Lagugné-Labarthet*, ACS Nano, 2022, 16, 12352-12363.

[2] "Periodic Spiral Ripples on VS2 Flakes: A Tip-Enhanced Raman Investigation", M. O. Avilés, J. Jelken, F.Lagugné-Labarthet*, J Phys. Chem. Lett, 2022, 13, 9771–9776.

[3] "Real-Time Observation of Photo-Oxydation of Single MoS2 Flakes using Stochastic Optical Reconstruction Microscopy", 2023, submitted.

23SPR04: Plasmonics and Catalysis, Cascade 4
Chair: Malama Chisanga, University of Montreal

(SPR-04.1)**Promoting Unique Molecular Processes with Plasmonics**
Matthew Sheldon, *Texas A&M University*

Vibrational Strong Coupling (VSC) is emerging as a potentially transformative tool for manipulating chemical reactions, yet its mechanistic understanding remains elusive, primarily due to limitations in experimental platforms. This study presents an innovative method using nanoscale plasmonic resonances to enhance the Local Density of Optical States (LDOS) over conventional Fabry-Pérot cavities, and uses confocal Raman spectroscopy to monitor a solid-state dehydration reaction. We demonstrate that VSC can reduce the temperature threshold for dehydration of copper sulfate pentahydrate [CuSO4(H2O)5] by up to 14 °C, with the effect scaling with the coupling strength. Additionally, we provide chemical mapping with sub-wavelength resolution, revealing that VSC-induced changes in the reaction are localized to areas with the highest LDOS. This research offers critical insights into the spatial specificity of VSC-modified chemistry, showcasing the potential of nanoscale plasmonic resonances for controlling chemical reactions, and providing a promising approach for unambiguous experimental design in 'polariton chemistry'.

(SPR-04.2)**Plasmonic Magnesium Nanoparticles for SERS and Catalysis**

Andrey Ten, Vladimir Lomonosov, Christina Boukouvala, Jean-Francois Masson, Emilie Ringe, *HORIBA Scientific*

Surface-enhanced Raman spectroscopy (SERS) and plasmon-induced photocatalysis are important applications of plasmonic materials. These phenomena have been mainly studied using nanostructures of noble metals such as gold and silver. Recently, alternative metals, either more abundant, biocompatible, or resonant in different ranges, have attracted much interest. Magnesium, one such alternative, has been identified as a potential noble metal replacement owing to its excellent optical behaviours across the UV, visible and NIR, low cost and inherent biocompatibility. Recent colloidal synthesis advances leading to monodisperse, shape-controlled nanoparticles enable the exploration of the applications of magnesium in SERS and photocatalysis.

This talk will begin by introducing magnesium nanoparticles and their plasmonic properties. We will use STEM-EELS and numerical methods to survey the distribution and intensity of the enhanced near-

field of magnesium nanoparticles and their native oxide layer. Will we then assess magnesium's performance as a SERS substrate by experimentally evaluating the SERS enhancement factor. Next, we will show how to harness the advantages of SERS to evaluate magnesium's photocatalytic activity using the well-established coupling reaction of 4-nitrothiophenol (4-NTP), leading to p,p'-dimercaptoazobenzene (DMAB). The talk will draw to a close with the prospect of the future of magnesium nanoparticles for use in SERS and plasmon-induced catalysis.

(SPR-04.3)**Investigating the Relationship of Electron Transfer and Heating of Nanomaterials with a Homemade Photothermal Heterodyne Imaging System**

Yechan Moon, Zachary Schultz, Susan Olesik, *The Ohio State University*

Due to the unique properties of polymer nanofibers like flexibility, mechanical strength, high surface area to volume/weight ratio, nanomaterials could be incorporated to be used in the fields of lithium ion batteries1, flexible electronics2, surface assisted laser desorption/ionization (SALD) substrates3–6, catalysts7, and solar cells8. These reports show incorporation of the nanomaterial additives alters the overall properties; however, further advances in these applications will benefit from improved understanding of the properties of the additives on the system. Plasmonic nanomaterials are known to have a localized surface plasmon resonance (LSPR), which is an oscillation of the conduction band induced by the electromagnetic fields propagated by an incident light. LSPR causes two things: a short-lived enhancement of the local electric field of the nanomaterial which allows for surface enhanced Raman spectroscopy (SERS)9, and hot carrier formation which is responsible for electron transfer10. The latter also contributes to the nonradiative decay of LSPR and is also responsible for the heating of the nanomaterials. To study the relationship of electron transfer mechanism and heating caused by LSPR of plasmonic nanoparticles, a homemade Raman spectrometer incorporated photothermal heterodyne imaging (PHI) system was devised. The change in PHI signal provides a spatial probe of relative temperature, while the electrospun polyacrylonitrile (PAN) has a nitrile residue whose Raman frequency can be used to assess electron transfer from the nanoparticles to the surrounding environment. Preliminary results show that the heating of plasmonic nanoparticles result in a higher population of ionized nitriles of PAN, but no ionization was observed when using silicon nanoparticles which heats but lacks a LSPR. This indicates that the LSPR of plasmonic nanoparticles are responsible for the ionization but is enhanced through heating of nanoparticles.

Plenary Sessions: SAS and Applied Spectroscopy William F. Meggers Award; Johannes Pedarnig, Sierra 5

(PLEN-L4.1)**LIBS and LA-SD-OES: Laser Ablation and Electric Sparks for Chemical Element Analysis**

Johannes Pedarnig, Nikos Giannakaris, Stefan Grünberger, *Johannes Kepler University Linz*

Optical emission spectroscopy of plasma induced by ultrashort and short laser pulses is employed to detect tiny masses of samples ablated from macroscopic specimens, to measure chemical images of micro-structured samples with spatial resolution of approx. 1 μm, and to determine the concentration of side elements in industrial materials. Ultrashort femtosecond laser-induced breakdown spectroscopy (fs-LIBS) of thin metal layers on glass enables us to detect 370 fg of Ag, 100 fg of Cu, and 8 fg of Na (ablated mass per laser pulse) [1]. Orthogonal fs-double-pulse excitation enhances the emission line intensities and improves the contrast of chemical images in comparison to single-fs-pulse measurements. Short nanosecond laser pulses are used for LIBS measurements of industrial steel samples (concentration $C(Fe) \geq 94$ wt% and $C(Si) \leq 4$ wt%). The LIBS spectra reveal a surprisingly strong matrix effect and the line intensities of analyte elements like Mn are cross-sensitive to the element Si. However, this detrimental matrix effect is overcome when the laser-ablated sample is re-excited by an electric spark discharge (Laser Ablation-Spark Discharge-Optical Emission Spectroscopy, LA-SD-OES) [2, 3]. The different behavior of LA-SD-OES and LIBS is probably due to different processes of sampling and excitation of analytical plasma. LA-SD-OES enables the

element analysis of industrial steel largely independent of the composition and structure of samples while in LIBS the matrix effect has to be taken into account.

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Plenary Sessions: Spectroscopy's Emerging Leader in Molecular Spectroscopy; Dmitry Kuroski, Sierra 5

(PLEN-L4.2)**Structural Characterization of Biological Systems at Macro and Nanoscale Using Vibrational Spectroscopy**

Dmitry Kurouski, *Texas A&M University*

Raman and Infrared (IR) spectroscopy can be used to determine energies of molecular vibrations in analyzed specimens. Using this information, chemical structure of the sample of interest can be revealed. In this talk, I will discuss the complementarity of Raman and IR spectroscopy in the structural characterization of biological systems. I will also show the advantage of Raman-based optical sensor for a label-free, non-destructive and confirmatory detection and identification of biotic and abiotic stresses in plants. Furthermore, I will discuss the extent to which Raman spectroscopy can be used for non-invasive plant phenotyping and nutritional analysis of fruits and vegetables. If coupled to scanning probe microscopy techniques, Raman and IR can be used to investigate the structure and composition of biological systems with nanometer spatial resolution. Using nano-Raman and nano-Infrared techniques, my group was able to resolve the secondary structure of amyloid oligomers, protein aggregates that cause a large number of neurodegenerative pathologies, including Alzheimer and Parkinson diseases. Our recent findings show that lipids can uniquely alter rates of protein aggregation, as well as change the secondary structure and toxicity of amyloid aggregates. These findings suggest that irreversible changes in lipid profiles of plasma membranes can trigger the abrupt protein aggregation that yields highly toxic oligomers, which, in turn, are responsible for the onset and progression of neurodegenerative diseases.

23AWD08: SAS and Applied Spectroscopy William F. Meggers Award Symposium Honoring Johannes Pedarnig, Cascade 3
Chair: Johannes Pedarnig, Johannes Kepler University Linz

(AWD-08.1)**Investigation of LIBS-RF Plasma for Analytical Spectroscopy**

Igor Gornushkin, Cristina Méndez-lópez, Nerea Bordel, *Department of Physics, University Of Oviedo, BAM*

Laser breakdown spectroscopy (LIBS) is a common tool for applications in various fields of science and technology. Originally an atomic analysis technique, LIBS was later extended to molecular

analysis due to the transient nature of the laser-induced plasma, which develops from a hot dissociation stage on a nanosecond to several microsecond scale to a relatively cold recombination stage on a scale of 10 to 100 microseconds after breakdown. Molecules formed during the recombination stage or incompletely dissociated after ablation can be efficiently detected, allowing the analysis of "difficult" elements or even molecular isotopes. However, with a small amount of ablated material and a short lifetime of the luminous plasma, analytical signals, especially molecular ones, can be very weak.
Several methods have been proposed for reheating the plasma and increasing its lifetime, for example, a two-pulse LIBS or a LIBS combined with microwave radiation or with an electric spark discharge. Here we propose another one, LIBS combined with a capacitively coupled RF discharge at 13.6 MHz. The advantages of this combination are an increase in the lifetime of atomic and molecular emission and operation in a low-pressure atmosphere, which significantly reduces pressure line broadening and allows high-resolution spectroscopy. Another major advantage is operating in a chemically controlled atmosphere that can predictably drive desired chemical reactions. In this presentation, we will show the first results obtained with RF-LIBS combination. These will include separate and joint characterization of LIBS and RF plasmas and evaluation of its potential for elemental and molecular analysis and for plasma enhanced chemical vapor deposition.

(AWD-08.2)**Remote detection of Li isotopes**

Vassilia Zorba, Kevin Touchet, Jose Chirinos, Changmin Kim, Zach Alvidrez, Xianglei Mao, *Lawrence Berkeley National Lab & Uc Berkelely*
Remote detection of Li isotopes in the solid phase under atmospheric pressure has remained a long-standing spectroscopy challenge. In this work we introduce a new capability for rapid measurements of Li isotopes in solid samples through a combination of femtosecond filamentation and Laser Induced Breakdown self-Reversal Isotopic Spectrometry which allows distinguishing small isotopic shifts of Li in plasmas remotely. The technique leverages self-absorption and the spectral shift of the central wavelength of the unresolved isotope peaks caused by isotopic ratio changes, combined with femtosecond laser filamentation for plasma generation at standoff distances. Here we report on Li isotope detection and quantification at 18 m. These results represent the basis of a new method for remote Li isotope quantification in the field.

(AWD-08.3)**On The Significance Of Laser Ablation Efficiency For LIBS Signal Enhancement.**

Alessandro De Giacomo, *University of Bari*

During LIBS the crucial question determining the emission intensity signal is the number of emitters produced during the laser ablation and the plasma temperature. In this frame it becomes important to determine the fraction of ablated matter the really atomizes with respect of the total mass ejected after the laser pulse. In this lecture it will be discussed with simple thermodynamics and kinetics the basic equations that allow the determination of the number of atomic species produced during laser ablation and the main parameters affecting the yield of atomization. On the basis of this considerations the main techniques exploited for enhancing the LIBS signal will be briefly commented, with particular emphasis on Double Pulse LIBS, Nanoparticle enhanced LBS, single particle LIBS and sample surface treatments.

(AWD-08.4) **LIBS and LIMS in the context of planetary/space research**
Jose M. Vadillo, Javier Moros, Javier Lasema, *University of Bari, Universidad de Malaga*

Astrochemistry and space exploration are hot topics in today`s resarch and laser-ablation based techniques - as laser-induced breakdown spectroscopy (LIBS) and laser-ionization mass spectrometry (LIMS) - are gaining kudos and speaking with loud voice withing the astrochemist community. The talk will show examples of the current role of LIBS and LIMS in past, present and future missions, as well as the lab-scale experiments performed by the authors on such topics, with the aim of helping in the seek of biosignatures and in a better understanding of the geochemistry of rocks and particulated material.

23AWD09: Spectroscopy's Emerging Leader in Molecular Spectroscopy Award Symposium Honoring Dmitry Kurouski, Sierra 5
Chair: Dmitry Kurouski, *Texas A&M University*

(AWD-09.1)**Measuring Chemical Composition, Optical and Thermal Properties at the Nanoscale with AFM Probes**

Andrea Centrone, *Nist*

Conventional Fourier-transform IR (FTIR) spectroscopy and time-domain thermoreflectance (TDTR) reliably measure, chemical composition and thermal properties, respectively but have insufficient spatial resolution (few μm) for many nanoscale applications. Furthermore, TDTR typically requires long measurement times (≈120 s/pixel) and suffers from high uncertainties (ΔG>35%).

Photothermal induced resonance (PTIR) [1,2,3], also known-as AFM-IR, is a scanning-probe technique that uses the tip on an AFM to transduce the sample photothermal expansion and measure IR (or visible) spectra at the nanoscale. Conventional AFM probes are kicked into oscillation (like a struck tuning-fork) with amplitude proportional to the sample absorption. This way, PTIR maps the sample composition, molecular conformation, and bandgap at the nanoscale. However, conventional AFM probes lack the sensitivity and bandwidth required to measure the fast time-domain sample thermalization linked to the sample thermal conductivity (η) and interfacial thermal conductance (G).

Here, I will introduce a custom PTIR setup based on new optomechanical AFM probes[4,5,6] that achieve low detection-noise (≈1 fm/Hz1/2) over a wide (>100 MHz) bandwidth. This way, the entire, time-domain, thermal expansion of the sample is measured at once with high spatial (≈10 nm) and temporal (≈ 4 ns) resolutions, yielding composition (IR absorption), η and G mapping, concurrently. Compared to conventional TDTR, these measurements achieve ≈6000x higher throughput (20 ms/pixel), >140x higher spatial resolution and record-low uncertainty (ΔG≈2%). Compared to conventional AFM-IR (ringdown), they achieve higher sensitivity (≈700x) and throughput (≈500000). Furthermore, the high resonance frequency of these custom probes (10 MHz) is ideal to improve the spatial resolution.[7]

Finally, I will introduce a method that exploits the time-correlated nature of thermal position fluctuations in AFM cantilevers within their dissipation timescale, to remove the extrapolated-in-time thermal noise from the PTIR signal, effectively reducing the cantilever temperature from 300 K to ≈12 K without cryogens, and improving precision by >2x. [6].

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(AWD-09.2)**Nano-Chemical Infrared Imaging and Spectroscopy from Supramolecular Protein Aggregates to Single Polymer Chains**

Francesco Simone Ruggeri, *Wagenigen University*

A fundamental objective of modern analytical methods in Physics and Chemistry is to unravel the heterogeneous chemical and physical properties of single biomolecules and relevant bio-surfaces at the nanoscale. While innovative imaging methods have been developed to characterise biomolecular processes at the nanoscale, imaging microscopies are to the most part chemically blind; thus hampering the characterisation of inhomogeneous and complex systems.
Here, we first demonstrate that Atomic Force Microscopy (AFM) to overcome the limitations of conventional imaging microscopies, we show a real breakthrough with the development and

application of Infrared Nanospectroscopy (AFM-IR) in bio- and materials science. AFM-IR combines the high spatial resolution of AFM (~1 nm) with the chemical analysis power of infrared (IR) spectroscopy to retrieve unprecedented correlative information at the nanoscale on the structural, mechanical and chemical properties of heterogeneous bio-molecular processes and functional (bio-)organic materials. As most recent advances in the field, we demonstrate the achievement of single protein molecule chemical identification and structural determination, which in turn now we demonstrate useful for single polymer chain analysis. Then, we show the application of this single molecule sensitivity to unravel the molecular interaction fingerprint between a small molecule and its target, the surface properties of artificial model membranes and polymeric coating, the structure of functional protein self-assemblies in living organisms to be exploited as a novel class of biomaterials in bioscience.

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(AWD-09.3)**Monitoring Chemistry on Plasmonic Nanoparticles**

Zachary Schultz, *South Dakota Mines*

The excitation of localized plasmon resonances has been shown to give rise to intense electric fields and energetic charge carriers on the surface of nanoparticles. Understanding of the electric fields and transfer of these energetic charge carriers can enable chemical reactions that are otherwise not observed. The channeling of energy on plasmonic particles also provides ways to create spatially discrete regions with charge differences that may affect chemical reaction products. Some of the electrons excited by the plasmon resonance can be transferred to nearby molecules, altering the response observed from the molecules. In this presentation we will examine both ways to monitor the effects of these energetic charge carriers as well as the impact on the signals observed from the nearby molecules. Materials that take advantage of these effects suggest new opportunities in chemical analysis and other applications.

(AWD-09.4)**Plasmon-enhanced Raman Spectroscopy Assessed by a Holistic Quantum Mechanical Approach**

Stephan Kupfer, Kevin Fiederling, Stefanie Gräfe, *Friedrich Schiller University Jena*

Recent TERS experiments suggest unexpectedly high lateral resolutions in the molecular or even sub-molecular regime, despite the size of the plasmonic particle.[1,2] To elucidate the interaction between the plasmonic nanoparticle and the immobilized substrate, i.e., in the scope of TERS, a quantum mechanical description is indispensable – addressing the so-called “chemical effect” as well as the “electromagnetic effect”.
In this contribution, we go beyond the computational description of the non-resonant chemical effect, as already introduced successfully by our group based on 3-dimensional grid calculations at the density functional level of theory (DFT),[3] while the immobilized molecule is mapped by the plasmonic “tip” mimicked by a single silver atom. Firstly, resonant effects as well as charge transfer states are incorporated by time-dependent DFT (TDDFT) simulations to cover the complete “chemical effect”, exemplarily for the surface-immobilized tin phthalocyanine (SnPc).[4] Secondly, complementary electrodynamic calculations based on the finite element method are performed to describe the electric field confinement in the near-field.[5] The static electric field, obtained in the vicinity of an atomistic sized feature – the “electromagnetic effect”, is then incorporated in the quantum chemical simulations. This way, TERS spectra and intensity maps of SnPc are

computationally evaluated under non-resonant and resonant conditions [6] Finally, our computational approach is applied exemplarily to elucidate the thermodynamics of plasmon-induced reactions.

