FACSS PRESENTS

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ABSTRACT BOOK

September 26, 2021

21SUNKEY01: Sunday Keynote

Chair: Jean-François Masson

(SUN-01.1) **Measuring Scientific Impact**

David R. Walt[1]; [1]*Harvard*

Translating discoveries from an academic lab to the commercial sector is rewarding but challenging. In this talk, I will describe two successful examples of how academic discoveries were translated and commercialized. The paths to each translation were nuanced. I will give specific examples of some of the challenges of starting a new company and will provide general lessons for science entrepreneurs to follow.

September 27, 2021

21ATOM05: Ramon M. Barnes and his Impact on Spectrochemical Analysis

Chair: Gary Hieftje
Co-Chair: R. Kenneth Marcus
On-site Chair: Gary Hieftje

(ATOM-05.1) **Ramon M. Barnes: Stalwart of Spectrochemistry**

Gary M. Hieftje[1]; [1]*Indiana University*

Several alternative albeit similar definitions can be found in dictionaries for the noun “stalwart”. Overall, they suggest someone who is faithful and committed to a concept or cause for an extended length of time. Professor Ramon M. Barnes clearly meets this definition. Over a period of more than a half century, he has contributed in many ways to the fields of plasma spectrochemistry, elemental and molecular spectroscopy, and fundamental atomic spectrometry. He has been engaged in the promotion of those fields, dissemination of information in and about them, and the mentoring of both senior and junior scientists active in the same areas. I have had the privilege of working with and under Prof. Barnes for most of this interval and have been witness to many of his achievements and a beneficiary of his contributions. In this presentation, a few of his many activities, accomplishments, and accolades will be highlighted. Examples include early work in time-resolved high-voltage spark spectroscopy, basic studies involving the inductively coupled plasma, mentoring outstanding students and co-workers, founding and maintenance of the most important international scientific conferences on analytical plasma spectrometry, and ongoing publication of an international newsletter on that subject. No mention will be made of Professor Barnes’s age, although rumors have it that he, like Jack Benny, remains stuck at 39. Moreover, as Jack Benny himself once said, "Age is strictly a case of mind over matter. If you don't mind, it doesn't matter."

(ATOM-05.2) **All I really needed to know I learned…in graduate school**

Gary A. Meyer[1]; [1]*Promerus, LLC*

Dr. Ramon M. Barnes was, and continues to be, a major influence in my life as a professional analytical chemist in industry. Concepts and attitudes that were learned under the watchful eye of Dr. Barnes have been a constant guide throughout my 40 years in the field. It's safe to say that my career was literally launched by my association with Dr. Barnes and the help I received along the way from his many other graduate students. The field of inductively coupled plasmas in chemical analysis was just in its infancy back in the late 70s when I joined his group. Being a graduate student in Dr. Barnes' group made it possible to be in the company of other

great scientists also studying ICPs and atomic spectroscopy. The tools and techniques learned in his lab gave us the chance to present unique solutions to the challenges of real time analysis. I am very grateful for the many opportunities Dr. Barnes has given me, and hope to extend his legacy and influence in the scientific community.

(ATOM-05.3) **Ramon Barnes: How a Sage of Atomic Spectroscopy Paved the Road Ahead**

Steven J. Ray[1], Christopher J. Brais[1], Williams Kelsey, Nicholas Hazel[2], Khue Nguyen, Caitlin Massimi, Eric Jensen; [1]*The State University of New York at Buffalo,* [2]*University at Buffalo*

The field of atomic spectrometry has often benefited from the presence of individuals with formidable foresight in their development of research themes, a dedication to teaching and nurturing new scientists, and a commitment to the larger international community. During the later twentieth and early twenty-first centuries, Prof. Ramon Barnes has served as one such exemplar for those researchers working in analytical atomic spectrometry and plasma spectrochemistry. In this presentation, we will evaluate the influence of Prof. Barnes in each of these categories, and how efforts sustained over multiple decades have helped to shape the research community today. We will also examine strategies and offshoots that have developed from some of the seminal research reports generated in his laboratory, in some cases by considering concepts that have been directly adopted into the author's laboratory. The development of novel sample introduction strategies for arsenic analysis, the development of new microwave-based ionization sources, and the fundamental consideration of analytical measurements will be traced through several historical iterations. Through these examples, the profound impact of Professor Ramon Barnes on the field can be appreciated.

(ATOM-05.4) **The Winter Conference: An Enabling Technology for My Career in Academia**

R. Kenneth Marcus[1], R. Kenneth Marcus[1]; [1]*Clemson University*

An enabling technology can be defined as "equipment and/or methodology that, alone or in combination with associated technologies, provides the means to generate giant leaps in performance and capabilities of the user." To me, the key part of the definition is "provides a means". Ramon Barnes initiation and sustainment of the Winter Conference on Plasma Spectrochemistry, affectionately referred to as the "Winter Conference" has indeed been a major "means" by which the field of atomic spectrometry grew since its inception in 1980. Technologies, ideas, and challenges have been described, debated, and advanced on an annual basis through oral and poster presentations, at coffee breaks, and in late night bar exchanges. Just as the Winter Conference has influenced the overall field of atomic spectroscopy, my career evolved through the opportunity to attend and participate with my colleagues and peers in the field. My first occasion to attend the Winter Conference was January, 1988, just 18 months after I started my academic career. I was totally out of my element, presenting work in glow discharge spectrometries at an "ICP" meeting, and meeting people whose work I had been studying for the last 6 years; Hieftje, Horlick, Blades, and Caruso. I have learned how to be a better spectroscopist at the conference. I have grown as a person, through the enriching interactions with persons from around the globe. As the field of atomic spectrometry has evolved, so too has my research expanded. Many changes were inspired by challenges and opportunities emanating from my attendance at the Winter Conference. From the old standard line "direct solids elemental analysis", our program has evolved to novel means of elemental speciation, LC/MS, and now to isotope ratio mass spectrometry with ultrahigh resolution Orbitrap instruments. While I have grown professionally and personally from my attendance at the Winter Conference, my students have gained immeasurably from attending the meeting, presenting their science and interacting with world leaders in the field. In my presentation, I will hopefully demonstrate the "means" by which the Winter Conference has influenced my science and indeed my life; all thanks to Ramon Barnes.