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(AWD-09.5)**Multimodal Spectral Nano-Imaging of Plasmonic Nanoparticles**

Patrick El-Khoury, *PNNL*

This talk will highlight recent work from our group that is aimed at characterizing bare and chemically functionalized plasmonic metal nanostructures and nanoparticles using various nano-optical approaches. Some of the methods discussed are well-established (e.g. TERS). In this case, we will described non-standard analyses aimed at extracting different properties of localized and enhanced optical fields via molecular TERS. Other methods will be shown in this talk for the first time. For instance, measurements that track plasmons with sub-nm resolution under ambient laboratory conditions will be described, with emphasis on visualizing the resonances that underpin TERS and other tip-enhanced (non)linear optical approaches. Overall, the emphasis will be on molecules, plasmons, and their interactions - all visualized on the nanoscale using well-known and novel tip-enhanced (non)linear optical approaches.

23FORENS05: Early Career Research in Forensic Science, Southern Pacific A/G
Chair: Alexis Weber, *University at Albany, SUNY*

(FORENS-05.1)**Chemical Anthropology: The Forensic Chemist's Perspective on Bones**

Kristen Livingston, *University Of Central Florida*

Communicating scientific results to non-experts in a meaningful way is a critical part of forensic science. Collaborative, interdisciplinary research is one avenue that enables scientists to engage with individuals outside of their own field and hone their communication skills. For the past three years, I have played the role of a forensic chemist and spectroscopist who studies chemical applications for bone analysis. A major focus in my PhD research is developing a chemical method to reassociate commingled human remains via Laser-Induced Breakdown Spectroscopy (LIBS). My work in this area routinely involves conversations with trained anthropologists, requiring a conscious shift in the scientific discussion.

My research on the LIBS sorting of commingled remains will be presented through a series of challenges I have encountered as a chemist in a unique field of study. Specific topics will include explaining spectroscopy to those with little to no chemistry background, addressing misconceptions about chemical analysis, and developing research projects that address explicit needs. I will share strategies that support effective communication and collaboration. Finally, this presentation will highlight the benefits of working on an interdisciplinary research team and how it provides opportunity for career growth.

(FORENS-05.2)**Multimodal Raman Spectroscopy For Comprehensive Forensic Analysis**

Matt Gabel, Sudhir Dahal, *Thermo Fisher Scientific*

Raman spectroscopy and microscopy have established themselves as prominent analytical techniques in forensic laboratories. Although there is little to no specific sample preparation required for general Raman analysis, the bigger challenge is often realized when introducing a variety of unique samples to the instrument. The numerous shapes, sizes, forms, and physical properties of many different materials inadvertently require diverse ways to excite and collect maximum Raman scattering for optimized analysis. Since forensic laboratories must regularly deal with diverse types of samples (such as solids, liquids, powders, smears, fibers, etc.) of different sizes and origins, understanding a comprehensive methodology to conduct the analysis can dramatically improve laboratory results. In this presentation, we will demonstrate numerous sampling modes and associated accessories to obtain optimal Raman signal from representative forensic samples.
A number of forensic samples (such as duct tape fibers, lipstick residue, mixed powders, alcohols, and ink writing samples) were analyzed under various collection modes and conditions. These collection modes included macro/bulk measurements, microscopic measurements, measurements using fiber optic probes, and measurements leveraging surface enhanced Raman spectroscopy (SERS). The optimal sampling method for each material will be highlighted and a comprehensive Raman solution for forensic laboratories will be presented.

(FORENS-05.3)**Near Infrared for rapid screening for oil type used in fried snacks manufacturing**

Alessandra Victorio, Luis Rodriguez-saona, Siyu Yao, *The Ohio State University*

As the trend toward wellness keeps gaining strength, the selection of oils used in fried snacks can add value as healthier alternatives are more appealing for the consumers. Fried snacks manufacturers are selecting oils with high-oleic traits but adulteration of high-price oils (e.g., sunflower) with canola, soybean, and palm oils is a common source of economically-motivated fraud. The objective of our research was to develop a rapid method to identify the type of oil used in fried snacks manufacturing and to predict the fatty acid profile using unique NIR spectral patterns. Samples (n=80) were obtained from local grocery stores in Columbus, OH. The oil (2-4 g) was obtained with a hydraulic press and analyzed by a handheld NIR sensor (1350 to 2500 nm). An aliquot of 0.3 mL of oil at 40°C was placed in a glass vial and measured by transflectance with 1 mm pathlength for 10 s. The fatty acid profile was determined by gas chromatography (GC-FID) by derivatized fatty acid methyl esters. Spectral data was analyzed by pattern recognition using a soft independent modeling of class analogy (SIMCA) and partial least squares regression (PLSR) algorithms. PLSR allowed to match the NIR spectra with oils fatty acids profiles, predicting the percentage of palmitic, oleic, and linoleic acids ($R>0.90$, $SEP<3\%$). The SIMCA models successfully classified the type of oil used for frying. The performance of an external validation set was superior/comparable with previous studies by using benchtop system, achieving excellent sensitivity and selectivity. Overall, our results demonstrated that NIR coupled with chemometrics would be able to screen the oil type and its fatty acid profiles with a timely manner. This non-destructive and fast technology needs only 10 s and few amount of sample (~0.3 mL) and can assure the authenticity of our food supply; thus, it can easily substitute labor-intensive, time-consuming, and hazardous reference methods for fatty acid identification.

(FORENS-05.4)**High Ionic Strength-Resistant Colorimetric Sensor for Lead Ion Quantification in Tap Water**

Hanwei Wang, Seo Won Cho, Craig Butler, Haoran Wei, *University of Wisconsin–Madison*

Lead poisoning has been associated with the impairments in brain function and nervous system, particularly affecting the development and growth of children. One significant source of lead exposure is the corrosion of lead service lines used in aging drinking water distribution infrastructures. However, conventional analytical techniques, such as inductively coupled plasma mass spectrometry, are often time-consuming and expensive. Therefore, there is an urgent need for a simple sensor that can rapidly detect Pb2+ in drinking water at minimal cost. In this study, a nonaggregation-based colorimetric sensing approach was developed to achieve sensitive and selective detection of Pb2+ in tap water samples collected from Madison, WI and Chicago, IL.

Gold nanoparticles (AuNPs) are commonly utilized as colorimetric sensors for lead analysis. However, these sensors typically rely on the aggregation of AuNPs induced by Pb2+, which is challenging to control and unsuitable for use in matrices with high ionic strength, such as tap water sourced from groundwater. To overcome these challenges, we present an innovative approach by employing Janus gold nanorods (Au JNRs) for Pb2+ detection, instead of the commonly used gold nanospheres (AuNSs). The high colloidal stability of Au JNRs is attributed to the additional steric hindrance provided by the partial silica coating. Compared to AuNSs at an equivalent concentration, Au JNRs exhibited significantly lower propensity for aggregation in both salt solution and groundwater-based tap water (conductivity = 752 μS/cm). The gold nanorods within Au JNRs were etched by thiosulfate and 2-mercaptoethanol, resulting in a decrease in localized surface plasmon resonance (LSPR) extinction. The presence of Pb2+ significantly enhanced the gold leaching rate, which was exploited for rapid quantification of Pb2+. The sensor demonstrated high sensitivity towards Pb2+ with a limit of detection (LoD) of 10 nM, well below the Maximum Contaminant Level (75 nM) regulated by US Environmental Protection Agency. Furthermore, its cost-efficiency, simplicity of operation, and remarkable resistance to complex water matrices offer promising potential for the development of a smartphone-based sensor for household lead quantification in tap water.

(FORENS-05.5)**Hyperspectral Threat Anomaly Detection (Hyper ThreAD) Technique for Potential Explosive Threats**

Eric Languirand, Matthew Collins, *DEVCOM CBC*

Potential chemical threats such as toxic industrial compounds (TICs), pharmaceuticals, explosives, or chemical warfare agents pose a risk as a surface-based threat if the material(s) are dispersed on a surface or on the ground. Rapid assessment of that risk requires sampling a large area (as to not miss the dispersed analyte) and quick evaluation of the data to make an appropriate decision. Large area sampling can be achieved via hyperspectral analysis, though this technique provides a large amount of data that often requires post-processing and therefore may delay a response. Employing a hyperspectral threat anomaly detection (Hyper ThreAD) technique can achieve real-time analysis for anomalies (i.e., threats) on a surface.

Herein we discuss our recent efforts with our ThreAD technique, which is a semi-supervised machine learning algorithm to identify anomalies on a learned surface. The time-series spectral content associated with a push-broom hyperspectral spectrometer can be statistically parameterized into higher order statistics and information-theory based entropy for rapid anomaly assessment. Our work with multiple surfaces and in wetted conditions is discussed here.

23IR11: IR Frequency Combs, Sierra 3
Chair: Pedro Martin Mateos, Universidad Carlos III de Madrid

(IR-11.1)**Frequency combs for precision hyperspectral imaging**

Pedro Martín Mateos, *Universidad Carlos III de Madrid*

Dual-comb spectroscopy is based on the use of two closely related optical frequency combs (ideally equal offset frequency and slightly different repetition rates) that, after characterizing the sample under analysis, are combined into a photodetector. This multi-heterodyne mixing process enables the direct mapping of the spectral information engraved into the combs from the optical to the RF domain, where it can be easily accessed by common electronics. These sources have absolutely revolutionized, between other fields, optical spectroscopy by providing an unmatched combination of high resolution, high measurement speed and frequency accuracy.
Until very recently, for the detection of these very high-performance spectroscopic sources, a high bandwith photodetector was required, as heterodyne signals usually covered RF spectral spans of many MHz. However, the development of new dual-comb architectures, such as electro-optic dual-comb generators, thanks to the extreme coherence between the frequency combs, made it possible, for the first time, to obtain heterodyne signals with bandwidths that can be compressed down to a few hertz. This obviously made it possible to detect the multi-heterodyne signal using very low bandwidth

detectors, but much more importantly, it opened the way for direct hyperspectral imaging using dual-comb sources. All in all, this allows many of the characteristics of these sources to be exploited in hyperspectral imaging.
Direct dual-comb hyperspectral imaging could be considered a reasonably mature technology at this point in time. By the simplicity of the architecture, first demonstrations were performed in the near infrared region, in the vicinity of 1550 nm, where molecules such as ammonia can be easily targeted. These first experiments demonstrated the unmatched capabilities of the method to provide very high optical resolution in combination with very short measurement times. Nonetheless, further developments of the method enabled dual-comb imaging in the mid-infrared to open the access path to the fundamental vibrations of most molecules of interest. Subsequent technological developments have made it possible to open new frequency ranges and interesting applications.

(IR-11.2)**Paradigm Changes in Time Resolved Infrared Spectroeletrochemistry**

Scott Rosendahl, Ian Burgess, *University Of Saskatchewan, Canadian Light Source Inc.*

Time-resolved vibrational spectroelectrochemistry (SEC) is a powerful tool for following dynamic processes occurring at, or near, electrode surfaces. When the thermodynamic perturbation that drives a kinetic process is the applied potential, temporal resolution in infrared SEC is limited by one of the following two factors; 1) the inherent electrochemical cell constant (RC) and 2) the speed of the instrument's interferometer. These factors have effectively limited the time resolution of IR SEC studies to the order of 10-4 s or higher. Herein, we describe two approaches to access microsecond resolved IR spectroelectrochemistry. The first approach uses a microband electrode printed on a silicon internal reflection element to decrease the critical dimension of the electrode to several hundred micrometers thus providing a sub-microsecond time constant in a Kretschmann configured spectroelectrochemical cell. The high brilliance of synchrotron sourced infrared radiation has been combined with a specially designed horizontal attenuated total reflectance (ATR) microscope to focus the infrared beam on the microband electrode. The first use of a sub-microsecond time constant working electrode for ATR surface enhanced infrared absorption spectroscopy (ATR-SEIRAS) is reported. The second approach employs a dual infrared frequency comb spectrometer with heterodyne detection. The measurement of the potential dependent desorption of a monolayer of a pyridine derivative (4-dimethylaminopyridine, DMAP) with time resolution as high as 4 μs was achieved without the use of step-scan interferometry. An analysis of the detection limit of the method as a function of both time resolution and measurement co-additions is provided and compared to step-scan experiments of an equivalent system. Dual frequency comb spectroscopy is shown to be highly amenable to time-resolved ATR-SEIRAS. Microsecond resolved spectra can be obtained with high spectral resolution and fractional monolayer detection limits in a total experimental duration that is two orders of magnitude less than the equivalent step-scan experiment.

(IR-11.3)**Frequency Combs for Spectroscopy and 3D Imaging**

Nathalie Picque, *MPI Of Quantum Optics*

A frequency comb is a broad spectrum of evenly spaced phase-coherent narrow laser lines. Initially invented for frequency metrology, such combs enable new approaches to interferometry. Exploiting time-domain interference between frequency combs of different repetition frequency has grown increasingly popular.
One of the most widespread applications has been dual-comb spectroscopy, which enables fast and accurate measurements over broad spectral bandwidths, of particular relevance to molecular sensing. Dual-comb spectroscopy is a new type of Fourier transform spectroscopy where the interferometer has no moving parts. Compared to conventional Michelson-based FTIR spectroscopy, recording times could be shortened from seconds to microseconds, with intriguing prospects for spectroscopy of short lived transient species. Accurate determination of all spectral line parameters and broadband detection in light-starved conditions become possible in regions of interest to sensing such as in the mid-infrared fingerprint region. Combined to nonlinear excitation of the samples, they open up new opportunities for precision spectroscopy and stringent comparisons with theories in atomic and molecular physics.

Concurrently, progress towards chip-scale dual-comb spectrometers promises integrated devices for real-time sensing in analytical chemistry and biomedicine.
Recently, dual-comb digital holography, another application of frequency-comb interferometry, has been demonstrated. The combination of broad spectral bandwidth and high temporal coherence opens up novel optical diagnostics, such as precise dimensional metrology over large distances without interferometric phase ambiguity, or hyperspectral 3-dimensional imaging with molecule-selective imaging of an absorbing gas.
With selected examples, I will illustrate the rapidly advancing field of dual-comb spectroscopy.

(IR-11.4)**High-resolution Quantum Cascade Laser Dual-comb Spectroscopy and Hyperspectral Imaging in the Mid-infrared**

Gerard Wysocki, *Princeton University*

Numerous molecules of environmental, industrial and security/safety importance have their strongest ro-vibrational bands in the mid-infrared spectral region that plays a key role in detection and quantification of many chemical species. In the recent years, quantum cascade laser frequency comb sources (QCL-FCs) operating in the mid-infrared have proved to be reliable spectroscopic sources for chemical sensing in the laboratory as well as in field-deployable sensing systems. These frequency combs also offer a tremendous potential for future monolithic/hybrid integration with photonic platforms to enable lab-on-chip applications.
We have developed a variety of chemical sensing techniques based on dual-comb spectroscopy (DCS) with QCL-FCs that can acquire mid-infrared spectra with temporal resolution down to 10 μs/spectrum while providing broadband spectral coverage (up to 90 cm^-1) and high spectral resolution (in the ~MHz or ~10^-5 cm^-1 range). In this talk I will discuss mid-infrared QCL-DCS systems used to perform both in-situ and stand-off spectroscopic detection of trace-gases and hyperspectral imaging of solids/liquids on surfaces. To enable absorption spectroscopy with MHz-level resolution and gapless optical frequency tuning, we implemented repetition rate (f_rep) control via precise radio-frequency (RF) injection, thermal/current control of offset frequency (f_ceo), and high-speed phase- and timing-correction algorithms to enable computational coherent averaging of the acquired DCS spectra for sensitivity improvements. Such a precise control of QCL-DCS system as well as other sensitivity- and selectivity-enhancement techniques such as wavelength modulation spectroscopy or Faraday rotation spectroscopy of paramagnetic molecules, provide a variety of broadband DCS tools for advanced chemical detection. Examples of QCL-DCS laboratory demonstrators as well as field-deployable remote detection systems for trace-gas detection and localization of chemical plumes will be presented in details.

(IR-11.5)**Ultraviolet Dual-Comb Absorption Spectroscopy of Neutral and Ionized Fe in a Laser-Produced Plasma**

John McCauley, Mark Phillips, Yu Zhang, Jason Jones, Sivanandan Harilal, Reagan Weeks, *Air Force Research Laboratory*, *University Of Arizona, Pacific Northwest National laboratory*

Absorption spectroscopy of laser-produced plasmas (LPPs) provides valuable and complementary information to optical emission spectroscopy (LIBS). In particular, high-resolution absorption spectroscopy is useful for resolving linewidths and lineshapes of atomic transitions without instrumental broadening and can probe transitions from low energy levels with large cross-sections. However, laser sources suitable for high-resolution spectroscopy of atomic transitions have been limited in wavelength coverage, especially in the ultraviolet region which contains strong resonance transitions for many elements. We have recently extended operation of Ytterbium-fiber laser frequency combs to access ultraviolet wavelengths via fourth-harmonic generation to 260-266 nm. Using this new system, we perform dual-comb broadband absorption spectroscopy of neutral and ionized Fe in an LPP. Absorption spectra of multiple transitions from Fe I and Fe II are measured from delays 5-150 us, with spectral resolution < 1 pm. Spectra are analyzed to determine quantitative column densities and excitation temperatures of both neutral and ionized atoms. Absorption from Fe ions is observed at delays up to 150 us, providing information on electron density at late times of LPP evolution beyond what can typically be probed using emission spectroscopy.

23IR13: Applications of Optical- and AFM-Photothermal IR Spectroscopy, Sierra 2
Chair: Minghe Li
Co-Chair: Curtis Marcott

(IR-13.1)**Correlative Fluorescent and Infrared Imaging and Spectroscopic Analysis of Human Primary Skin Fibroblasts**

Kathleen Gough, Sabine Mai, Matheus Fabiao de Lima, Benoit Girouard, Sheyenne Gamage, D University of Manitoba, Rohith Reddy, Chalapathi-charan Gajjela,Univ. Of Houston, arryl Dyck, Lumiere Microscopy, Winnipeg MB

Our long-term goal is correlate fluorescent, infrared and Raman spectra and images, at <300 nm spatial resolution, of human buccal cells obtained via standard cheek swabs, to improve our understanding of genomic instability and changes in nuclear architecture related to ageing and Alzheimer's disease. Currently, we are analyzing GL 51/92 primary human fibroblasts to explore multiple immunostaining strategies and establish protocols. Previously, proof-of-principle experiments were performed on Human Oral Mucosal Epithelial cells (H-6234, Cell Biologics Inc. USA), mounted on CaF2 windows or thin glass coverslips, suitable for spectroscopy and imaging. Here, GL 51/92 cells are chosen as they can be maintained and grown through 20 passages without aging, while the H-6234 could only be used once. Freshly thawed cells were plated onto sterile growth medium and allowed to grow to sub-confluence. Cells were gently harvested by removing most growth medium through suction, then gently scraping the cell-rich fraction into eppendorf tubes and washing in normal saline. A few microlitres of cell suspension were placed on the substrates, air dried and rinsed with a few microlitres of ultrapure water to remove salt residue. Infrared data were obtained with a mIRage™ IR Microscope operating under PTIR studio software (v. 4.3). Approximately 50 cells were imaged at single wavelengths, with >70 individual spectra from 1800-900 cm-1 for CaF2 windows or 1800-1350 cm-1 for glass coverslips. Hyperspectral images with 0.25 micron step size were obtained on select targets. Depth profiling on a spindle-shaped cell was achieved by collecting 10 z-stacked layers. Following spectroscopy with mIRage™, samples on CaF2 underwent immunostaining and correlative fluorescent imaging for nuclei (DNA stained with DAPI and Lamin A/C stained with anti-lamin A/C-Alexa 488); cells on glass coverslips were stained with Mitoview green for mitochondria with DAPI counterstaining DNA. Fluorescent images were deconvolved to ~200 nm in x-y; stacks were recorded at 102 nm in z. Additional fluorescent imaging was performed on these and on parallel cells to identify other organelle features. O-PTIR images revealed lipid content lost during subsequent fixation; correlations between OPTIR images and spectra and deconvolved fluorescence images facilitated establishment of IR spectroscopic biomarkers.

(IR-13.2)**Challenges and constraints of multimodal-multiscale vibrational spectroscopy imaging in biomedical: A breast microcalcifications case study**

Margaux Petay, Alexandre Dazzi, Dominique Bazin
Ariane Deniset-besseau, , Maguy Cherfan, *Institut de Chimie Physique, CNRS, Université Paris-Saclay, Université Paris-Saclay, Hopital NOVO, GHT Nord Ouest Val D'oise*

Breast microcalcifications (BMCs) are calcium-based mineral deposits in breast tissue. When observed on mammograms, these deposits can provide valuable information as their morphologies and spatial distribution can be marks of different pathologies. Over the past decades, several studies based on vibrational spectroscopy attempted to chemically characterize BMCs in breast tissue. So far, findings indicate that BMCs exhibit greater chemical diversity than historically acknowledged but also emphasize a potential link between BMC chemical speciation and the patient's clinical diagnosis[1,2,3]. However, the formation of microcalcifications in the breast is not fully comprehended. BMCs vary in size ranging from a few nanometers to several hundreds of microns and are distributed within breast biopsies which are millimeter-sized samples. Therefore, to comprehend

their formation and function, it is essential to adopt a multiscale approach that combines BMCs fine chemical analysis with consideration of the overall histopathological context of the biopsy. In that respect, we implemented a multimodal and multiscale approach that combines scanning electron microscopy (SEM), classic and super-resolved infrared (IR) microscopy that enables BMCs chemical description in breast biopsy up to the nanoscale[4]. SEM enables to morphologically characterized BMCs and to locate them in the sample, while IR microscopy provides their chemical composition. Finally, we look at their fine chemical speciation through AFM-IR analysis: a technique that combines an atomic force microscope and IR spectromicroscopy and provides a chemical description with a spatial resolution of about 20 nm. This multiscale strategy offers insights into BMCs complex chemical structure at both micrometric and sub-micrometric scales: all of which could contribute to expanding our understanding of biomineralization in breast tissue. Furthermore, this approach, initially developed for breast tissue, holds promise for adaptation to other biomedical applications requiring nanoscale understanding within a broader biomedical picture. In this presentation, we will introduce our multimodal-multiscale method, explore its significance, as well as discuss its experimental feasibility and limitations.

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(IR-13.3)**Advancing bottom-illuminated photothermal nanoscale chemical imaging - in air and liquid - by introducing cost-effective, flat silicon carriers**

Ufuk Yilmaz, Bernhard Lendl, Georg Ramer, *Technische Universität Wien*

INTRODUCTION
Infrared (IR) spectroscopy is a powerful method providing information about chemical compositions. However, it is limited by the Rayleigh criterium and thus the spatial resolution is limited by the wavelength used. Furthermore, measurements in solvents like water are challenging due to high absorption in the mid-IR. Atomic force microscopy infrared spectroscopy (AFM-IR) is a nearfield technique to overcome these drawbacks. A bottom-illuminated setup with attenuated total reflection (ATR) allows nanoscale measurements in liquids. Traditionally, high-refractive index ATR prisms (e.g., ZnSe, Ge) are used, but they have limitations, including unsuitability for acidic samples or liquids, challenging handling and sample preparation, difficulty in functionalization, and high cost.

METHODS
In this study, we introduce a novel sample carrier for liquid AFM-IR measurements using a flat, micromachined silicon (Si) ATR crystal. We make them available in a commercially available AFM-IR, normally employing conventional prisms. Our approach unlocks new possibilities and advancements for measurements in liquids. The sim-ple shape of the crystal facilitates easy handling and since it is made of Si, surface functionalization becomes simpler, benefitting from a wealth of existing literature. Prior to our experiments we conducted ray tracing and finite-difference time-domain method (FDTD) simulations. Our findings regarding beam intensity distribution on the crystal surface, as well as the development of the evanescent wave underline the excellent suitability of the flat, ATR crystals for AFM-IR. Of course, adapting instrumentation using conventional prisms required adjustments. Here, we employed rapid prototyping, making existing instrumentation accepting the new, flat-shaped crystals. To ensure high sensitivity, modifications to the beam paths were made to account for the change in material and geometry.

RESULTS
We successfully introduced a new type of ATR and demonstrated the feasibility using a polymer blend of polystyrene (PS) and poly(methyl methacrylate) (PMMA) in both, water and air. We recorded IR spectra at nanoscale and perform chemical imaging. Now, we are taking advantage of the functionalization ability of the Si-ATR crystal and demonstrating that our approach is a more flexible and more cost-effective method for AFM-IR measurements in liquids.

(IR-13.4)**Bond Selective Fluorescence Imaging with Single Molecule Sensitivity**

Dongkwan Lee, Haomin Wang, Lu Wei, *Caltech*

Bioimaging harnessing optical contrasts and chemical specificity is of vital importance in probing complex biology. Vibrational spectroscopy based on mid-infrared (mid-IR) excitation can reveal rich chemical information about molecular distributions. However, its full potential for bioimaging is hindered by the achievable sensitivity. Here, we report bond selective fluorescence-detected infrared-excited (BonFIRE) spectral microscopy. BonFIRE employs two-photon excitation in the mid-IR and near-IR to upconvert vibrational excitations to electronic states for fluorescence detection, thus encoding vibrational information into fluorescence. The system utilizes tuneable narrowband picosecond pulses to ensure high sensitivity, biocompatibility, and robustness for bond-selective biological interrogations over a wide spectrum of reporter molecules. We demonstrate BonFIRE spectral imaging in both fingerprint and cell-silent spectroscopic windows with single-molecule sensitivity for common fluorescent dyes. We then demonstrate BonFIRE imaging on various intracellular targets in fixed and live cells, neurons, and tissues, with promises for further vibrational multiplexing. For dynamic bioanalysis in living systems, we implement a high-frequency modulation scheme and demonstrate time-lapse BonFIRE microscopy of live HeLa cells. We expect BonFIRE to expand the bioimaging toolbox by providing a new level of bond-specific vibrational information and facilitate functional imaging and sensing for biological investigations.

23LIBS03: LIBS for Forensics and Security, Southern Pacific B/C

Chair: Tatiana Trejos
Co-Chair: Ruthmara Corzo

(LIBS-03.1)**A Two-Step Method for the Identification and Highly Specific Analysis of Organic Gunshot Residue Using Raman and Laser-Induced Breakdown Spectroscopies**

Igor Lednev, Shelby Khandasammy, Lenka Halámková, Matthieu Baudelet, *University at Albany, SUNY, University Of Central Florida, Texas Tech University*

A novel two-step method using Raman spectroscopy for the identification and laser-induced breakdown spectroscopy (LIBS) for highly specific analysis of individual organic gunshot residue (OGSR) particles will be discussed. In this study, organic gunshot residue (OGSR) was generated from three cartridges sharing the same manufacturer, caliber, firing conditions, and discharging firearm. These three cartridges shared many similarities overall, and only differed minimally based on the bullet type. GSR samples were generated by firing using a GLOCK Model 26 semi-automatic 9 mm firearm, which was discharged by our collaborators from the NY State Police Forensic Investigation Center from approximately 0.3 m away into a 9x9 inch cloth substrate. Raman spectroscopy was first performed to identify and confirm the identity of the OGSR particles. Then, LIBS was used to probe individual OGSR particles. A Support Vector Machine-Discriminant Analysis (SVM-DA) statistical model with 5 latent variables was created based on LIBS data to differentiate between OGSR generated from three different bullet types. LIBS lines for barium, lead, copper calcium, iron, sodium, potassium titanium, silicon, strontium, and the CN molecular band were notably identified as statistically significant lines. Overall, the model allowed for excellent differentiation between the three OGSR groups. This study demonstrates that the LIBS analysis of OGSR has the potential to point to specific ammunition type as a source of discharge and is not limited to broad identification of manufacturers. The two-step method presented has the potential to allow forensic examiners to use Raman spectroscopy to identify OGSR particles and then utilize LIBS for their highly specific analysis. Future work on exploring this methodology is needed and is intended to be performed with an expanded spectral dataset and a greater variety of ammunition samples including different manufacturers and calibers.
This project was supported by Award No. 15PNIJ-21-GG-04153-RESS (I.K.L.) and Award No. 2019-R2-CX-0035 (S.R.K.) awarded by the National Institute of Justice, Office of Justice Programs, U.S.

Department of Justice. The opinions, findings, and conclusions or recommendations expressed in this publication are those of the authors and do not necessarily reflect those of the Department of Justice.

(LIBS-03.2)**Development of a method for the forensic discrimination of solder by LIBS-LA-ICPMS**

Claude Dalpe,Nigel Hearns, Royal Canadian Mounted Police, Katie Moghadam, Diane Beauchemin, *Queen's University, Department of Chemistry*

This research focuses on the development of a unique analytical method by laser induced breakdown spectroscopy (LIBS) combined with laser ablation inductively coupled plasma mass spectrometry (LA-ICPMS). The tandem LIBS-LA-ICPMS technique is a desirable tool for the sensitive multi-elemental determination, characterization, and classification of alloyed forensic evidence (i.e., metal alloys such as solder, precious metal, and steel pipes) encountered in some high-profile caseworks. LIBS has been applied successfully for the quantitative analysis of complex solid matrices, namely solders [1], yet augmenting this technology with LA-ICPMS improves detection limits and enhances discrimination relative to either technique alone [2]. In this method, analysis is performed in-situ to provide direct solid sampling and eliminate the challenging sample digestion of solid matrices [3]. Impressively, the LIBS-LA-ICPMS technique achieves mere microscopic destruction of the sample, which is useful in preserving the exhibit and for analyses of evidence in exceedingly small quantities.

The application of this method will be demonstrated on lead-free solder alloys, which form valuable evidence from post-blast crime scenes involving improvised explosive devices (IEDs). Both the major (alloying metals) and trace elements (impurities or additives) were quantified in solders and utilized in multivariate chemometric analyses. The use of chemometrics provide the means to identify unknown materials with a high degree of discrimination, and most importantly enable associations between known and questioned materials seized from crime scenes and/or warrant searches. This holds both investigative and evidentiary value in establishing sample identity and provenance. For the first time ever, outcomes from this project will lead to the creation of the first database of solder metal alloys.

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(LIBS-03.3)**Advancement of LIBS Mobile Technology for the Detection of Firearm Discharge Residue from Various Substrates and Assessment at Mock Crime Scenes**

Kourtney Dalzell, Leah Thomas, Thomas Ledergerber, Courtney Vander Pyl, Tatiana Trejos, Luis Arroyo, Jhanis Gonzalez, Chunyi Liu, Miyeun Yoo, Richard Russo, *West Virginia University, Excimer Laser Ablation-Based Tandem Approach to the Analysis of Solid Samples*

In the last decade, our research groups have developed Laser-induced breakdown spectroscopy (LIBS) methods for gunshot residue (GSR) applications as a rapid, reliable technology that can streamline laboratory and crime scene processes. A laboratory LIBS unit was compared to a mobile instrument using authentic hand samples from shooters (100 samples) and non-shooters (200 background samples). A significant novelty of the portable instrument is its enhanced imaging, which allows quick searching and visualization of GSR particles for single-micron-sized particle examination. Both instruments obtained accuracy better than 98.8%, demonstrating their suitability for trace IGSR detection from skin specimens. This study evaluates LIBS capabilities for GSR detection in other substrates commonly encountered at a crime scene and is tested in mock crime scene situations.

Also, alternative substrates were exposed to a shooting at close range (10 fabrics, 7 wood, 7 drywall) for quick identification of entrance bullet holes and GSR residue around it. Performance rates for all substrate types resulted in 100% true positives and 100% true negatives demonstrating high accuracy and no potential interferences from the substrates. The mock crime scene scenarios encompassed 3 situations commonly involving a firearm, including arrests, homicides, and suicides. Two different arrest scenarios were assessed for detection of GSR from a known shooter and known non-shooter, as well as potential transfer from handling the firearm to and from the arresting personnel. The homicide

situation evaluated the shooter after a delayed arrest and a fabric sample from the victim. The suicide scenario evaluated the persistence of GSR from the suicide victims' left and right hand after 30 minutes and the potential transfer to a first responder or family member that had contact with the deceased. Conclusions from both studies highlight the LIBS applicability to provide fast investigative leads under realistic casework-like samples. Implementation of this methodology is anticipated to drastically speed up response times (i.e., from several hours per sample by standard SEM-EDS practice to a few minutes by LIBS.)

(LIBS-03.4)**Advances in Tandem LA – LIBS Technology and Data Analysis**

Jhanis Gonzalez, Charles Sission, Chunyi Liu, Steve Shuttleworth, Miyeun Yoo, Richard Russo, *Applied Spectra Inc, Excimer Laser Ablation-Based Tandem Approach to the Analysis of Solid Samples, Advances in Tandem LA – LIBS Technology and Data Analysis*

Since 2012, for more than a decade, Applied Spectra has led both introduction and advancement of tandem LA-LIBS instrumentation. This laser ablation chemical analysis technology combines LIBS and LA-ICP capabilities to address the needs of the analytical science community to analyze heterogeneous materials with higher productivity, superior sensitivity and enhanced precision, while delivering the maximum chemical information with each laser sampling. Applied Spectra continues to evolve this technology to exploit some unique advantages of LIBS and LA-ICP in the analysis of solid samples. This presentation highlights the advantages of the Tandem Instrument utilizing a high performance sample chamber capable of providing fast washout of ablated particles to ICP-MS/OES, while maintaining the optimum LIBS sensitivity for multiple LIBS sensors that may operate simultaneously. Examples of rapid and high-resolution Tandem LA - LIBS imaging for spatially structured (2D and 3D) or zoned samples will be discussed. Finally, new data analysis capability for processing and fusing LIBS and LA-ICP-MS is discussed as a part of data software advances (ClarityNeXtTM) for the Tandem Instrument. The software advance brought by ClarityNeXtTM significantly enhances the way chemical images are visualized for both LIBS and LA-ICP-MS data and sample classification power by incorporating and weighing the unique chemical fingerprinting data that LIBS and LA-ICP-MS provide.

(LIBS-03.5)**Performance of μXRF and LIBS for the forensic analysis of small and irregular glass fragments**

Ruthmara Corzo,Nist Oriana Ovide, Tatiana Trejos, *West Virginia University*

Glass is a common type of trace evidence that is often submitted to forensic casework. The elemental analysis of glass, using analytical techniques such as Laser Induced Breakdown Spectroscopy (LIBS) and micro-X-ray Fluorescence Spectrometry (μXRF), has been shown to provide excellent discrimination of glass samples that originate from different sources. Many studies involving the analysis of glass using LIBS or μXRF have focused on large, full-thickness glass samples. However, glass evidence submitted to forensic laboratories are typically small and irregularly shaped. This study aimed to assess the performance of LIBS and μXRF when analyzing small (< 1 mm) and irregular fragments, compared to full-thickness fragments. One hundred glass fragments from the inner and outer pane of a single windshield were analyzed to evaluate the false exclusion rates. An additional 100 fragments from different windshields were also analyzed to evaluate the discrimination potential and false inclusion rates. This study demonstrated that precision deteriorated when analyzing small fragments compared to full-thickness fragments. Moreover, the study supports the ASTM E2926 recommendation to perform comparisons between fragments similar in shape and size. When comparing one questioned (Q) fragment to four known (K) fragments, LIBS resulted in a false exclusion rate < 3% for full-thickness fragments and < 10% for small fragments. However, increasing the number of small K fragments reduced the false exclusion rate to < 4%. LIBS resulted in a false inclusion rate < 6% for full-thickness fragments and < 23% for small fragments. Micro-XRF also resulted in better false exclusion rates for full-thickness fragments compared to small fragments (< 12% and < 25%, respectively) when comparing one Q fragment to four K fragments. Increasing the number of small K fragments decreased the false exclusion to < 16%, and the μXRF false inclusion rates for both full-thickness and small fragments were < 1%. Despite the poorer precision observed

when comparing small fragments, the within-source variability remained smaller than the between-source variability. This study demonstrates the continued utility of LIBS and μXRF for forensic glass examinations; however, care must be taken to account for the higher variability and to reduce the error rates.