(ATOM-05.5) **San Diego 1992 to Tucson 2020 - a ride through (LA-)ICPMS**

Detlef Guenther[1], Thomas Vonderach, Christoph Neff, Pascal Becker, Bodo Hattendorf; [1]*ETH Zurich*

Attending the US Winter Conference on Plasma Spectrochemistry for the first time in San Diego 1992 was an eye-opening event for me. I got the unique chance to see and meet all those researchers and authors of the papers I was reading to get familiar with inductively coupled plasma mass spectrometry. Thanks to the support by Ramon Barnes, I was able to continuously attend that conference, and it always has been a distinct exchange place for many new ideas and developments over almost 40 years. Many approaches, which had been proposed and evaluated in the late 80s and early 90s have now become mature. Laser ablation inductively coupled plasma mass spectrometry has become one of these examples. Today it provides capabilities for high spatial resolution imaging (2-D and 3-D on a micron scale) and some of our recent results will be shown. Furthermore, we coupled the new nitrogen-based MICAP-MS to laser ablation sampling to assess the figures of merit and compare them to conventional argon-based LA-ICP-MS. Finally, after having known ICP-MS for 40 years in horizontal and vertical upwards configurations, we realized a downwards oriented ICP-MS. The first version using a quadrupole mass spectrometer already indicated that aligning the trajectories of droplets and particles with gravity reduces size-related losses and can lead to increased transport efficiencies. Most recently, we combined this new configuration with TOFMS detection and initial experiments studying the evolution of ion signals from individual droplets at high temporal resolution will be discussed.

21BIM03: Vibrational Spectroscopy for Cancer Screening and Diagnostics

Chair: Fay Nicolson
On-site Chair: Fay Nicolson

(BIM-03.1) **Pharmacokinetic Tomography: Mapping Drug Uptake in Tissue with Coherent Raman Imaging**

Conor Evans[1]; [1]*Wellman Center for Photomedicine / Massachusetts General Hospital*

Coherent Raman scattering (CRS) imaging is capable of directly imaging the diffusion and uptake of drugs into tissue, enabling the direct quantification of pharmacokinetics. The ability to quantitatively visualize pharmacokinetics on the cellular and subcellular scale has opened new windows into studying previously inaccessible pharmacokinetic information. Importantly, the microscale information gleaned from CRS imaging can be processed to extract key macroscale pharmacokinetic parameters, including Tmax, Cmax, and partition ratios. We will present our work translating this toolkit to the study of percutaneous pharmacokinetics in human skin and clinical studies. Areas of investigation include the quantification of dermal pharmacokinetics in multiple skin tissue types, the combination of CRS with macroscale skin pharmacokinetic techniques, and the development of CRS methods aimed at determining both pharmacokinetics and pharmacodynamics in the same tissue. Our ongoing work incorporates new machine learning models to map drug PK to human skin morphology, as well as portable coherent Raman imaging tools for pharmacokinetic studies directly in diseased skin.

(BIM-03.2) **Estimating the depth of inclusion and the optical properties of biological tissues using Spatially Offset Raman Spectroscopy**

Sara Mosca[1], Priyanka Dey[2], Marzieh Salimi[3], Ben Gardner[4], Francesca Palombo[4], Nick Stone[5], Pavel Matousek[6]; [1]*CLF, RAL, STFC,* [2]*Teesside University,* [3]*Exeter University,* [4]*University of Exeter,* [5]*Biomedical Physics, School of Physics and Astronomy, Uni of Exeter, UK,* [6]*STFC Rutherford Appleton Laboratory*

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 in biological tissues. Spatially offset Raman spectroscopy (SORS) allows chemical characterisation of biological tissues at depths of up to two orders of magnitude greater than conventional Raman spectroscopy. Here we propose a new method for estimating the depth of inclusion within turbid media (e.g. biological tissues) using SORS with the aid of external calibration data only. This new approach facilitates a fully non-invasive methodology potentially applicable for in vivo medical diagnosis without any a priori knowledge of the sample. The concept of depth prediction is based on relative changes in

Raman band intensities of the inclusion that are directly related to the pathlength of Raman photons travelling through the medium, thereby encoding information on the depth of the inclusion(1,2). Monte Carlo simulations of photon propagation were used to gain an insight into the relationship between the spatial offset and the photon pathlengths inside different tissues, enabling one to derive a general scaling factor to be used in SORS measurements for depth prediction. The approach was validated by predicting the depth of surface-enhanced Raman scattering (SERS) labelled nanoparticles (NPs) acting as an inclusion inside ex vivo porcine tissue with an average root mean square error of prediction of 7.3 % of the overall tissue thickness(3). These results pave the way for future 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. References: (1) Mosca, S.; Dey, P.; Tabish, T. A.; Palombo, F.; Stone, N.; Matousek, P. Anal. Chem. 2019, 91 (14), 8994–9000. (2) Mosca, S.; Dey, P.; Tabish, T. A.; Palombo, F.; Stone, N.; Matousek, P. J. Biophotonics 2020, 13 (1), 1–7. (3) Mosca, S.; Dey, P.; Salimi, M.; Palombo, F.; Stone, N.; Matousek, P. Analyst 2020, 145 (23), 7623–7629.