23PAT06: PAT in Petroleum and Refinery Industries, Southern Pacific E
Chair: Toni Miao, Research Chemist

(PAT-06.1)**Detailed PIONA of Mogas built by GC and predicted using Multivariate IR**

Bryan Bowie, Aditya Shetkar, Payman Pirzadeh, *Exxonmobil*

Traditionally when a detailed characterization of Mogas is needed a GC or series of GC runs would be performed. The data would then be typically tabulated into a paraffins, olefins, naphthenes and aromatics (PIONA) by carbon number. More detail could also be gleaned by combining the results of multiple GC rund including a PIONA/Reforulizer. The data from these can then be combined to give information on more than 225 compounds or a PIONA+. If a PIONA is needed in a real-time setting, online PIONA GC's can be more difficult to maintain than lab systems and the time between samples can be large. Spectroscopy can be more stable online and easier to maintain. However, this would imply the need for building hundreds of multivariate models for all the individual species or dozens for different chemical classes. This can be circumvented by regressing the spectra of the unknown against a library of knowns which contain spectra and a detailed PIONA analysis as described above. Thus, with one model, the composition or PIONA with chemical speciation can be predicted. This makes spectroscopy a more viable option to ensure mogas composition online.
Over 100 samples of finished mogas were collected from more than 5 different refineries spanning all north America over a year period. These data include all seasonal and grade variations as well as refinery feed variations. A three-part GC run was performed on all samples, as described above, to obtain a detailed PIONA with chemical speciation. A leave-one-out (LOO) cross-validation was performed using least squared regression to estimate the error. The calculated regression vector from the least squares operation was used to calculate the 225+ molecule composition or PIONA with chemical speciation. This means that one model can be used to predict composition of a mogas blend. Furthermore, the composition can also be used to calculate an estimate of some performance properties such as RON or MON.
1. "SYSTEMS AND PROCESSES FOR PERFORMANCE PROPERTY DETERMINATION USING OPTICAL SPECTRAL DATA". US patent No. 62/740,032 (2018)

(PAT-06.2)**The Use Of Static Optics FTIR For Controlling Petrochemical And Refinery Operations With Mid Infrared**

Victoria Grigson, Jonathon Speed, Adam Wilson, Kiran Haroon, Carolina Cruz, *Keit Industrial Analytics*

Mid infrared spectroscopy is full of information that would be useful for petrochemical and refinery applications – it is arguably the most informative type of spectroscopy available for modern chemistry. Traditional instrumentation requires the use a Michelson interferometer built upon moving mirrors, which are inherently sensitive to vibration and movement, making them unsuitable for industrial installations. Fiber optic probes can be used to move the spectrometer some distance from the process, but these fibers are significantly lower performance than near infrared comparatives, and are often so fragile they are also unsuitable for use.

Here we present the use of static optics FTIR – a spectrometer based on a Sagnac interferometer – specifically designed for industrial use. We will explain the fundamental optical design and how it differs to conventional instrument design, as well as some of the benefits and drawbacks. We will explain some of the learnings discovered in the design of the instrument, and modifications made to the design over time to optimize stability and reliability, even at the cost of performance if required.

We will also demonstrate some applications of the instrument ranging from renewable diesel feedstock monitoring, through to contaminant formation and catalyst monitoring in refining processes.

(PAT-06.3)**Portable FT-NIR spectral sensing for instant and global insights**

Bob Schumann,s Mostafa Medhat, *Si-Ware System*

We present a multidisciplinary platform seamlessly integrating advanced optical MEMS, rugged device engineering, smart application interfaces, and cloud-based SaaS. This convergence enables advancing portable analytical technology to unprecedented heights, offering unprecedented performance and flexibility to on-site analysis.

This fusion of cutting-edge components creates a platform capable of providing instantaneous insights with implications reaching far beyond its initial application. In contrast to conventional methodologies, this platform empowers users with the ability to conduct real-time, non-destructive analyses at the point of need, significantly improving the dynamics of decision-making processes.

In comparison to other portable spectrometers, the inclusion of Optical MEMS to create FT-NIR spectrometers provides unique features and performance. Furthermore, the integration of engineered rugged devices extends the platform's applications across a diverse range of industries, bridging the gap between laboratory-grade analyses and in-field requirements. The integration of intuitive smart apps ensures reliable measurements from non-technical personnel, while a cloud-based SaaS portal provides seamless evaluation and insights into critical parameters from anywhere.

This transformative technology is used in a wide variety of industries, impacting sectors that span the breadth of agriculture and food to industrial and chemical. In this presentation, a spotlight is cast on the field of soil analysis, with a specific focus on the determination of Total Petroleum Hydrocarbons (TPH).

(PAT-06.4)**Site-Independent Determination of Total Petroleum Hydrocarbons in Soil by Handheld NIR Spectroscopy: Locally Weighted Regression**

Marina De Gea Neves, Toni Miao, Natasha Sihota, Cory McDaniel, Heinz Sieslerm, *University Duisbr-essen, Department of Physical Chemistry, Chevron Technical Center*

Total petroleum hydrocarbons (TPH) are one of the possible contaminants in soils and it is of utmost importance to identify and quantify them for soil remediation. Standard methodologies are based on the collection of environmental samples, followed by laboratory analyses resulting in a time consuming and costly delaying management decision. The use of handheld NIR spectroscopy can dramatically decrease the time/cost of analyses. Once the calibration model has been developed, new samples can be predicted in short time.
With the recent development of lightweight (<500 g) and more cost-effective (<$10,000) handheld NIR spectrometers, that are optimized for practicality and have greater flexibility in operation, on-site measurements for various analytes using this technique have become very popular.
Partial Least Squares (PLS) is an efficient method for developing predictions based on NIR spectroscopic data when the relationship between the spectral information and the chemical or physical properties to be determined is linear. However, soil samples, for the most part, exhibit great variability and heterogeneity in composition, especially when they have different geographic origins, resulting in nonlinearities. This can significantly alter the predictions if nonlinear methods are not applied to correct this effect. In this work, PLS and LW-PLS (Locally Weighted - PLS) were applied to develop global calibrations that included three continents (US-North America, Kuwait-Asia, and Australia-Oceania) for the quantification of TPH in soil.
To evaluate the performance of the regressions, a calibration set was used with 89 samples from US (site 1), 56 from US (site 2), 44 from Australia, and 20 from Kuwait, for a total of 209 samples with a TPH content range of 0 to 30,000 ppm, and a test set with 15 samples from US (site 1) and US (site 2), 5 from Australia, and 3 from Kuwait, for a total of 23 samples. With the PLS model RMSE and R^2 values of 3263 ppm and 0.708, respectively, where achieved, while the LW-PLS model yielded RMSE

and R^2 values of 1417 ppm and 0.944, respectively. Thus, the use of the LW-PLS algorithm has significantly increased the quality of the calibration and prediction results compared to the PLS.

23PMA09: Analysis of Proteins, Antibodies, Biologicals and Nucleic Acids, Southern Pacific D
Chair: Jaimie Dufresne, YYZ Pharmatech
Co-Chair: John Wasylyk, Bristol Myers Squibb

(PMA-09.1)**Protein kinase A displacement and aberrant interactions underlie adrenal Cushing's syndrome**

Mitchell Omar, *University Of Nevada Reno*

Mutations in the catalytic subunit of protein kinase A (PKAc) drive the stress disorder adrenal Cushing's syndrome, a disease of chronic hypercortisolism. Patients suffer from symptoms such as cardiovascular disease, depression, obesity, and osteoporosis. We define mechanisms of action for two Cushing's mutant kinases, PKAc-L205R and W196R. Proximity biotinylation and photoactivation microscopy demonstrate that both variants are mislocalized in mutant adrenal cells and excluded from their normal signaling complexes. Rescue experiments that tether the mutants into PKA signaling complexes abolish cortisol overproduction, indicating that kinase anchoring can restore normal endocrine function. Proximity phosphoproteomic experiments reveal the surprising finding that these highly similar mutants engage mutually exclusive mitogenic downstream signaling pathways, with upregulation of YAP/TAZ by PKAc-L205R and ERK kinase activation by PKAc-W196R. Analyses of adrenal-specific PKAc-W196R knock-in mice and Cushing's syndrome patient tissue corroborate this evidence. Finally, unpublished proximity labeling experiments identify a new model of PKAc pathophysiology as observed with the novel Cushing's variant PKAc-W196G. Overall, we find that aberrant spatiotemporal regulation of adrenal Cushing's syndrome kinase mutants drives disease and promotes distinct pathogenic signals.

(PMA-09.2)**Enzyme Linked Mass Spectrometric Assay (ELiMSA) for Sensitive Detection of DNA from SARS-CoV2, HIV and pUC19**

John Marshall, **Ming Miao**, *YYZ Pharmatech*

An ultrasensitive technology for DNA detection has developed through combining hybridization, signal amplification using an enzyme conjugate with quantification of the enzyme product by mass spectrometry, named Enzyme Linked Mass Spectrometric Assay (DNA ELiMSA). DNA hybridization assays are quantitative, linear and continuous with a high degree of freedom but poor sensitivity compared to PCR that has great sensitivity but inferior linearity over ~5 to15 amplification cycles. The COVID-19 pandemic has shown that a sensitive and accurate identification of pathogen like SARS-CoV2 is of vital importance to diagnosis, treatment, and monitoring epidemics. Specific DNA capture sequences immobilized on a 96-well plate were incubated with DNA targets, PCR products or sheared plasmid. The powerful enzyme alkaline phosphatase conjugated with the high affinity biding reagent streptavidin (APSA) was used to amplify signal from biotinylated DNA detection probes after hybridization. The signal was generated by APSA that converted adenosine monophosphate (AMP) to adenosine that can be sensitively and specifically analyzed by liquid chromatography electrospray ionization and tandem mass spectrometry (LC-ESI-MS/MS) alongside a heavy adenosine internal standard. The study of a synthetic SARS-CoV2 DNA target (155mer) showed detection above background to 10 fM with safe quantitation from 100 fM – 10 nM with good linearity (R2 = 0.99). A comparison with the gold standard of gel electrophoresis and GelRed fluorescence detection showed ELiMSA detection was more sensitive by 2-3 orders of magnitude for specific SARS-CoV2 PCR products. The detection of SARS-CoV2 nucleocapsid plasmid after sonication (shearing) followed by DNA ELiMSA showed a sensitivity through the 100 pM - 100 nM concentration range with good linearity (R2 = 0.98). In addition, ELiMSA was applied to other DNA sequences that the safe detection of HIV PCR products reached 100 fM while safe detection of pUC19 reached10 fM. Here we demonstrate that greater sensitivity in buffer or serum can be achieved by the combination of DNA

hybridization assay with APSA enzyme amplification for detection of the adenosine product by mass spectrometry (ELiMSA) than traditional methods.

(PMA-09.3)**Plate-based Infrared Spectroscopy for Rapid Peptide Quantitation: A Potential Drug Discovery Workflow**

Raffeal Bennett, Donovan A. Adpressa, Alexey A. Makarov, Merck, Nathaniel Hendrick, Kaitlyn Corazzata, Aaron Beeler, Douglas Fraser, *Department of Chemistry, Boston University, Tornado Spectral Systems*

Pharmaceutical discovery efforts toward finding new, efficacious peptide-based therapeutics have increased the throughput of peptide development, allowing the rapid generation of unique and pure peptide samples. However, establishing analytical workflows that match the speed of modern synthetic processes for peptides remains a difficult challenge in drug discovery. We report herein a fit-for-purpose method to quantify peptide concentration through the mode of high-throughput infrared spectroscopy (HT-IR). Multiple critical method parameters were optimized including solvent composition, droplet deposition size, plate drying procedures, sample concentration, and internal standard. The relative absorbance of the amide region (1600-1750 cm-1) to the internal standard, $K_3Fe(CN)_6$ (2140 cm-1), was determined to be most effective at providing the most specificity for the analyzed peptide. The best sample dissolution solvent was a 50:50 v/v allyl alcohol/water mixture for deposition of peptides on 96-well plates with an analysis rate of 22 minutes per plate. Calibration curves of sample concentration versus relative IR response were generated for three peptides and showed sufficient linearity ($R^2 > 0.95$). The linear dynamic range of the method was determined to be between 1 and 5 mg/mL. The repeatability and wide applicability were demonstrated with eighteen peptide samples that were measured with most values below 20% relative standard deviation. This developed HT-IR methodology could be a useful tool in peptide drug candidate lead identification and optimization processes.

23RAM08: Raman Imaging, Cascade 1
Chair: Katsumasa Fujita, Osaka University

(RAM-08.1)**Raman-fingerprint-activated cell sorting**

Kotaro Hiramatsu, *The University of Tokyo*

Cell sorting serves as a fundamental tool in biological research and medicine, frequently employed to separate diverse cell populations based on their inherent characteristics. Recently, Raman-activated cell sorting (RACS) has garnered significant attention due to its capacity to distinguish cells based on their intracellular chemical composition without the need for labeling. However, the widespread application of RACS has been limited by a fundamental trade-off between throughput and measurement bandwidth, or cellular information content.

In this study, we successfully address this trade-off by demonstrating broadband RACS in the fingerprint region (300 - 1,600 cm-1) with a record high throughput of approximately 50 cells per second. This achievement marks a 25-fold increase in throughput compared to previous broadband fingerprint-region RACS implementations outside the resonance Raman regime.

To showcase the practicality of our RACS, we conduct real-time label-free sorting of microalgal cells based on their accumulation of carotenoids and polysaccharide granules. The outcomes of this research have promising implications for medical, biofuel, and bioplastic applications, potentially broadening the reach and impact of RACS technology.

(RAM-08.2)**In search of chemoresistance markers of acute lymphoblastic leukemia: Raman-based in vitro evolution of drug-induced metabolic changes**

Katarzyna Majzner, Patrycja Dawiec, Adriana Adamczyk, Anna M. Nowakowska, Justyna Jakubowska, Marta Ząbczyńska, Agata Pastorczak, Kinga Ostrowska, Wojciech Mlynarski, Malgorzata Baranska, *Jagiellonian University In Krako*

B-cell acute lymphoblastic leukemia (B-ALL) represents the most common childhood malignancy and is the most common subtype of ALL. Different genetic changes impact not only the development and progression of B-ALL but also the final outcome of the treatment. Choosing novel targeted treatment strategies with inhibitors or monoclonal antibodies over or together with the chemotherapy can improve the overall effectiveness of the treatment.
To address the issue of chemoresistance in B-ALL, it is important to identify the molecular differences that occur at the subcellular level. Therefore, a specific, sensitive, and label-free molecular imaging method is needed to capture the fingerprint of drug-induced alterations. Raman spectroscopy (RS) is a label-free and non-destructive method which provides information about the cellular chemical composition, reflecting its metabolic state. Due to the latest developments in RS, we can conduct ground-breaking metabolic research on biological systems at the subcellular and molecular levels. By utilizing Raman imaging, it is possible to profile drug-treated cells to identify biomarkers for the chemoresistance of B-ALL.
Here, we present preliminary results from Raman-based studies conducted in vitro on B-ALL cell lines sensitive (BV173) and resistant (SD-1) for venetoclax (VEN). VEN is a first FDA-approved inhibitor of the anti-apoptotic B-cell lymphoma-2 (Bcl-2) protein. The Bcl-2 protein family plays a key role in mitochondrial-mediated apoptosis and overexpression of Bcl-2 in some lymphoid malignancies has been linked to increased resistance to chemotherapy. [1] In our studies, both control and VEN-treated cells were imaged using the WITec Alpha300 confocal Raman system. The analysis protocol included unsupervised (e.g., principal components analysis, PCA) and supervised (e.g., partial least squares discriminant analysis, PLS-DA) chemometric methods that were used to identify the molecular fingerprint of VEN-cell interactions. We have found that VEN induces biochemical changes in both cell lines, but their profile is different when compared with resistant and sensitive cells.

Acknowledgments
The „Label-free and rapid optical imaging, detection and sorting of leukemia cells" project is carried out within the Team-Net programme of the Foundation for Polish Science co-financed by the EU.

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(RAM-08.3)**The Development and Implementation of Spectroscopic Coherent Raman Imaging as a Discovery Tool for Biology**

Marcus Cicerone, *Georgia Institute Of Technology*

Spectroscopic coherent Raman imaging (CRI) methods allow label-free, chemically specific imaging of materials and biological systems and open many exciting possibilities for understanding phenomena in these systems. I will introduce spectroscopic CRI, focusing on broadband coherent anti-Stokes Raman scattering (BCARS) microscopy. I will discuss approaches we have adopted to optimize signal generation with a limited photon budget and approaches we use to extract information of interest from our high-dimensional image data. I will also report on our use of BCARS to identify and explore a previously unknown biogenesis pathway in viral replication and our early efforts to develop it as a situational metabolomics surrogate.

(RAM-08.4)**Stimulated Raman Photothermal Microscopy towards Ultrasensitive Chemical Imaging**

Yifan Zhu, Xiaowei Ge, Hongli Ni, Jiaze Yin, Haonan Lin, Le Wang, Yuying Tan, Chinmayee Prabhu Dessai, Ji-Xin Cheng, *Boston University*

Stimulated Raman scattering (SRS) microscopy has shown enormous potential in revealing molecular structures, dynamics and coupling in a complex system. However, the bond-detection sensitivity of SRS microscopy is fundamentally limited to milli-molar level due to the shot noise and the small modulation depth in either pump or Stokes beam. Here, to overcome this barrier, we revisit SRS from the perspective of energy deposition. The SRS process pumps molecules to their vibrational excited states. The thereafter relaxation heats up the surrounding and induces a change in refractive index. By probing the refractive index change with a continuous wave beam, we introduce stimulated Raman photothermal (SRP) microscopy, where a ~500-fold boost of modulation depth is achieved on dimethyl sulfide with conserved average power. Versatile applications of SRP microscopy on viral particles, cells, and tissues are demonstrated. With much improved signal to noise ratio compared to SRS, SRP microscopy opens a new way to perform vibrational spectroscopic imaging with ultrahigh sensitivity and minimal water absorption.

(RAM-08.5)**Multimodal imaging analysis using Stimulated Raman Scattering Microscopy and Secondary Ion Mass spectroscopy to study drug penetration into skin**

Vasundhara Tyagi, Jean-Luc Vorng, Alex Dexter, Panagiota Zarmpi, Natalie Belsey, Dimitrios Tsikritsis, Begona Delgado-Charro, Richard Guy, *National Physical Laboratory*

Evaluating drug permeation into the skin is fundamental to the assessment of topical formulation performance but current techniques to study the skin pharmacokinetics of drugs can be laborious, time-consuming, and destructive. Stimulated Raman scattering (SRS) microscopy offers rapid, non-invasive, and label-free chemical imaging to map drug distribution in skin. However, the SRS signal sensitivity is limited due to attenuation with depth into the skin and has a poor signal-to-noise ratio. Here, secondary ion mass spectroscopy (SIMS), a more sensitive technique, has been used to cross-validate the SRS signal detection limit of 4-cyanophenol (CP) (a model 'drug'). In-vitro permeation studies were performed on porcine skin after application of different concentrations of CP solutions. Untreated skin provided a control. After skin treatment, complementary SRS and SIMS images were acquired from the same tissue sections. Feature-based image registration and line profile generation of average intensities across respective SRS-SIMS images were performed. To inform the results observed and to evaluate the detection limit and signal to noise ratio, SRS-SIMS data were also obtained from (a) a skin homogenate (including connective tissues such as collagen and elastin), and (b) isolated skin epidermis, both of which had been spiked with known CP concentrations. Line intensity profiles of the registered images showed that, while SRS was able to detect CP ∼150 μm into the epidermis, SIMS could extend this depth to ∼500 μm. In the skin homogenate and in isolated epidermis, the SRS signal of CP was linearly proportional to concentration up to ~ 0.04 mM. The signal-to-noise ratio was significantly higher in skin homogenate than isolated epidermis due to structural and optical heterogeneities. Overall, multimodal image registration with SRS and SIMS demonstrated that the Raman technique has real potential for the assessment of drug permeation into skin and to compare the performance of different topically-applied formulations.

23RAM17: Bioanalytical Applications of Raman Spectroscopy, Cascade 4
Chair: William Tipping, The University Of Strathclyde
Co-Chair: Gregory Wallace, The University Of Strathclyde

(RAM-17.1)**Molecular Insights into the Binding of Linear Polyethyleneimines and Single-Stranded DNA Using Raman Spectroscopy: A Quantitative Approach**

Rusul Mustafa, David Punihaole, *University of Vermont*

Establishing how polymeric vehicles bind and package their nucleic acid cargo is vital towards developing more cost-effective gene and drug therapies. Polyethyleneimines (PEIs) are a class of polymers that are commonly used in gene delivery applications. Utilizing Raman spectroscopy and MD simulation, we developed a quantitative approach to obtain a molecular-level picture of DNA

cargo binding. We find that the intense Raman bands located in the CH_2 bending modes are very sensitive reporters of PEI interactions with their local environment. We use these bands as spectroscopic markers to assess the binding between low molecular weight PEIs and single-stranded DNA (ssDNA). Analysis of the Raman spectra suggest that PEI primarily binds via electrostatic interactions to the phosphate backbone, which induces the condensation of the ssDNA. We show that the analysis conducted here is critical to investigate the behavior of PEI vectors in the extracellular matrix including their binding to sugars attached to glycoproteins.

(RAM-17.2)**On the Potential of Stable Isotope Raman Microspectroscopy for Analysis of Microbial Degradation of Microplastics**

Natalia Ivleva, Kara Müller, Martin Elsner, *Technical University of Munich, Chair of Analytical Chemistry and Water Chemistry, University of Münster*

Biodegradable polymers are designed to reduce environmental plastic pollution, but it is of great importance to show their complete mineralization by microorganisms to avoid assimilation of inert micro- and nanoplastics in soils or waterbodies. Conventional methods either analyze changes in polymer properties during degradation (e.g., mass loss) or microbial activity (e.g., O2 consumption or CO2 production). However, these methods lack a direct relation between plastic and microbes, to clarify which species are responsible for degradation and to determine (micro)plastic fate. Therefore, stable isotopes can be used to trace elements from the polymer into biodegradation products (e.g., microbial biomass). Here, a combination of Raman microspectroscopy with the stable isotope approach – stable isotope Raman microspectroscopy (SIRM) which enables for nondestructive, quantitative spatially-resolved analysis of microorganisms at the single-cell level can be applied to study biodegradation of (micro)plastics labeled with 13C or D. SIRM provides characteristic fingerprint spectra containing information on stable isotope-labeled substances and the amount of a label (based on red-shift of bands of labeled substances). Furthermore, this method requires no or limited sample preparation, and can be performed in situ without interference of water.
Here we illustrate the potential of SIRM for quantitative analysis of (micro)plastic biodegradation. Since 13C-labeled polymers are expensive or even unavailable, alternative D-labeling approach is applied. Deuterated polylactic acid (d-PLA) microplastic particles are incubated with Sphingomonas koreensis (which was isolated from an environmental sample and can produce carotenoids). Those pigments dominate Raman spectra due to the resonance Raman effect. Besides those carotenoid spectra, we obtain full biomass spectra after photobleaching. Depending on the cellular carotenoid concentration, we use two different strategies to determine the degree of deuteration: i) the estimation of peak area ratio CD/(CH+CD) for the microbial biomass (since CH str. vibrations are strongly red-shifted from 2990 cm-1 to 2145 cm-1, i.e., Raman silent region); ii) the evaluation of the band position for v1 (C=C str.) vibration of carotenoids (Weng et al., 2023 doi.org/10.1039/D2AN01603F). Our results show that SIRM enables for the reliable analysis of D-flow into microbial biomass at the single-cell level and has a high potential for the monitoring of (micro)plastic biodegradation.

(RAM-17.3)**Ensuring Optimal Detection Outcomes in Handheld Raman Spectroscopy**

Luisa T.M. Profeta, Brian L. Bures, John T. Wiesemann, Christopher Langford, Michael D. Hargreaves, *Rigaku Analytical Devices*

Over the last 20 years, the rise and prevalence of the portable analytical equipment has created a significant impact in the forensic, defense and security industry. No longer is the typical end user of Raman spectroscopy equipment a subject matter expert with a comprehensive background on deciphering complicated spectra of chemical mixtures. This shift from the technical user group to a first responder, military or quality assurance user demands development of equipment with a balance of analytical rigor and non-expert usability. In this presentation, the authors will discuss the impacts of data quality and robustness on the outcomes of unknown materials. Data collection methodology, tackling noisy backgrounds, fluorescence and optimization of weaker Raman signals are some of the areas that Rigaku has continued to improve upon with it's CQL Raman instrument. These inherently are interwoven with continued algorithm refinements and advancements. Examples of these changes will be exhibited with explosives, narcotics and other CBRNE materials of interest, with the goal of

comparing the desired outcomes and outputs for the non-expert user with those expected by an expert audience.

(RAM-17.4)**A study on relationship between development of neuronal cells and susceptibility to bisphenol A (BPA) using Raman spectroscopy**

Kosuke Hashimoto, Shogo Sato, Hidetoshi Sato, *Kwansei Gakuin University*

The final goal of this study is to develop an Raman spectroscopic manner that can track molecular dynamics and action potential dynamics to evaluate developmental neurotoxicity of chemical substances during long-term culture. Bisphenol A (BPA) is a popular chemical used in plastic products such as PET bottles. There is concern about its effects on the brain development of fetuses and children because BPA is an endocrine disrupter chemical to induce developmental disorder such as ADHD. However, the mechanism of developmental disorder under exposure of BPA is still unclear because it is difficult to trace molecular dynamics and function in live neurons for long term. The aim of this study is to analyze the response of neurons at various developmental stages to BPA in individual living neuronal cells using Raman spectroscopy. Rat hippocampal neurons were exposed to 0 (Control), 25, and 100 μM BPA on 15, 30, and 60 days of culture. Raman measurements were performed using a confocal Raman microscope equipped with a CO2 incubator at 30 min, 1, 2, 3, 4, and 5 h after exposure of BPA. Principal component analysis (PCA) was performed with Raman spectral dataset. The result shows that differences in Raman spectra are observed between the BPA-treated group and the control group on 15 and 30 days of culture. The spectral difference can be attribute to tyrosine which is the source of noradrenaline synthesis. In contrast, Raman spectra obtained from cells at day 60 of culture did not discriminate between the BPA-added and control groups. Additionally, we demonstrated fluorescent imaging for quantitative analysis of action potential of neuron using FluoVolt (a voltage-sensitive dye). The spontaneous firing intervals of neurons at 15 and 30 days in culture become shorter by about 2 seconds after 100 μM BPA treatment than before BPA treatment, indicating that BPA treatment induced high-frequency firing. In contrast, the interval of spontaneous firing in 60 days cultured neuron does not change after the BPA treatment. This study suggests that the cause of developmental neurotoxicity by BPA exposure is a gradual and long-lasting excitotoxicity to neurons in the immature stages.

(RAM-17.5)**Reaching stars: unique characteristics of gold nanostars for sensing and targeting applications**

Anastasiia Tukova, Alfonso Garcia-Bennett, Alison Rodger, *Yuling Wang, Macquarie University*

Anisotropic gold nanoparticles have recently been garnering increasing interest due to their sensitivity and selectivity enhancement of diagnostical methods. One such usage is on the detection and identification of biomarkers present in trace amounts using surface enhanced Raman spectroscopy (SERS). However, its properties and interactions with analytes vary from widely studied spherical nanoparticles.

Our study delves into the properties of gold nanostars that set them apart from spherical nanoparticles and showcases their potential in biomedicine. It covers topics such as gold nanostars distinctive plasmonic properties, hot-electron generation, surface energy, that effect their interactions with analytes and biomolecules. The differences in optical properties and interaction with biomolecules on spherical and anisotropic nanoparticles (nanostars in particular) were studies via joint experimental (extinction spectroscopy, nanoparticles tracking analysis [NTA], zeta potential and Raman spectroscopy) and computational methods (the finite element method [FEM] and molecular dynamics [MD] simulations).

Understanding the distinctive properties of anisotropic gold nanoparticles is essential for unlocking their potential in a variety of applications. Our results indicate that gold nanostars have high surface energy due to their complex morphology and large surface area-to-volume ratio, affecting their interaction with different analytes. We demonstrated a drastic difference in the adsorption behaviour of common analyte molecules and biomolecules (proteins) on gold nanoparticles with different morphologies. Our observations will help the design and development of new nanostructures with improved sensing and targeting ability.

23SPECIAL01: Skilled: Scientific Discoveries and Professional Lessons After Academia, Southern Pacific F
Chair: Kristy Mckeating, Google
Co-Chair: Sam Hinman

(SPEC-01.1)**Rapid Discovery of Functional Human Memory B Cell-Derived Antibodies for SARS-CoV-2 Using the Beacon® Optofluidic System**

Sam Hinman, *PhenomeX*

The COVID-19 pandemic highlights the urgent need for rapid discovery and development of therapies against life-threatening viruses such as SARS-CoV-2. The human immune response to an infectious agent provides effective antibodies with high specificity and low risk of immunogenicity. Hence, human memory B cells from survivors and immunized donors are a potential source of therapeutic antibodies that can be used to treat or prevent viral infections. The Beacon® optofluidic system enables the functional screening and characterization of human memory B cells for the discovery of unique antibodies against SARS-CoV-2 variants in under one week from blood draw. Antibodies were selected based on their ability to bind to the SARS-CoV-2 Spike protein, in addition to their ability to block the binding of the Spike protein to the human ACE-2 receptor, an essential interaction for infection of human cells by the SARS-CoV-2 virus. The antibodies discovered from work like this have the potential to become critical antibody therapies in the struggle to contain COVID-19 or future pandemic causing viruses. Moreover, this developed workflow may have applications to other areas of infectious disease research, as it enables the screening of antibody secreting cells derived from primary human memory B cells using sequential functional assays where the assay antigens and characterizations are defined by the user.

(SPEC-01.2)**Publish and/or Flourish: A National Lab Perspective**

FREDERIC POITEVIN, *SLAC National Accelerator Laboratory*

I will share my experience transitioning from an early career in academia to a career path in a National Laboratory, where I split my time between doing exploratory research and contributing to the operation of the Linac Coherent Light Source (LCLS) at SLAC National Accelerator Laboratory. I will highlight the benefits and challenges offered by this line of work and hopefully inspire some of you to consider it as a third possible science career option, besides industry or academia.

(SPEC-01.3)**Apparently Healthy: Biosensors for Wellness Applications**

Kristy Mckeating, *Google*

In recent years there has been a significant increase in consumer adoption of health monitoring devices, predominantly due to the advent and increased popularity of wearable trackers and smart watches. These devices have advanced from counting steps to monitoring health metrics previously confined to a clinical setting such as heart rate, ECG, and sleep stages. In the in vitro diagnostic field the rise of viral testing kits to be used at home expanded a market previously dominated by pregnancy tests and glucose monitors. As consumers become more conscious of the benefits of tracking different biomarkers, how should the field of biosensing adapt? What considerations need to be taken into account when designing a platform intended to be used on a healthy population for trend tracking as opposed to diagnostics? During this talk I will discuss my own experience in moving biosensor research from academia to industry, specifically a consumer facing industry, with a focus on the regulatory considerations required when designing devices for an apparently healthy population.

(SPEC-01.4)**Careers beyond the bench: Publishing, Product Management and Pivots**

Marshall Brennan, *Cell Press*

Students are often told that jobs away from the research lab are "alternative" careers compared to academia; however, this is often the reverse of the situation encountered by jobseekers in the real-world, where academic positions are scarce compared to "alt chem jobs". The speaker, Marshall Brennan, has made a career out of seizing these non traditional opportunities in publishing and product management and will discuss the importance of being well-rounded, well-networked and open to solving problems that present themselves as a core driver of professional growth. This presentation will describe careers specifically in the publishing and product management industries as well as a general mapping of scientific skill sets onto non-academic job opportunities. Topped off with personal stories of growth (including some behind-the-scenes looks at the development of ChemRxiv.org), attendees can expect to come away with a sense for how to look at their career trajectory not as a straight path but rather an often curving road traveling through myriad opportunities — if you're open to seeing them.

FACSS Innovation Award Finalists Plenary Session, Sierra 5

Real-time Controlling a Single DNA in Hotspot for Programmable Surface-enhanced Raman Spectroscopy Scanning in Solution

Jinqing Huang, *The Hong Kong University Of Science And Technology*

Single-molecule surface-enhanced Raman spectroscopy (SERS) could directly probe intrinsic molecular vibrations beyond the optical diffraction limit for spatial recognitions at a nanometer scale in aqueous conditions, since water generates weak Raman signals as background. However, it is unavoidable to encounter target molecules in undesired conformations, such as intermolecular stacking and intramolecular coiling, which might hinder the accurate detections of their buried sites. From the fundamental perspective, SERS effect mainly arises from the excitation of localized surface plasmon resonance near metal surface in a nanometer range, thus the signal is subject to the quantity, the location, the retention time, and the exposed site of the target molecule in this highly confined local electromagnetic field, known as "hotspot". Due to limited molecular control, there are long-standing concerns about the credibility, sensitivity, repeatability, and accuracy of the existing single-molecule SERS measurements, in particular, for biomacromolecules with conformational complexity in solutions.

Here, we developed a novel optical tweezers-coupled SERS platform integrating single-molecule manipulations and site-specified SERS characterizations. As a proof of concept, we characterized the spatial coordinate of the bound and the unbound states between a single DNA and (dpy)$PtCl_2$, a chemotherapeutic compound interacting with DNA to induce cell death. Our results demonstrated that the correlative spatial and temporal control between the single (dpy)$PtCl_2$-bound DNA and the SERS-active hotspot could significantly improve detection sensitivity, stability, and reproducibility. More importantly, this stretched single (dpy)$PtCl_2$-bound DNA was translocated through hotspot in a programmable back-and-forth manner for rereading the specified sites of the same single molecule multiple times to ensure detection accuracy. Without massive parallel detections on the small fragments of numerous individual molecules or complex population-scale data analysis, it showcases a new strategy to achieve real-time sequential reading and multiple rereading at sub-nanometer precision for the direct spatial characterization of a "real" single biomacromolecule. Since the nucleobases were evenly separated in alignment under stretching force, it would be possible to link the spatial distribution of the drug binding sites to the nucleotide sequence. With precise molecular control, the platform holds the promise to develop wide applications in molecular interactions, encryption, and sequencing.