(BIM-03.3) **Nanoscale plasmonics for cancer lipid biopsy: practical aspects of detecting extracellular vesicles amidst the nano-junk**

Randy Carney[1], Hanna Koster[1], Tatu Rojalin[1], Mariss Taub[1], Andrew Birkeland[1]; [1]*UC Davis*

Extracellular vesicles (EVs) are nanoscale biomolecular packages of variable size and composition readily found in all biofluids and shed by every cell type measured to date, thus are highly attractive for non-invasive liquid biopsy for many diseases. New analytical approaches are needed that account for the vast molecular heterogeneity of EVs amongst the milieu of other bioparticles and assemblies in complex biofluids. In particular, lipoprotein subclasses can outnumber EVs by orders of magnitude, are co-isolated to various extent by any single purification method, and are largely unpredictable and uncontrolled at any given point in time (e.g., from blood draw to blood draw). The influence of non-EV contaminants is of wide concern to the EV research community, particularly as they relate to the choice of isolation methodology, yet contemporary characterization methods are largely unsuitable to either account for such contamination in precious biofluids, or better, to even "see through" it for effective diagnostics. We present here a platform that both accounts for and overcomes lipoprotein contamination, by utilizing label-free plasmonic enhancement of the Raman scattering inherent to the analyzed EV preps. Our major findings are: (1) Surface enhanced Raman scattering (SERS) can accurately fingerprint various relevant lipoprotein subclasses, and distinguish them from EVs according to their inherent relative chemical content. (2) Using simple principal component analysis (PCA) of SERS spectra generated from just a few microliters of human clinical biofluids, we demonstrate that different subclasses of lipoprotein are co-isolated with EVs depending on the particular isolation methodology used. A combination of isolation methods is needed to completely remove all lipoprotein from EV preps. (3) Despite the previous finding, the major driver of chemical distinction as observed in SERS spectra across a cohort of clinical samples from head and neck cancer patients vs. non-cancer controls is the disease state itself, and not the influence of variable lipoprotein contamination. We believe our work could have important consequences in basic EV research as well as their application in therapeutics and diagnostics. Robustly producing highly pure EV preparations and assessing their level of purity, e.g., by SERS, are the cornerstones of such developmental steps.

(BIM-03.4) **Toward developing a new technology for real time histopathology of endometrial tissue using chemical Imaging**

Ghazal Azarfar[1], Rebecca Sinkes[2], Ike Uzoaru[2], Georgina Cheng[2], Rohit Bhargava[3]; [1]*University of Illinois,* [2]*Carle Health,* [3]*University of Illinois at Urbana-Champaign*

In fifteen words or less, explain the significance of this contribution (Novel Aspect).: This work is an advance over the traditional examination of the surgical sections in clinics.

Abstract Text: Cancer of the endometrium is reported as the most common cancer of the female reproductive

system in 2021 by the American cancer society. Irregular chemical and morphological alterations of endometrial glands are correlated with the risk of progression to endometrial carcinoma. There is a significant limitation in diagnosing the severity of the alteration and assigning a risk factor to an H&E-stained biopsy. IR chemical imaging can capture both morphological changes and chemical alterations associated with the disease. We imaged 182 paraffin-embedded endometrial tissue microarray samples and 18 human surgical resections using an IR chemical microscope. A pathologist used a parallel H&E-stained slide to annotate the IR chemical images into eight pathologically significant tissue classes. Five million IR spectra were extracted from the chemical Images, and the corresponding second derivative IR spectra were calculated. The second derivative spectra were examined by principal component analysis followed by linear discriminant analysis to identify the spectral chief contributory variables resulting in endometrial tissue variation. Twenty-one spectral variables were selected as the main features for the classification, and a cascade artificial neural network was trained to classify the condition of the endometrial glands. The classifier has more than 80% accuracy in classifying the tissue and assigning one of the conditions of benign, hyperplastic, atypical hyperplastic, and cancer to the biopsy.

(BIM-03.5) **ATR-FTIR Spectroscopy for Clinical Translation: Detection of Pancreatic Cancer in over 200 Patients.**

Alexandra Sala[1], James M. Cameron[2], Cerys A. A. Jenkins[3], Hugh Barr[4], Loren Christie[5], Justin J. A. Conn[2], Jeff Evans[6], Dean A. A. Harris[7], David S. S. Palmer[5], Christopher Rinaldi[1], Ashton G. Theakstone[1], Matthew J. Baker[2]; *[1]University of Strathclyde, [2]ClinSpec Diagnostics Ltd., [3]Swansea University, [4]Gloucestershire Hospitals NHS Foundation Trust, [5]ClinSpec Diagnostics Ltd. and University of Strathclyde, [6]CR-UK Beatson Institute, [7]Swansea Bay University Local Health Board*

In fifteen words or less, explain the significance of this contribution (Novel Aspect).: Earlier diagnosis of pancreatic cancer to enable curative surgery and/or advanced treatment for patient management.