Acoustic Ion Manipulation: A Novel Approach to Enhance Ion-based Spectroscopies

Jacob Shelley, Yi You, Julia Danischewski, Jens Riedel, *University of Utah, Rensselaer Polytechnic Institute, Federal Institute for Materials Research and Testing (BAM)*

Approaches to control the motion and direction of ionized particles and molecules have been pursued for more than three centuries. Because these species are inherently charged, most methods for ion manipulation rely upon electrostatic and/or Lorentz forces in electric fields and magnetic fields, respectively. Measuring the responses of gaseous charged species within a controlled external forcefield led to the development of mass spectrometry (MS) and ion mobility spectrometry (IMS) which have become cornerstones in contemporary chemical analyses. The ability to produce intact gaseous ions for these techniques is often performed at atmospheric-pressure (AP) due ease of sample introduction, high ionization efficiencies, and minimal fragmentation. However, diffusion and electrostatic repulsion between ions hinders the transport of gaseous ions into the lower-pressure environment of the IMS or MS. Conventional ion optics, that use electric or magnetic fields, can guide ions at AP, but require high field strengths to overcome the dominating aerodynamic effects.
Here, we describe a remarkable phenomenon whereby low-power acoustic fields are used to move, shape, gate, and separate beams of gaseous ions at atmospheric pressure. We refer to this approach as Acoustic Ion Manipulation Spectrometry (AIMS). Gaseous ions at AP are directed towards and separated by the presence of the acoustic field. To better understand the phenomenon, an ion-detector array provided a measure of bulk ion movement, while mass spectrometry (MS) offered chemical-specific information. As one example of an AIMS setup, a standing acoustic wave was formed with two ultrasonic speakers and placed between an ionization source and ion detector. Ion beams preferentially travel through regions of stable pressure gradients (i.e. nodes) and deflect from unstable regions (i.e. antinodes). Shadowgraphy revealed that the ions are separated from a neutral gas stream. Specific examples of ion focusing, gating, and separation (based on ion size) will be shown. In addition, experimental findings will be used to postulate a theory to develop a better understand of the behavior of gas-phase ions in acoustic fields. This discovery could have profound impacts in IMS/MS instrumentation as well as materials processing and characterization.

Unveiling Superior Spectroscopic Precision: A Shoebox-Sized, Low-Cost Spatial Heterodyne Spectrometer with 1-pm Resolution

Yi You, Xunyu Li, Steven Ray, *, J*ens Riedel*, SUNY Buffalo Dept of Chemistry, Federal Institute For Materials Research And Testing (bam)*

Spectroscopy has long been a cornerstone of scientific exploration. Among approaches striving for a balance between spectral resolution, spectral range, and sensitivity, the Spatial Heterodyne Spectrometer (SHS) offers many theoretical advantages over the most commonly used Czerny-Turner and Echelle types. The SHS, tracing its origins back to the ingenious Modulation Amplitude Selection Interference Spectrometer (SISAM) developed in 1958 by Connes, leverages the unique combination of interferometry and diffraction. However, past iterations of such spectrometers have often over-promised and under-delivered. In the present work, we challenge the status quo by bridging theoretical idealism with engineering reality, offering a practical SHS platform characterized by unparalleled performance, cost-effectiveness, and compactness.
The key features of our SHS prototype were exposed by a spectrum containing baseline-separated spectral peaks corresponding to ^{6}Li and ^{7}Li ^{2}S1/2 ← ^{2}P1/2,3/2 transitions. Specifically, this preliminary prototype can achieve 1-pm resolution and 200,000 resolving power at 670 nm, while demonstrating a 10-pmol limit-of-detection for lithium. Importantly, this platform utilizes an industrial camera without the need for active cooling or an intensifier. This small leap in performance surpasses prior benchmarks and propels us into a new era of practical applications involving reduced-pressure glow discharge, solution cathode glow discharge, atomic absorption, and more.
In this presentation, we will delve into the theoretical and hardware frameworks of the SHS platform, illustrating how the current iteration shatters the long-standing limitations of the SHS with consumer-grade hardware. Moreover, we will explore often-overlooked information inherent in the interferograms, which carry crucial instrument functions and source-specific spectral contributions. We will also discuss methods that leverage modern computational power, leading to the one-image derivation of absorbance spectra and the determination of the instrument response function from a single image.

New Opportunities for Mass Spectrometry in Nanocrystal Surface Chemistry

Mengliang Zhang, Kevin Cavey, P. Gregory Van Patten, *Middle Tennessee State University*

Colloidal nanocrystals (NC), such as quantum dots and Au/Ag nanoparticles, hold great promise to enable or advance various emerging technologies. Understanding the details of NC surface chemistry is vital to understanding and controlling their behavior. Improper surface coordination can diminish or destroy otherwise excellent catalytic activity, colloidal stability, chemical stability, electronic and optical properties, programmed self-assembly, and biocompatibility. Current methods, such as NMR, photoluminescence, and thermogravimetric analysis, have been used, but few mass spectrometry-based methods were implemented, mainly as a supplement technique to analyze ligands on NC. Thermal desorption direct analysis in real-time mass spectrometry (TD-DART-MS) is employed in our research to study the surface chemistry of NC. A temperature program is applied to desorb the ligands, then analyzed by DART-MS. This approach is highly sensitive, allowing analysis of sub-microgram samples, including even minor components in the ligand coatings. The method requires a small sample size (i.e., 5 μL) and minimal sample preparation and is fast (i.e., <10 min/sample). The technique also allows rapid discrimination of bound vs. unbound ligands. Unlike NMR, our method does not require reporter moieties, which opens opportunities for many molecules (e.g., stearic acid and stearamine) as ligands, which can significantly expand the NC material family. The quantitative analysis of ligands on NC surfaces was introduced in this study and applied to explore the ligand exchange process, which provides critical insights into the fundamental structural and thermodynamic properties of NC materials. The effects of ligand structure and binding geometry, including the length, bending, and saturation of the organic ligand tail group, can also be elucidated with this approach.

The project opened possibilities for MS-based techniques in nanoscience, and MS could eventually be a valuable tool in the routine analysis of NC. The unique capabilities of TD-DART-MS for detecting ligands on NC will enable the investigation of the critical surface chemistry of NC that are extremely difficult to study, if not impossible, with the existing techniques. The small sample size required for the analysis makes it an excellent tool for monitoring the process of nanomaterial synthesis and providing additional insights into the reactions.

Poster Presentations
Thursday Poster Session

(Thurs-P1) **On the Behavior of Microplastics Subjected to AC Insulator-based Dielectrophoresis**

Shulin Bu, Domin Koh, Alexandra Ros, *Arizona State University*

Microplastics have become an emerging threat to terrestrial and marine systems contaminating oceans, lakes and soils impacting biodiversity and the ecosystem. Referred to small plastic particles with diameter <5 mm, microplastics are now recognized as a significant threat to the environment and humans. Nanoplastics with dimensions <1 μm pose a potential high risk to animals and humans, as these particles have been found in blood and other body fluids [1]. However, there are limited methods to detect and quantify nanoplastics.
Here, we propose studying the AC-electrokinetic effects of nanoplastics in body fluids using insulator-based dielectrophoresis (iDEP), which we further aim to utilize for the separation and detection of nanoplastics from biological fluids. Polystyrene (PS) and polymethyl methacrylate (PMMA), as commonly discovered microplastic types in the environment, were selected and subjected to varying experimental conditions such as suspended medium conductivity and applied frequency in iDEP devices. We hypothesize that nanoplastics occur in body fluids coated with high abundant biomolecules and chose the protein bovine serum albumin (BSA) as a model to study the influence of protein coating on PS particles. To gain insight into the low-frequency dielectric properties, coated and non-coated PS and PMMA beads were characterized using dynamic light scattering. PS beads reveal a decrease in zeta potential upon protein adsorption, whereas PMMA beads exhibit an opposing behavior. While PS particles in both sizes displayed nDEP in high and and pDEP in low ionic strength buffer, quadrupolar vortices were observed around the insulating posts in low-conductivity buffer at 1 kHz arising from concentration-polarization electroosmotic effects. Despite these non-linear effects, our results reveal that the iDEP behavior of particles could switch from nDEP to pDEP with a decrease of medium ionic strength as well as Zeta potential, which is in excellent agreement with an iDEP model based on surface conduction. This knowledge will aid in developing highly effective analytical tools for pre-concentration, analysis and separation of nanoplastics in the future.
References
[1] Leslie, H. A et al. (2022). Environment International, 163, 107199.

(Thurs-P2) **Dielectrophoresis as a detection tool for Rickettsial diseases**

Negar Farhang Doost, Soumya K Srivastava, *Department Of Chemical And Biomedical Engineering, West Virginia University*

Tick-borne diseases pose significant public health challenges worldwide. Tick-borne diseases are caused by various pathogens including Rickesttsia species are on the rise, with increased incidence reported in multiple regions. The ricketssiae are a diverse group of intracellular Gram-negative bacteria found in ticks and can cause infections that spread in the blood to other organs. These infections include Rocky Mountain spotted fever (RMSF), other spotted fevers, epidemic typhus, and murine typhus.
Approximately 4,000-6,000 tickborne spotted fevers, including RMSF, are reported in the United States annually. RMSF is the most severe and fatal ricketssial infection with a 5-10% fatality rate in the United States that would increase to 40-50% if the treatment is not started. Early signs and symptoms of RMSF are usually non-specific such as fever and headache. Non-specificity in clinical presentations underscores the importance of accurate and timely diagnosis. The common diagnostic methods such as serological tests and polymerase chain reactions have limitations in terms of specificity and sensitivity. Therefore, there is a clinical need to develop a diagnostic tool that will provide sufficient accuracy and sensitivity to detect rickettsial infections.

We proposed a novel approach for diagnosis using an electrokinetic, dielectrophoresis (DEP) technique. DEP is based on the interaction between polarizable particles and non-uniform electric fields. This technique utilizes the electrical property of biological samples to offer label-free and rapid detection of pathogens. By subjecting samples to non-uniform electric fields, the method can selectively manipulate and concentrate target pathogens to enhance detection sensitivity.
Vero cells were cultured as host cells and were inoculated by Rickettsia montanensis. After reaching the confluent level, the cells were resuspended in the buffer of interest in order to maintain desired conductivity and pH. Properties of infected and uninfected cells were obtained using the 3DEP dielectrophoresis system. The differences in the dielectric properties of Rickestssia monranensis–infected Vero cells versus healthy Vero cells were exploited to design a fast and cost-effective diagnostic tool that would improve patient outcomes and public health surveillance.

(Thurs-P3) **Self-focusing DLP-based 3D Printing Enables On-chip Integration Of Soft Functional Materials In Electrokinetic Microfluidic Devices**

Guillermo Ramirez, Diego Cabello, Gongchen Sun, *University Of Texas At San Antonio*

The development of 3D printing technologies has heralded a new era in microfabrication, enabling complex designs inaccessible to conventional soft lithography methods. Among various 3D printing techniques, Digital Light Processing (DLP) is the most popular method to fabricate microfluidic devices due to its capability to create high-resolution microchannel features (tens of micrometers) at a much lower cost. DLP-based techniques have been reported to create microfluidic devices with complex 3D structures and fluid actuating parts such as on-chip pumps and valves. However, most studies focus on the manufacturing of mechanical structures which are typically made by a photo-curable resin. Electrokinetic microfluidic devices with on-chip functional components have not been widely achieved by DLP-based 3D printing yet, because such devices require in situ integration and patterning of multiple materials beyond one single resin. It is challenging to integrate other materials, such as conductive polymer and ion-conducting hydrogels, inside enclosed microchannels. This is because conventional DLP methods have only one fixed focal plane. As a result, the DLP process is limited to starting at the bottom of the print, outside of any existing microchannels.

To address this challenge, we develop a novel DLP-based 3D printing technique which allows for on-demand adjustment of the printing focal plane. Our technique introduces a self-focusing step which combines image-based feedback and precision z-axis control to calibrate the focal plane in a microchannel. Combined with automated exchange of printing reagents, our technique enables the sequential DLP printing of different soft materials inside any given microfluidic device.

We further demonstrate the utility of our technique by designing a field-flow fractionation device for cell sorting applications. A pair of line electrodes are fabricated by patterning conductive polymer to introduce a uniform electric field in a main microchannel, which separates cells based on their surface charges. The electrodes are isolated by a hydrogel salt bridge which allows the electric field to penetrate but shields any side electrode reactions from the main channel to protect the cells. The on-chip integration offers a more efficient coupling of electric field into the microchannel than bulk electrodes and therefore improves the sorting throughput drastically.

(Thurs-P4) **3DEP Characterization of Human Mesenchymal Stem Cells and the Development of DEP-based Cell Sorting Strategies**

Zuri Rashad, Anthony Tsai, Kiara Lacy, Sune Terbush, Lexi Crowell, Stephany Alonso, Tayloria N.G. Adams, *University of California, Irvine*

Every year, thousands of patients depend on stem cell therapy and tissue regeneration to deal with various ailments, such as heart disease, scarring of internal organs, or autoimmune disorders [1]. However, due to the heterogeneity that exists within stem cell samples, there are often inconsistencies in clinical outcomes for mesenchymal stem cell (MSC) transplantations [1]. Characterization of the biological and electrical properties of MSCs can lead to the development of cell sorting strategies to

generate homogeneous sub-populations of cells using microfluidic platforms. Dielectrophoresis (DEP) is a cell sorting technique that uses a non-uniform electric field to generate homogeneous populations of cells [2]. Our hypothesis is that homogeneous sub-populations of MSCs will have a more predictable cell behavior than heterogeneous MSCs. Our cell sorting strategy is based on three steps: 1) characterize the electrical profile of MSCs using DEP, 2) characterize the gene expression of the MSCs using qPCR, and 3) correlate the electrical profile to the gene expression profile using a statistical software. Our DEP characterization of MSCs' electrical and the gene expression profile revealed that there is in fact a strong correlation between membrane capacitance and collagen, as well as cytoplasm conductivity and fatty acid binding protein. These correlations suggest that membrane capacitance and cytoplasm conductivity are good label-free biomarkers for cell sorting. Using this three-step cell sorting strategy, we successfully generated two sub-populations of MSCs.

Reference:
[1] D. Phinney, Cell Biochem. J. 113(9) (2012) 2806-2812.
[2] K. F. Hoettges, Y. Hubner, L. M. Broche, S. L. Ogin, G. E. Kass, M. P. Hughes, Anal. Chem. J. 80(6) (2008) 2063-2068

(Thurs-P6) **Human Mesenchymal Stem Cell Sorting with Dielectrophoretic-based Microfluidic Devices**

Sune Terbush, Tayloria N.G. Adams, *University of California Irvine*

HMSCs are significant in clinical research for their regenerative and proliferative properties. HMSCs are a heterogeneous cell population able to differentiate into osteoblasts, chondroblasts, adipocytes, and more, though it is hypothesized that only one fate will be useful at a time. HMSCs can be sorted with methods like fluorescent activated cell sorting, which must label cells and affect their functionality. Dielectrophoresis (DEP) is an alternative cell sorting method that employs a nonuniform electric field that causes cell movement towards or away from the field maximum at specific frequencies based on electrophysiological cell properties, eliminating the need for biomarkers. The electrophysiological properties are different for each hMSC progenitor. We have implemented two DEP-based microfluidic devices for sorting hMSCs. The hydrophoretic oblique angle parallel electrode sorting (HOAPES) device uses the traditional DEP approach with electrodes across a microchannel. Uniquely, the device uses a cell aligner to push cells to the sides of the chip before sorting in the focusing region. The parallel electrodes in the focusing region continuously sort cells by attracting one population towards the center and creating two distinct populations flowing into three outlet microchannels. The cytochip device uses a contactless DEP mode with electrodes on the edges of the chip, creating local field maximums with insulating pillars in the microchannel to generate two populations of cells: one trapped to the posts while the second continues to flow through the microchannel. These devices aim for higher throughput and homogeneity of sorted populations. The HOAPES device has been used to generate two flowing undifferentiated subpopulations of hMSCs. The cytochip has sorted undifferentiated AT-hMSCs at low frequencies (200-600 kHz), trapping a population over 20 minutes that was differentiated into osteoblasts. In conclusion, both DEP-based microfluidic devices can be used to create enriched populations of hMSCs in a label-free manner.

(Thurs-P7) **Dielectric characterization of ductal adenocarcinoma using murine PyMT+/- model**

Raphael Oladokun, **Sai Deepika Reddy Yaram**, Timothy Eubank, Soumya K Srivastava, *West Virginia University*

The growing characteristic of a cancer cell is one of the features that encourage the use of the dielectrophoresis technique to manipulate and carry out electrokinetic separations of normal or healthy cells from cancer cells and vice versa, as they tend to behave differently under a non-uniform electric field. In this paper, we use dielectrophoresis techniques to distinguish the distinct stages of tumor progression.
Animal models are powerful tools to analyze the mechanism of the induction of human breast cancer. This proposal applies transgenic technology in mice (MMTV-PyMT model) to study mammary cancer

progression between 4-14 weeks. Our overall objective is to develop a diagnostic tool that detects the early stages of breast cancer via a non-invasive label-free electrokinetic technique, dielectrophoresis (DEP). This will be achieved by probing the electrical properties of the peripheral blood mononuclear cells (PBMCs) from whole blood and primary tumor sources of MMTV-PyMT mice at weeks 4 (stage I) and 12+ (stage IV) of infiltrating ductal adenocarcinoma on a microfluidic platform. The central hypothesis of this research is that the changes triggered in the subcellular components, such as the cytoskeleton, lipid bilayer membrane, cytoplasm, focal adhesion proteins, and extracellular matrix (ECM) at the onset of carcinoma regulate dielectric (conductivity, σ and permittivity, ε), thus affecting the bioelectric signals that aid in the detection of breast cancer. This hypothesis is developed based on our preliminary published data demonstrating: 1) unique dielectric properties of PBMCs under healthy and early stages of infiltrating ductal adenocarcinoma (ADCs), and 2) label-free sorting. The results obtained here will identify the bioelectric signals that regulate human adenocarcinoma cells. This novel tool is label-free, rapid (~2 min.), and low-cost cell sorting technology that detects early and late stages of breast cancer. This work will lead to preclinical development and future clinical trials of the developed detection platform.

(Thurs-P8) **Investigation of Zinc Soap Formation and Damage Mechanisms in Oil Paintings**

Klavdija Bukovec, Stephanie Zaleski, *California State University East Bay*

Degradation due to the formation of zinc soaps is one of the most challenging issues for paintings conservation. Zinc soaps, also called zinc carboxylates, causes degradation in oil paintings due to the formation of protrusions under the surface or delamination of paint layers. Zinc soaps are formed from carboxylic acids from the oil medium and zinc ions from dissolved zinc oxide pigment particles. Unfortunately, no treatment exists to prevent or stop zinc soaps' degradation. Previous research has proposed that there are two different types of carboxylates based on Fourier-transform infrared (FTIR) spectroscopy. FTIR measures the absorbance of infrared radiation for the sample due to different molecular vibrations. The two types of zinc carboxylates (disordered and ordered) each have a unique FTIR signal. Researchers propose that over time, disordered carboxylates are converted into ordered carboxylates and that the ordered, crystalline carboxylates are what cause physical damage in oil paintings. However, researchers still have not discovered to what degree of crystallization causes physical damage in a paint film. The most common factors that lead to observed crystalline zinc soap formation are the type of oil used and environmental conditions. This study investigates how environmental conditions (temperature, relative humidity) affect the rate of damaging zinc soap formation of artificially aged samples using a combination of FTIR and microscopy data. When zinc white oil paints are aged under different conditions, high humidity conditions should have the most significant impact on the formation rate of zinc soaps. In contrast, high temperature has been expected to be a less critical factor. Paint films that cure more slowly are more likely to susceptible to the formation of damaging zinc soaps.

(Thurs-P10) **Parametric Evaluation of Femtosecond Laser Ablation Utilizing Molecular Dynamics Simulations**

Zachary Karg, Prasoon Diwakar, Jospeh Thalakkottor, *South Dakota Mines*

There is an ongoing need for new homogeneous reference materials suitable for method development and quality control applications relevant to the analysis of glassy post-detonation nuclear debris. We describe a method of fabricating silica-based surrogate debris reference materials (SDRMs) using the Stöber process to synthesize uniform nanoparticles with diameter of 500 ± 40 nm and doped with approximately 1 ppm (w/w) of various actinides, fission products, and activation products. We characterized the dopant distribution in two sample sets utilizing feedstocks from similar but distinct synthesis methods. Each sample set comprises samples from the same feedstock, first consolidated either by electrophoretic deposition (EPD) or by mechanical compaction via die-pressing (DP), then densified by sintering, mounted in epoxy, and polished flat for analysis. Laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) was used to quantify the spatial uniformity of dopant distribution in the samples, measured by relative standard deviation (RSD) of integrated LA-

ICP-MS counts at various locations across the sample surface. The newer synthesis outperformed the original in minimizing spatially-resolved RSD, while EPD outperformed DP. Mean RSD of dopant distribution in samples from the improved feedstock synthesis was 0.11 using EPD and 0.36 using DP, while the mean RSD of the original feedstock was 0.51 and 1.18, respectively.

(Thurs-P11) **Electrophoretic Deposition for Improved Trace Element Homogeneity in Silica Reference Materials**

Peter Boone, Tashi Parsons-Davis, Sharee Harris, Christina Ramoon, *University Of California, Berkeley And Lawrence Livermore National Laboratory,*

There is an ongoing need for new homogeneous reference materials suitable for method development and quality control applications relevant to the analysis of glassy post-detonation nuclear debris. We describe a method of fabricating silica-based surrogate debris reference materials (SDRMs) using the Stöber process to synthesize uniform nanoparticles with diameter of 500 ± 40 nm and doped with approximately 1 ppm (w/w) of various actinides, fission products, and activation products. We characterized the dopant distribution in two sample sets utilizing feedstocks from similar but distinct synthesis methods. Each sample set comprises samples from the same feedstock, first consolidated either by electrophoretic deposition (EPD) or by mechanical compaction via die-pressing (DP), then densified by sintering, mounted in epoxy, and polished flat for analysis. Laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) was used to quantify the spatial uniformity of dopant distribution in the samples, measured by relative standard deviation (RSD) of integrated LA-ICP-MS counts at various locations across the sample surface. The newer synthesis outperformed the original in minimizing spatially-resolved RSD, while EPD outperformed DP. Mean RSD of dopant distribution in samples from the improved feedstock synthesis was 0.11 using EPD and 0.36 using DP, while the mean RSD of the original feedstock was 0.51 and 1.18, respectively.

(Thurs-P12) **GC–MS and GC–IR of Regioisomeric 4-N-Methoxy- and Dimethoxybenzyl Derivatives of 3-Chlorophenylpiperazine**

Randall Clark, Mohammad Almalghrabi, Younis Abiedalla, *Auburn University*

A series of N,N-disubstituted piperazines were synthesized containing the structural elements of 3-chlorophenylpiperazine (3-ClPP) in combination with monomethoxybenzyl-, and dimethoxybenzyl substituents to yield nine N,N-disubstituted piperazine compounds. These nine potential designer-like drug analogs were prepared based on common designer modifications of the known novel psychoactive substance 3-ClPP and compared in GC–MS and GC–IR studies. While the compounds in this study have not been reported as drugs of abuse at this time, incorporating the two major structural features of two drugs (substituted phenylpiperazines and benzylpiperazines) already used in combination is an obvious direction for future designer development. Thus, a proactive study of this kind would provide data for the timely identification of these potential designer-like drug analogs. The GC separation on an Rxi®-17Sil MS stationary phase showed the regioisomers of the methoxybenzyl to elute according to the position of aromatic ring substitution with the 2- isomer eluting before the 3-isomer and the 4- methoxybenzyl isomer eluting last. The six regioisomeric dimethoxybenzyl analogs eluted according to the degree of substituent crowding with the 2,3- and 2,6-isomers eluting first and the 3,5-isomer last. Numerous EI mass spectral fragment ions occur via processes initiated by one of the two nitrogen atoms of the piperazine ring. The major EI-MS fragment ions are at m/z 195 observed in all nine spectra occurs from the loss of the substituted benzyl radical and the m/z 56 cation (C3H6N)+ originates from the piperazine ring. Other characteristic fragments containing the benzyl portion of these analogs show a 30 Da variation based on the number of methoxy group substituents. The relative intensity of ions in the methoxybenzyl series as well as some unique ions in the dimethoxybenzyl series provides initial points of preliminary differentiation. These

results coupled with characteristic vapor phase infrared spectra in the 1600-700 cm-1 region allow for the differentiation and specific identification of each regioisomer. This report will include the GC separations, EI-mass spectra, fragmentation pathways/mechanisms and vpIR spectra for these nine disubstituted piperazine analogs.

(Thurs-P13) **The Application of Particle-Correlated Raman Spectroscopic Analysis of Soils to Mock-Casework Scenarios**

Samantha Gong, Brooke Kammrath, University of New Haven, Marisia Fikiet, Peter De Forest, *Forensic Consultants*

Soil is a continuous, complex mixture, reaching across geological bodies. Although it is continuous, soil is distinctly variable and differentiable, based on aspects like geographic location, seasonal factors, and human interference. In combination with its highly transferable nature, the complexity of soil makes it valuable for forensic trace evidence and object-to-scene association. The traditional tool for forensic mineralogy is polarized light microscopy (PLM). Developments in forensic mineralogy focus on employing elemental analysis, most often using scanning electron microscopy with energy-dispersive X-ray spectroscopy (SEM-EDX), to simultaneously visualize and analyze mineral grains. Other methods, like Raman spectroscopy, have also been explored for forensic soil analysis. Raman spectroscopy has a demonstrated history of use for mineral identification, thus its application to forensic soil analysis is a logical extension.

Particle-correlated Raman spectroscopy (PCRS) is a non-destructive, novel analytical method, combining automated image analysis with Raman spectroscopy, to provide morphological and chemical information about a sample. PCRS provides information like mineral identification, microscopic morphological characteristics, and particle size distributions. This information is valuable for forensic soil comparisons, but more research is needed to understand the significance of an association of these properties, given the complexities of transfer and persistence.

In this project, PCRS is used to analyze soil particles collected from simulated evidence. Shoes and shovels were used to collect mock evidence from three geologically distinct locations. Known soil samples were also collected from these locations. The mock evidence was prepared in a method detailed by Stoney et al. for the analysis of very small particles. The collected adhering soil was then cleaned to isolate the mineral grains per the method described by Palenk. The particles of size 90nm-180nm in diameter were then dispersed onto a Raman-inactive microscope slide and analyzed using PCRS. The results were compared to the reference samples that were treated and analyzed with the same method. Source consistency could then be determined using a set of match criteria.

Analysis of the soil samples showed it was possible to determine the source of soil collected from mock evidence by using PCRS when reference samples from suspected sources are available for appropriate comparison.

(Thurs-P14) **Bioprocess Projections Using Multi-Layered Bioprocessing Analytical Technologies**

Christopher Brown, Sam Stewart, 908 Devices, Colin Gavin,ULRI FSRI, Steve Driscoll, *National Physical Laboratory*

Over the last 20 years the industry has achieved remarkable success with mAb-based therapeutics, and extraordinary advances in RNA, cell- and gene-based therapeutics are becoming everyday news. But predictive bioprocess development and control is (still) hard, and is about to get harder as the industry grapples with patient-specific therapies. Technologies for near real-time bioprocess characterization have rapidly advanced in recent years, expanding the panel of readily accessible process and product attributes. Fundamental ChemEng factors (dissolved gases, pH, temperature) have been measurable in/online for some time. Miniature mass spectrometry platforms with high speed separations are able to characterize a very broad panel of core metabolites, amino acids, and other endogenous media factors. And core metabolites, waste components and bioprocess proliferation attributes (e.g. glucose,

lactate, and biomass, protein productivity) now have in-situ possibilities with different solid-state, membrane, and optical transducers. In this talk we'll discuss recent advances in these bioprocess analytical technology regimes, approaches to de novo analytical modeling/calibration of these systems, and how their informing power can be leveraged with the increasing sophistication of bioprocess metabolic models for predictive bioprocess optimization.

(Thurs-P15) **Enhancing Forensic Science: Urine Stain Analysis with Raman Spectroscopy and Chemometrics for Race Identification**

Bhavik Vyas, Igor Lednev, Lenka Halamkova, *Texas Tech University,* University at Albany

Trace bodily fluid evidence is vital in modern criminal investigations as it is the DNA source. Forensic scientists can identify suspects using DNA profiling. One body fluid that is commonly discovered during investigations is urine. However, developing a donor profile is generally difficult due to the small amount of DNA in urine stains. It is worth noting that urine stains may contain a lower quantity and quality of DNA than other biological materials, such as blood and semen. Additionally, urine can contain inhibitors that can interfere with DNA extraction, making isolating high-quality DNA from urine stains more challenging. So, there is an immense need for a non-destructive method that provides vital information about urine trace evidence. Raman spectroscopy can solve this problem as it is rapid, non-destructive, and offers high sensitivity and specificity for trace evidence analysis. In this study, Raman spectroscopy was applied with an advanced classification technique, Random Forest (RF), to distinguish between Caucasian American (CA) and African American Descent (AA) donors based on dried urine traces. Raman spectra were collected from samples of 28 donors varying in age and gender using automatic mapping to cover the heterogeneity of the dry traces of urine. For statistical analysis, we set aside ten donor samples out of 28 for external validation. The created RF model successfully identified 9 out of 10 donors from the external validation dataset offering 90% accuracy. This proof-of-concept study has shown promising results in distinguishing between the two races of human urine samples. The method has great potential for real crime scene investigation as it is non-destructive, rapid, and requires little to no sample preparation.

(Thurs-P16) **Identifying the effects of Bluestar Forensic Spray on the ability to identify bloodstains using Raman spectroscopy**

Alexis Weber, Igor Lednev, *University at Albany*

The identification, testing, and collection of biological evidence at crime scenes is a complex multi-step process, even when samples are apparent. This process is made more difficult when the biological samples are not visible to the naked eye. When body fluid samples are not visible, crime scene investigators must use biochemical enhancement techniques to search for stains. The interaction of these reagents with the body fluids commonly causes the stained areas to luminesce. These reagents are sprayed over large areas at once. This means that most, if not all, of the biological material in each area will interact with the biochemical reagents. Thus, the body fluid samples that are collected for confirmatory analysis will be contaminated. Therefore, the purpose of this work was to determine if a previously developed body fluid identification model can discriminate between body fluids exposed biochemical enhancement reagents.

This goal will focus on the identification of the most common body fluid encountered at crime scenes: blood. When bloodstains are suspected of being present but not visible, biochemical reagents are used to detect them. Bluestar and luminol are primarily used by crime scene investigators for the detection and enhancement of latent bloodstains. Approximately 20 μL of the body fluids will be deposited onto the non-interfering aluminum substrate. We used increasing dilutions of blood and semen test with the biochemical enhancement reagents, starting from neat samples down to 1:200 (blood: water) dilutions. The stains were left to dry overnight under ambient conditions. The following day they were sprayed with Bluestar. The mixture of body fluid and reagent was then set to dry overnight as well. The samples were then be analyzed using Raman spectroscopy, mapping a minimum of 12 points per sample. This allowed us to account for the heterogenous nature of the mixture and categorize the entire

sample. After a large sample set has been obtained, we will import the spectra into the PLS Toolbox in MATLAB for analysis and model deployment. The capabilities of our current body fluid identification model and identification results will be discussed.

(Thurs-P17) **Single Particle Interaction With Short Focus and Filament Laser Produced Plasmas**

Thiago Arnaud,Digital Surf, Kyle Latty, Kyle C. Hartig, *University Of Florida*

This work examines the interaction of single particles in a laser produced plasma with narrow-bandpass, fast-gated imaging of the particle vaporization and excitation. The initial investigation into the laser and plasma particle interaction effects were accomplished with carbon microspheres that are individually levitated using a novel hollow "cone" beam laser particle levitation setup that previously controls the position of the particle in relation to the laser pulse and expanding plasma. Particles are manipulated by the size of the hollow core of and power of the cone beam. The energy transfer between the laser produced plasma or laser filament and the particle during the particle ablation is achieved through use of a combination of laser shadowgraphy for hydrodynamic phenomena as well as fast-gated narrow bandpass imaging and high-resolution emission spectroscopy. Lastly, contaminants are added to the graphite microspheres to simulate trace analysis of materials of interest to nuclear debris fallout in single particles entrained in the atmosphere for potential application to wide area environmental sampling.

(Thurs-P18) **Laboratory time-resolved LIBS to support the SuperCam analyses on Mars.**

Bruno Bousquet, Elise Clavé, Sylvestre Maurice, *IRAP, University Of Bordeaux, DLR*

The SuperCam instrument enables to perform LIBS analyses on Martian rocks, hence characterizing their chemical composition. Previous studies showed that the characterization of specific elements, like C or H content, may require the use of internal standards for normalization; in particular, the O signal is often used. However, the use of such internal standards requires to understand the origin of the emitting species. The Martian atmosphere being composed of 96% of CO2, the atmosphere may contribute to C and O emissions from the ablation plasma. Differentiating between C and O coming from the atmosphere or the sample can then be complex, although it is key, for example, in the search for carbonates.
In the present study, time-resolved LIBS (TR-LIBS) has been investigated in the laboratory through a comparison between two experimental conditions, namely plasma expansion in a chamber containing either nitrogen or Mars simulant gas mainly composed of CO2, both of them under 7 mbar pressure. We will present a selection of TR-LIBS data including atomic emission lines and molecular emission bands, observing the interactions between the atmosphere and expanding plasma, and the contribution of the atmosphere to plasma emissions, and we will discuss the potential consequence for SuperCam.

(Thurs-P19) **Heavy Metal Quantitation Comparison of Hand-Held Laser Induced Breakdown Spectroscopy to Other Analytical Instrumentation**

Jay Clausen, Sam Beal, Michael Bishop, Patrick Sims, *Naval Information Warfare Center Pacific, US Army Corps of Engineers, Engineer Research And Development Center,* Corteva Agriscience

Handheld laser-induced breakdown spectroscopy (HH LIBS) is an emerging analytical technology with potential to replace X-ray fluorescence spectroscopy (XRFS) for field characterization of soils containing heavy metals. This study explored the accuracy and precision of HH LIBS for analyzing soils containing copper and zinc in comparison with XRFS and inductively coupled plasma–optical emission spectrometry (ICP-OES). Comparison involved the analysis of 108 soil samples from eight military installations as well as NIST certified reference materials. A 2-gram aliquot of material was utilized for digestion and analysis by ICP-OES. Samples were also analyzed with the field instruments (HH LIBS and XRFS) after pressing the material into 13-mm pellets as well as after milling the soil to reduce particle size and then pressing into pellets. Precision and accuracy results for HH LIBS non-processed NIST samples was well within typical laboratory acceptance criteria. However, non-

processed soil sample results for both LIBS and XRFS yielded poor precision and accuracy due to heterogeneity issues. Increasing the shot count proved unsuccessful in overcoming the heterogeneity issue. However, particle size reduction combined with increasing the shot count for HH LIBS yielded results well within typical laboratory acceptance criteria and greatly improved the correlation (<15 to > 80%) between field HH LIBS and laboratory ICP-OES results.

(Thurs-P20) **Feasibility of laser-induced breakdown spectrometry for determination of neodymium in magnet alloys**

Aline de Carvalho Elias, Maciel Santos Luz, Ivanise Gaubeur, Juliana Naozuka, Fábio Rocha, Cassiana Nomura, *University Of Sao Paulo*

The quantification of rare earth elements in (Nd-Pr)-FeB alloys used in the production of super magnets is required to ensure the quality of the raw material and a final product with suitable properties. Laser-induced breakdown spectroscopy is attractive to this aim by avoiding the need for decomposition of the refractory material, but quantitative analysis requires proper calibration approaches to overcome matrix effects. This work proposes a novel analytical method for Nd determination in (Nd-Pr)-FeB alloys after sample fusion pretreatment. Samples submitted to a simples fusion process was analyzed by LIBS using the Applied Spectra J200 Tandem system, with a Q-switched Nd:YAG laser operating at 266 nm was used. The instrumental parameters were optimized aiming at higher sensitivity (delay time = 0.50 µs, pulse energy = 20 mJ, number of laser pulses = 401, and spot size = 65 µm) and the performance of three different calibration approaches, namely external standard calibration (EC), multi-energy calibration (MEC), and slope ratio calibration (SRC) was evaluated. EC based on standards spiked with increasing amounts of Nd_2O_3 on Al_2O_3 as diluent yielded accurate results (relative errors < 20 %), whereas accuracy of MEC was impaired (relative errors > 57 %), because of the lack of emission lines with suitable sensitivity. SRC stood out due to calibration with a single standard and accurate results, as demonstrated by agreement with reference values at 95% confidence level and relative errors < 3 %.