Abstract Text: In 2020, pancreatic cancer has been the seventh most deadly cancer worldwide with over 460,000 victims. Carbohydrate Antigen (CA) 19-9 serum test is the first method used for detection of pancreatic cancer in the current diagnostic pathway; although, previous studies have reported poor positive predictive values in classifications involving symptomatic patients, showing strong limitations in providing certain information about the presence of a pancreatic tumour. Attenuated total reflection – Fourier transform infrared spectroscopy (ATR-FTIR) has demonstrated exceptional potential in human blood serum analysis for cancer diagnostics, and the detection of pancreatic cancer has the strong need to see the clinical implementation of a new diagnostic technique such as ATR-FTIR in order to increase both the detection rates and the survival rates of this invasive and dreadful disease. The proof-of-concept study presented here, focused on the discrimination between both cancer versus healthy control samples, and cancer versus symptomatic control samples from patients with comorbidities and/or confounding diseases; it aimed to investigate the use of ATR-FTIR spectroscopy on dried human blood serum deposited onto optical sample slides, which utilise a silicon internal reflection element (SIRE) as a novel and cost-effective approach for pancreatic cancer diagnosis. Different machine learning algorithms were applied to discriminate between cancer and healthy control samples. Partial least squares-discriminant analysis (PLS-DA) achieved results amounting to sensitivity and specificity of 92.3% and 90.3%, respectively; an optimal accuracy of 91.3 was also reported. Moreover, an area under the curve (AUC) equal to 0.9626 was obtained through receiving operating characteristic (ROC) analysis, highlighting the outstanding degree of the classification model's diagnostic separability. The same algorithms were also applied to discriminate between cancer and symptomatic control samples achieving balanced sensitivity and specificity over 75% with AUC of 0.8436. Both discriminations underwent bootstrapping validation and were proven statistically significant. Herein, we present these results and demonstrate that ATR-FTIR spectroscopic analysis of serum has an excellent potential to become a cost-effective, minimally invasive, highly sensitive, specific,

and accurate diagnostic test for detection of pancreatic cancer.

21FORENS01: Nuclear Forensics

Chair: Robert Lascola
On-site Chair: Robert Lascola

(FORENS-01.2) **Using a Quadrupole ICP-MS for Isotope Ratio Measurements**

Derek McLain[1], Donald Graczyk, Yifen Tsai, David Chamberlain, Jennifer Steeb; [1]*Argonne National Laboratory*

Argonne National Laboratory has developed a two-parameter modeling formula for quadrupole ICP-MS (ICP-QMS) data, which corrects for nonlinearity with a "dead-time" function and makes subsequent weighted mass-bias corrections to normalize isotope ratio data to pertinent isotopic reference materials. The model has been applied to isotopes spanning a wide mass range and a comprehensive strategy for performing isotope ratio measurements or trace-element concentration determinations has been developed. This strategy takes advantage of the model's strengths and accommodates observed run-to-run variations in ICP-QMS signal to improve short term and long term precision and accuracy of measurements. Supporting data from real use of the strategy and model is presented to demonstrate the improvements attained with the widely available ICP-QMS system.

(FORENS-01.3) **Matrix-Assisted Ionization Mass Spectrometry for the Detection and Characterization of Uranium Species**

Danielle Mannion[1], Joe Mannion, Wendy Kuhne, Matthew Wellons[2]; [1]*Savannah River NL,* [2]*Savannah River National Laboratory*

Mass spectrometry is considered the "gold standard" in many nuclear fields for the analysis of long-lived actinides such as uranium and plutonium; however, these analyses often require time consuming sample preparation or analytical methodologies. Matrix-assisted ionization (MAI) is a recently discovered and poorly understood ambient ionization technique. MAI generates gas phase ions without the application of heat, electrons, photons, or high voltage. Our team at SRNL was the first to utilize MAI for mass spectrometry analysis of inorganic analytes including a selection of molecular uranium species. Our findings demonstrate that MAI-MS enables rapid isotope ratio analysis (on the order of seconds) of uranium at nanogram levels with minimal sample preparation. Current efforts are focused on investigation of the gas phase species to interrogate ion formation mechanisms in MAI.

(FORENS-01.5) **Exploration of Trace Elements in Pu Using Hand-Held LIBS**

John D. Auxier[1], John D. Auxier[1], Dung M. Vu[1], Elizabeth Judge, James Colgan; [1]*Los Alamos National Laboratory*

Past efforts to perform analysis on Pu metal or Pu containing materials has historically been performed using NDA methods such as gamma-ray spectroscopy. Analysis methods for the composition of Pu metal for impurities such as Ga and Fe or other compounds has been performed with x-ray fluorescence (XRF) or has required dissolution and subsequent measurement with UV-vis spectroscopy methods. Similarly, Pu containing materials for the determination of Pu have historically been performed using gamma-ray spectroscopy, followed with XRF or similar technique. It is the focus of this project to explore hand-held laser induced break-down spectroscopy as a possible candidate as a technique for the determination of Pu, its impurities, as well as the identification of Pu in a variety of compounds. LIBS and HH-LIBS have been used previously to identify U in material. This work will present the efforts relating to initial development of a useful library of relevant emission lines for the identification of Pu using this technique. Furthermore, this work will highlight initial efforts into using Chemometric methods to improve the data which results from this technique.

21IR01: Nanoscale IR

Chair: Georg Ramer
On-site Chair: Dmitry Kurouski

(IR-01.1) **Infrared Absorption Nanospectroscopy at the Single Molecule Scale**

Francesco Simone Ruggeri[1], Francesco Simone Ruggeri[1]; [1]*Wageningen University*

Biological processes rely on a wide class of biomolecular and macromolecular machines that have nanoscale physical dimensions and whose function emerges from a correlation between their chemical and structural properties. A fundamental objective of modern analytical methods in physics, chemistry and biology is the comprehension of how physical-chemical properties and heterogeneity of single biomolecules underlie their role in cellular function and disease. While innovative nanoscale imaging methods have been developed to characterise biomolecules, imaging microscopies are to the most part chemically blind; thus hampering the characterisation of inhomogeneous and complex systems. Here, we show the application of infrared absorption nanospectroscopy (AFM-IR) as a real breakthrough for the analysis of heterogeneous biomolecules and their interactions from the single molecule scale to several multiple biological length scales in air and liquid environment. As a major advance in the field, we demonstrate the achievement of single protein molecule detection of infrared absorption spectra and maps by introducing off-resonance, low power and short pulse ORS-nanoIR. OUr approach enables the accurate determination of the secondary structure elements of single proteins in the amide band I region, such as alpha-helices and beta-sheets. 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 the structure of functional protein self-assemblies to be exploited as a novel class of biomaterials in bioscience. Overall, our aim is to expand the capabilities of analytical nanoscience to shed light on the structure-activity relationship of biomolecules for nano- and bio-science applications.

(IR-01.2) **Nanoscale imaging (PTIR) and Engineering mid-IR Hyperbolic Phonon Polaritons in 2D Materials.**

Andrea Centrone[1]; [1]*National Institute of Standard and Technology*

Hyperbolic phonon polaritons (HPhPs) are hybrid excitations of light and coherent charge oscillations that exist in strongly optically anisotropic 2D materials (e.g., hBN, MoO3 etc). HPhPs attract interest because of their long lifetimes (3 order of magnitude longer than plasmons) and because they confine light to sub sub-diffraction dimensions, thereby enabling novel mid-infrared nanophotonic applications. Photothermal induced resonance (PTIR),[1,2] also known as AFM-IR, couples the resolution of atomic force microscopy (AFM) with the richness of information of IR spectroscopy. Beyond nanoscale chemical imaging and material identification, PTIR also enables measurement of the sample optical properties in the near-field and mapping highly confined optical modes (i.e. HPhPs).[3,4] In this work, the dispersion relation and HPhP lifetimes in single-crystal α MoO3 are determined by Fourier analysis of real-space, nanoscale-resolution polariton images obtained with the PTIR technique. Measurement of MoO3 crystals deposited on periodic gratings show longer HPhPs propagation lengths (≈ 2 ×) and lower optical compressions in suspended regions compared to crystals in direct contact with the substrate as well as record long (≈ 12 ps) polaritons lifetimes.[3] PTIR measurements on hBN frustum nanostructures reveal high quality factors (Q ≈ 280) HPhPs that contrary to plasmon are preserved in high density arrays, a promising property for sensing and quantum emission applications.[4] This work enhances the ability to engineer nanophotonic devices by leveraging nanostructuring and substrate morphology to control phonon-polariton propagation and lifetimes. [1] A. Centrone, Infrared Imaging and Spectroscopy Beyond the Diffraction Limit, Annual Review of Analytical Chemistry (2015) 8, 101-126. [2] D. Kurouski, A. Dazzi, R. Zenobi, A. Centrone, Infrared and Raman Chemical Imaging and Spectroscopy at the Nanoscale, Chemical Society Reviews (2020), 49, 3315-3347. [3] J.J. Schwartz, S.T. Le, S. Krylyuk, C.A. Richter, A.V. Davydov, A. Centrone, Substrate-mediated hyperbolic phonon polaritons in MoO3, Nanophotonics (2021) 10,

1517–1527. [4] G. Ramer et al. High-Q dark hyperbolic phonon-polaritons in hexagonal boron nitride nanostructures, Nanophotonics (2020) 9, 1457–1467.

(IR-01.3) **Advanced AFM-IR Studies of Functional Nanomaterials: When Scanning Probe Microscopy Teams up with Vibrational Spectroscopy**

Bert M. Weckhuysen[1]; [1]*Utrecht University*

Recent decade has witnessed the emergence and use of novel analytical methods in which vibrational spectroscopy can be exploited to study functional nanomaterials, including solid catalysts, at the nanoscale, and even in well-defined cases under in-situ conditions. One of these methods is infrared spectroscopy, which in combination with atom force microscopy (AFM), allows to obtain single point IR spectra over a wide spectral range with ~ 5-10 nm spatial resolution. In this invited talk, I will provide an overview of the capabilities of the AFM-IR methodology, and demonstrate with different showcases the challenges, limitations and opportunities. The selected showcases include zeolites, supported metal catalysts as well as metal organic frameworks (MOFs). These materials, which are known to be solid catalysts, can be studied to learn more about their synthesis, activation, reaction and deactivation mechanisms. Examples include the Fischer-Tropsch synthesis over Co/TiO2 catalysts, in which we are able to discern between different mechanistic routes for CO activation in the presence of H2. We also show how surface-anchored MOFs can be studied with AFM-IR to elucidate their growth- and guest-host interaction mechanisms. Examples include HKUST-1 and ZIF-8; and we have used NO and formaldehyde as probe molecules to study their heterogeneities at the nanoscale. Zeolite thin-films allowed us to study the methanol-to-olefins (MTO) reaction at the nanoscale and investigate the relationship between Al zoning and hydrocarbon pool formation. The lecture will end with a perspective on what will be possible in the near future when using AFM-IR to study functional nanomaterials at work.

(IR-01.4) **Analytical measurements of extraterrestrial material: what do we expect from AFM-IR analysis?**

Jérémie Mathurin[1], Emmanuel Dartois[2], Cécile Engrand[3], Jean Duprat[4], Ariane Deniset-Besseau[5], Alexandre Dazzi[5], Yoko Kebukawa[6], Takaaki Noguchi; [1]*Institut de Chimie Physique, CNRS, Université Paris-Saclay,* [2]*Université Paris Saclay, CNRS, ISMO, France,* [3]*Université Paris-Saclay, CNRS, IJCLab, France,* [4]*CNRS, IMPMC, France,* [5]*Universite Paris-Saclay, CNRS, ICP, France,* [6]*Faculty of Engineering, Yokohama National University, Japan*