(Thurs-P21) **Distribution Of Rare-Earth Elements In Metamorphic Rocks : New Contributions From Micro Laser Induced Breakdown Spectroscopy Imaging**

Cecile Fabre, Alexandre Tarantola, Behzad Monfaredi, Lucas Marulier, Vincent Motto-Ros Georessources

This study focuses on several metamorphic rock samples from the Sanandaj-Sirjan area in Iran, where deposits of Rare Earth Elements (REEs) (La, Ce, Y, Gd, ...) have already been located. The thin sections of rock studied, in andalusite or sillimanite zones, show different generations of garnet, biotite and staurolite. µLIBS imaging with a spatial resolution of 15 µm, allows us to rapidly identify classic paragenesis minerals (quartz, andalousite, garnet...) and numerous small minerals of rutile, zircon and pyrite scattered in the matrix.
µLIBS imaging quickly reveals not only yttrium enrichment in the garnets, but also the presence of numerous La-Ce-Y minerals, enabling us to better constrain the location of rare earths, whose contents are only known from total analyses. Unexpectedly, by looking closely at the pixel recorded near the REE minerals, luminescence bands in some LIBS spectra suggest the presence of other unidentified REEs (i.e. Dy or Gd).

(Thurs-P22) **Identification of Fluoroquinolone-Resistant Mycobacterium Tuberculosis through Random Forest-Support Vector Machine Combined with Intensity Ratio Analysis using Laser-Induced Breakdown Spectroscopy**

Hohyun Keum, **Gookseon Jeon**, Soogeun Kim, Kyunghwan Oh, Heejoo Lee, Janghee Choi, *Korea Institute of Industrial Technology*

Tuberculosis (TB) is a communicable disease that poses a grave threat to public health and remains one of the leading causes of mortality worldwide. Drug resistance in TB significantly affects treatment procedures and success rates. Moreover, suboptimal treatment may lead to the development of drug resistance and various negative side-effects. Therefore, determining drug resistance is crucial aspect in ensuring effective treatments. Particularly, the urgent need for the development of rapid diagnostic tests arises from the extended diagnosis time, which can lengthen the treatment period and result in inappropriate therapeutic approaches.
Laser-induced breakdown spectroscopy (LIBS), which is an atomic emission spectroscopy, provides elemental composition information of samples. Due to its several beneficial advantages, such as rapid analysis and minimal or no sample preparation, LIBS has been considered a useful technique in a variety of fields, including biomedical applications. This study has verified the potential of LIBS as a diagnostic method for drug resistance in TB. To investigate the ability of LIBS to distinguish drug resistance in TB, Mycobacterium tuberculosis with mutated gyrA and gyrB genes, which indicate fluoroquinolone resistant TB, were used as experimental groups, while Mycobacterium tuberculosis H37Rv was used as a positive control group. To improve the diagnostic accuracy, a random forest-assisted support vector machine was combined with intensity ratio analysis of LIBS. The presented model showed considerable accuracy, reaching 91.6%.

(Thurs-P24) **Hybrid Raman spectroscopy and laser-induced breakdown spectroscopy system applied to food safety**

Sungho Shin, Iyll-Joon Doh, Kennedy Okeyo, Euiwon Bae, J. Paul Robinson, Bartek Rajwa, *Purdue University*

Food safety assurance systems are growing more rigorous as a response to the rising occurrence of food contamination and food frauds in the food industry. These factors pose significant threats to human health and well-being. Food contamination refers to the presence of harmful substances in food, including biological, chemical, and physical contaminants. Food frauds have also become a significant global concern including adulteration, substitution, and dilution. Traditional detection methodologies, such as microbiological and chemical testing, have limitations in terms of sensitivity and comprehensive analysis.
Here we present a combined Raman spectroscopy and laser-induced breakdown spectroscopy (LIBS) system which may offer a lower-cost alternative method for real-time and field-portable analysis of food contamination and adulteration. Both optical techniques offer comparable advantages, such as simple sample preparation, real-time detection, remote detection, and portability. When used together in a Raman/LIBS device, the elemental and molecular analysis can be performed simultaneously, making it suitable for applications such as pesticide detection or identifying the presence of heavy metals.
In this study, the feasibility of a combined Raman spectroscopy and LIBS system for detecting food contamination and food authentication were demonstrated. Quantitative analysis of pesticides on fruit peel are confirmed and it was demonstrated that surface enhancement using nanoparticles improves both Raman and LIBS signals together. In addition, we also demonstrate that the accuracy of classification improved by approximately 10% when utilizing the hybrid Raman/LIBS spectra, as opposed to the analysis of spectra from the individual methods. Thus, the successful implementation of multimodal optical systems is expected to contribute in agricultural as well as another environmental research.

(Thurs-P25) **High-resolution high-speed LIBS imaging**

Elena Vasileva

Laser Induced Breakdown Spectroscopy (LIBS) is becoming increasingly prevalent as an analytical technique for material content due to its high sensitivity and the capability to detect any element of the periodical table with nearly no requirements or limitations on sample dimensions, consistency, and/or surface quality. LIBS imaging has recently been introduced to expand the capabilities of the LIBS technique even further. LIBS imaging enables the reconstruction of elemental or mineralogical maps

which show the distribution of the elements or minerals within an analyzed sample. Typically, excitation sources, such as lasers, with a repetition rate between 10-100 Hz are used for LIBS analysis. These systems have traditionally provided a sufficient balance of sampling frequency and laser pulse energy. However, for high-resolution µm-scale LIBS images of large sample areas (several cm2) a higher repetition rate is required to avoid unreasonably long acquisition times.
This work demonstrates an approach to reduce the acquisition time for high-resolution µ-LIBS imaging by using a laser operating in the kHz frequency range. The faster laser repetition rate enabled the development of a µ-LIBS imaging microscope capable of resolving images with about 10 µm resolution at < 20 min /cm2. For the first time, images of nearly 4K resolution showing detailed elemental distribution within an analyzed sample have been achievable. The capability of the system to perform fast LIBS analysis over large samples and to reveal the spatial distribution of elements within the analyzed area opens opportunities for application within a wide variety of fields of research including biomedical and geological material analysis, as well as in industrial settings such as mining.

(Thurs-P26) **Excimer Laser Ablation-Based Tandem Approach to the Analysis of Solid Samples**

Jhanis Gonzalez, Charles Sisson, Excimer Laser Ablation-Based Tandem Approach to the Analysis of Solid Samples Chunyi Liu, Steve Shuttleworth, Miyeun Yoo, Applied Spectra Inc,Richard Russo, *LBNL/ASI, Applied Spectra Inc*

Laser Ablation-Based techniques for direct solid material analysis have become routinely used in research labs and industry alike due to their capability to provide a wide range of chemical information, from elemental and molecular data to isotopic information. In 2014 Applied Spectra, Inc. published the first paper [1] on the use of the Tandem LA-LIBS approach (simultaneous LIBS and LA-ICP-MS) and since then, increasing number of articles have been published on various analytical topics. The unparallel advantage of Tandem LA – LIBS is the ability to perform both measurements simultaneously and from the same ablation event.

Applied Spectra has extended the capability for Tandem LA-LIBS measurement to the excimer (193 nm) laser ablation. The excimer LA has been a primary workhorse of direct soild sampling for geoscience community. Furthermore, the excimer lasers have been used increasingly for LA-ICP-MS analysis in mining and other material industries due to high laser energy couping efficiency for the wide bandgap materials. The Tandem instrument development for the excimer LA required engineering and analytical solutions related to hardware synchronization, LIBS-compatible fast washout sample cell development, experimental conditions optimization, data reduction, and processing of the large amount of data obtained. In this presentation, we will highlight hardware engineering advances of Applied Spectra excimer LA instrument, RESOlution-SE and new instrument capabilities enabled by Tandem LA - LIBS technology (via Tandem PrismTM). We will also discuss our advanced software platform, ClarityNeXtTM, for processing and analyzing Tandem data to perform powerful imaging, classification, and quantitative analysis.

(Thurs-P27) **Optimization of Cannabinoid Extraction Conditions Including Solvent Choices Using Microwave and Ultrasonication Methods**

Joshua Animasaun, Ngee Chong, Beng Ooi, *Middle Tennessee State University*

Cannabinoid compounds extracted from Cannabis sativa have demonstrated high therapeutic potential. Hence, efficient extraction together with reliable determination of these compounds is crucial for utilization of hemp extract. In this research, optimal extraction conditions for hemp samples were developed, which involves the comparison of ultrasound-assisted extraction (UAE) and microwave-assisted extraction (MAE) using ethanol as a primary solvent. The effects of varying parameters such as extraction time, temperature, solvent-to-sample ratio and the ratio of mixed solvents were evaluated to determine their impact on the yield and quality of extracted cannabinoids. The extraction solvents evaluated for UAE and MAE are ethanol, limonene, olive oil, propylene glycol, and glycerol mixed in different volume ratios. The ultrasonication accessory called VialTweeter was used to evaluate UAE

conditions and solvent ratios to achieve optimal extraction efficiencies. The ultrasonic cell lysis and disruption of cell membranes by the sonomechanical shear forces contribute to shorter extraction times of UAE relative to MAE.

The comparison of the percent yields of cannabinoid compounds between cannabis flower and cannabis stem were also examined. GC-MS analysis shows that cannabis bud contains more cannabinoid compounds with the amount of CBD present in the cannabis bud five times greater than that of cannabis stem. The cannabinoid profile of the extracts is dependent on the extraction mode, solvents used, extraction temperature, and extraction time. The decarboxylation of the carboxylic acid functional group among some cannabinoid compounds are evaluated for GC-MS as the post-extraction analytical technique.

(Thurs-P28) **Cationic Isotachophoresis of Engineered Gold Nanoparticles For Enhanced Lateral Flow Assay**

Devon McCornack, Wen-Ji Dong, *Washington State University*

Lateral flow assays (LFAs) are widely used for point-of-care diagnostics due to their simplicity and rapid results. However, their sensitivity is often limited, particularly when detecting low-concentration targets. In this study, we investigated the use of cationic isotachophoresis (+ITP) to enhance the sensitivity of LFAs. +ITP is a technique that uses an electric field to separate positively charged analytes based on their electrophoretic mobility. By encapsulating Gold Nanoparticles (GNPs) with avidin, a highly basic (pI ~10.5) biotin-binding protein, a positively charged and modifyable probe is synthesized for facile attachment of antibodies. When integrated into paper-based lateral flow platforms, +ITP has the potential to enhance the performance of LFAs for a range of diagnostic applications, particularly in resource-limited settings.

(Thurs-P29) **Scandium Stabilized Lipid Bilayer Coatings**

Christopher Harrison, Juliette Gonzales, *San Diego State University*

The use of phospholipid bilayers to modify channel and capillary surfaces for electrophoretic separations has been well established. The phospholipid bilayers provide excellent protection for the capillary surface, as well as the potential to tune the magnitude and even the direction of the electroosmotic flow (EOF). However, the use of such bilayers is limited by their intrinsic longterm instability. The bilayers can remain virtually intact for a few separations, but invariably require some form of reconditioning to retain consistent function over time.

Attempts have been made previously to improve the stability of lipid bilayers, typically through the use of unique lipids which can undergo covalent reactions with each other in order to crosslink and stabilize the bilayer. Though these are effective, they are costly, require specific lipids, and the preparation process can be time consuming.

This work explores how the choice of the metal cation used in the buffer to prepare the phosphocholine based lipid bilayers can play a significant role in the stabilization of the structure. We have identified scandium as a remarkably effective cation to include in the bilayer. This trivalent cation yields a surprisingly fast reversed EOF when it is present in the BGE, and the EOF remains reversed and stable once scandium is removed from the BGE. Bilayers formed with scandium are shown to be resistant to the methanol washes typically used to remove bilayers from the capillary. We will present our explorations of the stability and utility of scandium in capillary electrophoretic separations with phosphocholine coated capillaries.

(Thurs-P30) **Increasing DEP-based separation to generate multiple A single-cell transcriptomic study of DEP-sorted mouse neural stem cell fractions**

Alan Jiang, Jazmine Moore, Nicole Lav, Lisa Flanagan, *University Of California, Irvine*

Dielectrophoresis (DEP) has been widely used as a label-free method to separate heterogeneous cell types based on innate differences in dielectric properties. One of the valuable attributes of DEP-based sorting is that sorted cells are viable, which allows downstream studies to further assess the characteristics of enriched cell types and enables mechanistic studies aimed at deciphering cell function. Based on this approach, we optimized the parameters of our DEP-based cell sorting (HOAPES) device to separate mouse neural stem and progenitor cells into cell fractions that differ in whole-cell membrane capacitance (Cspec), a dielectric property found to correlate with cell fate in human and mouse NSPCs. We increased the number of outlets in the sorting device to yield greater separation of mouse NSPCs. Post-sort quality assessment assays were performed to confirm the predicted difference in Cspec among the sorted cell fractions using the Deptech 3DEP reader. Differences in NSPC fate were determined using immunocytochemistry of differentiated cells and newly developed assays using qRT-PCR to detect distinct progenitor populations. Sorted cell fractions were subjected to single-cell RNA sequencing (scRNA-seq) to compare the cellular composition and transcriptomic differences among the sorted cell fractions. Increasing the number of cell fractions sorted by DEP enables analyses that could uncover molecular signatures defining distinct cell types, link molecular profiles to cell electrical properties, and lead to a better understanding of the genetic programs governing cell fate.

(Thurs-P32) **Determination of Chloride Ions in Water via Fluorescence Quenching of Rose Bengal**

Sulayman Oladepo, *King Fahd University of Petroleum and Minerals*

High concentrations of chloride ions are known to quench the fluorescence of quinine in acidic medium. In the work presented here, this quenching phenomenon is now being explored with other dyes such as Rose Bengal, in the author's laboratory. Since chloride ions are able to quench the fluorescence of quinine, we hypothesized that a similar quenching can take place with common dyes. So, in order to test this hypothesis, aqueous solutions of Rose Bengal were prepared in the presence of varying concentrations of chloride ions supplied by NaCl. Thereafter, the fluorescence spectra of the solutions were then measured. The resulting fluorescence spectra show concentration-dependence in such a way that the measured fluorescence of Rose Bengal decreased with increasing chloride ion concentration, which suggests fluorescence quenching by chloride ions. The fluorescence data were then used to obtain Stern-Volmer plots, which gave the Stern-Volmer constant and an intercept value of 1, which agrees with theory. When the experiments were repeated with KCl and MgCl2, similar Stern-Volmer plots were obtained, giving different quenching constants and an intercept of 1 in all cases. This approach was then used to determine the ppm concentration of chloride ions in a sample of bottled water, and the value obtained is consistent with that obtained with an independent analytical method. This shows that fluorescence quenching of Rose Bengal afforded by chloride ions can be used as a detection tool for chloride ions in aqueous media. Similar experiments with other halide ions are on-going in the author's laboratory, while other dyes are also being explored in a similar fashion.

(Thurs-P35) **Lithium-Ion Battery Recycling with Field-Portable Handheld LIBS**

Brendan Connors, *Sciaps, Inc.*

Global demand for lithium has increased sharply in recent years, driven by its value in energy storage. Much of this recent growth has been driven by the need to supply the growing demand for electric vehicles (EVs), and by some estimates annual lithium production must grow by five-fold over the next decade to meet demand. In addition to newly mined lithium, some demand will be met by recycling lithium and other critical elements from older batteries that have reached their end of life.
Field-portable handheld LIBS analyzers have been available commercially for several years and have proven themselves well for applications in metals, geochemistry, recycling, and other areas. The ability of LIBS to analyze light elements such as lithium, carbon, and fluorine have made them particularly useful to the lithium-ion battery recycling industry. The analysis involves direct testing of

so-called “black mass” material, which is a graphite-rich shredded cathode material. In this paper we will show qualitative and quantitative results for these and other elements in black mass from a test method developed for portable LIBS analysis.

(Thurs-P36) Comparing Crater Masses with Spectroscopic Masses In Laser-Ablation Analysis

Jonathan Merten, Shealyn Chestnut, Kyle Hartig, **Mary Foster**, *Arkansas State University*

Global demand for lithium has increased sharply in recent years, driven by its value in energy storage. Much of this recent growth has been driven by the need to supply the growing demand for electric vehicles (EVs), and by some estimates annual lithium production must grow by five-fold over the next decade to meet demand. In addition to newly mined lithium, some demand will be met by recycling lithium and other critical elements from older batteries that have reached their end of life. Field-portable handheld LIBS analyzers have been available commercially for several years and have proven themselves well for applications in metals, geochemistry, recycling, and other areas. The ability of LIBS to analyze light elements such as lithium, carbon, and fluorine have made them particularly useful to the lithium-ion battery recycling industry. The analysis involves direct testing of so-called “black mass” material, which is a graphite-rich shredded cathode material. In this paper we will show qualitative and quantitative results for these and other elements in black mass from a test method developed for portable LIBS analysis

Friday, October13, 2023

Oral Presentations

Closing Plenary Session including Special Speakers, Announcement of 2023 FACSS Innovation Award Winner and SciX 2024 Preview

(SciFri-1)**Should Anyone Care about Scientific Instruments (Besides Us)?**
Roger Turner, *Science History Institute*

Elegant, persnickety, frustrating, astonishing: instruments provoke powerful feelings. They enable great discoveries and valuable businesses as part of the scientific enterprise that creates the modern world. So how do we get other people to care about them? This presentation takes you behind the scenes at a history of science museum where we grapple with “the grey box problem.” We’ll meet a spectrometer so cantankerous that its operators swore it detected their desperation and malfunctioned accordingly. We’ll encounter glassware helpfully labeled “This is not a bong” as well as the mass spectrometer built from spare parts that led to a Nobel Prize. Throughout, we’ll ponder what makes an instrument historically significant, examining how museums decide what to put on display and how to tell the story of science. It’s not Antiques Roadshow, but if you’re curious if that thing in the corner of the lab should be in a museum, this is the talk for you.

(SciFri-2) **Scix and SERS - what lies ahead!**
Duncan Graham, *University Of Strathclyde*

A light hearted look ahead at the possible future of Scix and SERS.