Infrared (IR) vibrational microscopy provides insights on the chemical composition of organic matter (OM) in interplanetary samples (meteorites and micrometeorites) [1]. If it provides a global view of the dust grain chemical structure content, IR microscopy remains limited by the diffraction, with typical spot sizes sampling of a few micrometres in the mid-IR range. Such IR diffraction limit can be circumvented using AFM-IR microscopy. We report here recent results obtained on two UltraCarbonaceous Antarctic MicroMeteorites (UCAMMs) using AFM-IR [2]. UCAMMs are interplanetary dust particles in the 20-500 μm size range with extreme concentrations in OM. These UCAMMs exhibit large deuterium anomalies and most likely originate from the surface of small icy bodies residing in the outer regions of the solar system [1], [3]. We evaluated two different AFM-IR setups to study UCAMMs: i) the contact mode setup and ii) tapping mode setup. The latter mode appears well adapted to the AFM-IR imaging on loosely bound samples such as (micro)meteoritic samples. Based on these results obtained during UCAMMs' samples analysis and AFM-IR study of the Murchison and Bells meteorites from Y. Kebukawa et al. [4], we more recently successfully applied AFM-IR in tapping mode to carbonaceous chondrites which were prepared without the needs of chemical pretreatment. One of the main objectives of these investigations is to develop an upfront and dedicated expertise on complex materials such as interplanetary samples, to fully apply the AFM-IR technique high resolution capabilities on the samples recently returned by the Hayabusa 2 mission. [1] E. Dartois et al., "Dome C ultracarbonaceous Antarctic micrometeorites," Astron. Astrophys., vol. 609, p. A65, Jan. 2018. [2] J. Mathurin et al., "Nanometre-

scale infrared chemical imaging of organic matter in ultra-carbonaceous Antarctic micrometeorites (UCAMMs)," Astron. Astrophys., vol. 622, p. A160, Feb. 2019. [3] J. Duprat et al., "Extreme Deuterium Excesses in Ultracarbonaceous Micrometeorites from Central Antarctic Snow," Science (80-.)., vol. 328, no. 5979, pp. 742–745, May 2010. [4] Y. Kebukawa et al., "Nanoscale infrared imaging analysis of carbonaceous chondrites to understand organic-mineral interactions during aqueous alteration," Proc. Natl. Acad. Sci. U. S. A., vol. 116, no. 3, pp. 753–758, 2019.

(IR-01.5) **nano-FTIR Spectroscopy and Imaging for Material Analysis and Chemical Identification of Organic Nanomaterials**

Tobias Gokus[1], Andreas Huber[2], Artem Danilov[2]; [1]*neaspec GmbH,* [2]*attocube Systems AG*

In fifteen words or less, explain the significance of this contribution (Novel Aspect).: Correlative optical, mechanical and electrical material property imaging and infrared spectroscopy with nanoscale spatial resolution.

Abstract Text: Scattering-type Scanning Near-field Optical Microscopy (s-SNOM) is a scanning probe approach to optical microscopy and spectroscopy bypassing the ubiquitous diffraction limit of light to achieve a spatial resolution below 20 nanometers. s-SNOM employs the strong confinement of light at the apex of a sharp metallic AFM tip to create a nanoscale optical hot-spot. Analyzing the tip-scattered light enables the extraction of the complex dielectric function of the sample directly below the tip. Utilizing tunable monochromatic infrared laser sources, nanoscale resolved near-field reflectivity and absorption maps simultaneous to topography are obtained [1]. In addition, the technology has been advanced to enable Fourier-Transform Infrared Spectroscopy on the nanoscale (nano-FTIR) [2] utilizing broadband radiation from the visible spectral range to THz frequencies. Recently, the combined multimodal analysis of complex nanoscale material systems by correlating nano-FTIR data with information obtained by other optical, electrical and mechanical SPM-based measurement methodologies has gained significant interest. Owing to its order of magnitude higher detection sensitivity compared to conventional FTIR spectroscopy and extremely high spatial resolution of 10-20 nm nano-FTIR has been successfully employed for determining the chemical composition of multiphase thin films and polymer blends [3,4] and determining the molecular orientation and cluster size in ultrathin polymer brush layers and membranes [5,6]. In this presentation, we introduce the working principle and latest developments of nano-FTIR and highlight recent advances of the technique for characterization of organic nanomaterials. Due to its sensitivity to the local dielectric environment with high spatial resolution, we demonstrate nanoscale chemical identification of complex polymer systems. Further, we will discuss the inherent sensitivity of nano-FTIR to conformations and molecular orientations in the context of analysis of nanometer sized macromolecular clusters, as well as small molecular assemblies. 1. F. Keilmann, R. Hillenbrand, Phil. Trans. R. Soc. Lond. A 362, 787 (2004). 2. F. Huth, et al., Nano Lett. 12, 3973 (2012). 3. I. Amenabar et al., Nat. Commun. 8, 14402 (2017) 4. M. I. Penas et al., Polymer, 226, 123812 (2021) 5. A. de los Santos Pereira et al., Anal. Chem. 92, 4716 (2020) 6. A. Cernescu et al., Anal. Chem. 90, 10179 (2018)

(IR-01.6) **Miniature NIR Spectrometers: Possibilities and Pitfalls**

Richard Crocombe[1]; [1]*Crocombe Spectroscopic Consulting, LLC*

In the early 1950s the development of commercial (research grade) infrared spectrometers spurred the foundation of professional societies devoted to vibrational spectroscopy: The Coblentz Society in the USA and the Infrared Discussion Group (IRDG) in the UK. These societies had a mission to educate practitioners in the art and science of spectroscopy. In the mid-1950s, commercial compact benchtop spectrometers emerged, typified by the Perkin-Elmer 237 and Beckman IR-5, democratizing the technique further and putting these spectrometers in the hands of bench chemists. As these instruments were democratized, two themes were apparent: widespread use of the technique, coupled with a concern from the establishment about whether the

instruments are being used appropriately and their data being interpreted correctly. If the 1950s represented one turning point in optical spectroscopy, we are now at another with the wide availability of extraordinarily low cost spectroscopic components operating in the visible and shortwave near-infrared regions of the spectrum (~400 – 1000nm). Via physical spectrometers or smartphone cameras, these devices are now available and affordable to the general public, and so the question from the establishment noted above is reoccurring. This talk will explore these themes, explore the possibilities and outline the pitfalls of very low cost optical spectrometers.

21LIBS02: Consolidation of LIBS Methodology

Chair: Alessandro De Giacomo
On-site Chair: François Doucet

(LIBS-02.1) **Getting LIBS results outside the laboratory: lessons from the field**

Steven G. Buckley[1]; [1]*University of Washington // Ocean Insight*

The number of great applications for LIBS in the scientific literature is mind-boggling. LIBS has been used for everything from analysis of trace elements in biological material to chemical composition of coal on conveyor belts. However, the number of applications that have successfully been fielded outside of the laboratory and at scale is much smaller. This talk covers some of the prerequisites for field success with LIBS, based on over 20 years of experience. We will cover hardware, analysis methods, and ultimate expectations for LIBS in the field.

(LIBS-02.2) **Application of Laser-Induced Breakdown spectroscopy in Environmental and Biological areas of Research**

Madhavi Martin[1]; [1]*Oak Ridge National Laboratory*

A plethora of laser-based techniques are employed to understand the chemistry of biological and environmental matrices. In this presentation the research that has been conducted in the last 10 years will be presented. The focus will be on the application of laser-induced breakdown spectroscopy (LIBS) which has been used for the elemental analysis of a number of biological and environmental samples, for example: Various soil samples have been used to demonstrate the quantification of soil carbon using MVA analysis, in addition the elemental characterization of 73 samples of switch grass for ash characterization has been shown using the LIBS technique. The extension of LIBS to poplar hardwood samples where elements of interest that were detected were, silicon. potassium, calcium, magnesium, phosphorus, and sulfur. The authors have also demonstrated that forest fire events were identified by scanning the fire affected wood to detect the changes in dendro-chemistry. Weather conditions have been successfully detected at the treatment plots at throughfall displacement experimental site, Oak Ridge. It has been shown recently that when ionomics is combined with new genotyping technologies it provides a rapid way to identify genes that control elemental accumulation in plants. It is very important to find the genes that control the accumulation and distribution of each element by understanding the complex regulation of the ionome Hierarchical models using principal component analysis (PCA) and partial least square analysis (PLS) was used to determine the presence of the specific elements mentioned above. 1) To determine the characteristic spectra of switch grass containing different amounts of these elements and 2) To examine the viability of this technique for determining the quality of the feedstock in terms of the chemical composition. Previous work in climate change application, has shown that LIBS is an accurate and reliable approach to measuring critical nutrients in woody biomass for a 13-year weather treatment study. We obtained the LIBS validation prediction for the micronutrient elements mentioned here. Furthermore, these examples demonstrate an advance in LIBS-based techniques to determine the viability of switchgrass as a biomass in the production of biofuels and in the determination of climate change.

(LIBS-02.3) **LIBS Intelligence: autonomy and decision-making from spectral data.**

Pablo Sobron[1], Daniel Van Hoesen; [1]*Impossible Sensing*

A key advantage of LIBS is the ability to obtain compositional information in real time. Powerful new computational abilities and machine learning tools now have the potential to bring real autonomy to LIBS applications. A combination of advanced optoelectronics; cloud computing and deep learning; and data processing, display, and visualization can enable predictive and prescriptive analytics in real-time, autonomously, thus helping decision makers —human and robot— to rapidly optimize decisions and maximize operational, scientific, and economic objectives via continuous awareness and real-time response, transforming LIBS field data into real-time actionable insights. Our team at Impossible Sensing has developed neural networks such as CNNs and other statistical techniques and demonstrated unique capabilities to generate usable information in real time. We have trained multiple networks and deployed them in at-the-edge detection use cases, eliminating the need for laborious data processing guided by a domain specialist. Instead, our neural networks, built using domain-specific knowledge, learn an efficient way to interpret data providing the user with high-level actionable information. Building on these developments, our team has tailored spectroscopic instruments to specific applications. At the conference we will review select at-the-edge use cases and describe efficient and effective computing, compression, and transmission using DREAM, our Data Reduction Efficiency AlgorithMs. DREAM utilizes autoencoders to simultaneously denoise spectral data and reduce data volume. First, a neural network (CNN or ANN) is trained to encode and decode data separately. Then the encoder part is deployed in the field to reduce the data for in-the-field computation and decision making or for transmission. The user then recovers the data using the decoder where more resources are available. Combining our advances in LIBS hardware and software with other techniques such as Raman spectroscopy, LiDAR and optical imaging, we are building an integrated solution for 3-dimensional geochemical and mineralogical mapping, allowing for autonomous targeted multipoint measurements from multipoint data at various scales – mm scale for science discovery and km scale for survey-type field applications.

(LIBS-02.4) **Auto-focus LIBS applications for the process control using long and short laser pulses**

Yoshihiro Deguchi[1], Deguchi Yoshihihro, Zhenzhen Wang, Takahiro Kamimoto, Minchao Cui; [1]*University of Tokushima*

Recently, as a measurement technique with high sensitivity and fast response, laser diagnostics has been developed and applied to the actual industrial fields. Laser-induced breakdown spectroscopy (LIBS) is an analytical detection technique based on atomic emission spectroscopy to measure the elemental composition. Signal enhancement to improve the accuracy and detection ability of LIBS has been investigated in many fundamental and applied researches, as well as an understanding of basic plasma physics. DP-LIBS is an important way to enhance the emission intensities to improve LIBS analytical capability. a collinear long and short DP-LIBS method (LS-DP-LIBS) was developed to improve the detection ability and measurement accuracy by the control of the plasma cooling process using the long pulse-width laser radiation. The plasma generated by the short pulse-width laser is stabilized and maintained at high temperature during the plasma cooling process by long pulse-width laser radiation. The auto-focus technique is also one of the key technologies for LIBS applications to practical fields. The distance to the measurement target moves in many LIBS applications, which makes stable measurement difficult. The development of autofocus technology has been performed to automatically adjust the laser and LIBS signal focuses by controlling the automatic motorized stages based on the distance measurement result of using a two-dimensional rangefinder. In this study, LS-DP-LIBS with the auto-focus technique was investigated for the industrial applications of LIBS to demonstrate the LIBS capabilities of the process control.

(LIBS-02.5) **Listening for rock coatings on Mars: Understanding acoustic signals from laser-induced breakdown spectroscopy**

Nina Louise Lanza[1], Baptiste Chide[2], César Álvarez[3], Stanley M. Angel[4], Pernelle Bernardi[5], Olivier Beyssac[6], Bruno Bousquet[7], Alexandre Cadu[2], Elise Clavé[7], Erin Dauson[8], Olivier Forni[9], Thierry Fouchet[5], Olivier Gasnault[9], Xavier Jacob[10], Gaetan Lacombe[11], Carene Larmat[8], Javier Laserna[3], Jeremie Lasue[11], Ralph

Lorenz[12], Pierre-Yves Meslin[9], Franck Montmessin[13], Javier Moros[3], Naomi Murdoch[2], Ann Ollila[8], Paolo Pilleri[11], Pablo Purohit[14], Adriana Reyes-Newell[8], Susanne Schroder[15], Shiv K. Sharma[16], Alexander Stott[2], James Ten Cate[8], David S. Vogt[17], Maurice Sylvestre[18], Roger C. Wiens[1], David Mimoun[2]; *[1]Los Alamos National Laboratory, [2]ISAE-SUPAERO, Toulouse, France, [3]University of Malaga, [4]The University of South Carolina, Department of Chemistry and Biochemistry, [5]LESIA, Meudon, France, [6]Sorbonne University, [7]University of Bordeaux, [8]Los Alamos National Laboratory, NM, USA, [9]Institut de Recherche en Astrophysique et Planétologie (IRAP - CNRS), Toulouse, France, [10]IMFT, Toulouse, France, [11]IRAP-CNRS, Toulouse, France, [12]APL, MD, USA, [13]LATMOS, Guyancourt, France, [14]Universidad de Malaga, Malaga, Spain, [15]DLR, Berlin, Germany, [16]Hawaii, [17]German Aerospace Center, [18]Institut de Recherche en Astrophysique et Planétologie*

The goal of the NASA Perseverance rover mission is to assess the geology and past habitability of Mars to identify and cache samples with a high likelihood of preserving biosignatures. Perseverance carries the SuperCam instrument, which combines several analytical techniques including a laser-induced breakdown spectroscopy (LIBS) instrument for chemical analysis and a microphone for acoustic studies. The SuperCam microphone is a commercial off-the-shelf electret (based on Knowles EK-23132) and is designed to record sounds in the audible range from 100 Hz to 10 kHz. There are three main science investigations of interest for the SuperCam microphone: 1) Analysis of the LIBS acoustic signal; 2) study of atmospheric phenomena; and 3) examination of rover and Ingenuity helicopter mechanical sounds. Here we will focus on how the LIBS acoustic signal may be used to better understand geologic targets. Each time the LIBS laser is pulsed on a target under atmosphere, an acoustic signal is generated from the shock wave produced by the expanding plasma. This acoustic signal can provide critical information about a target's hardness and ablation depth, and whether there are coatings or thin layers present. Rock coatings are an important class of analysis targets for Mars because on Earth, they are associated with microbes and may preserve biosignatures. The LIBS laser ablates a small (nanograms) amount of material, with each subsequent pulse on the same location ablating deeper into the target surface. This produces a shot-to-shot depth profile of composition and acoustic data from the rock surface to the interior. Previous work has shown that rock coatings may be discerned in LIBS spectral data if the compositional change between coating and rock are sufficiently distinct. However, if the coating and rock substrate are similar in composition, it is challenging to discern the coating using spectral data alone. By examining both the chemistry and acoustic signal obtained by LIBS, the presence and nature of a rock coating may be determined. Here we describe LIBS acoustics experiments on Earth and Mars that provide insight in to the unique acoustic signal that can identify rock coatings and similar thin layers on martian rocks.

(LIBS-02.6) **Laser Ablation Plasma Spectroscopy for Nuclear Material Analysis**

Kyle C. Hartig[1], Emily Kwapis; *[1]University of Florida*

Rapid, in-field, and standoff analysis of radiological materials is extremely important to nuclear nonproliferation and forensics applications. Currently, analyses for these and many other fields are performed in laboratory settings and involve extensive sample preparation. Rapid, in-field, and standoff analysis of solid material is possible with optical spectroscopy tools when combined with laser ablation (LA); however, applying optical spectroscopy to the measurement of radiological materials presents numerous challenges (e.g., reactive chemistry). Improvements in spectroscopic techniques have allowed for measurement of radiological materials 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. Laser ablation plasmas are characterized by a rapid decay of temperature, density, and shockwave formation that significantly impacts the hydrodynamics and chemistry of the evolving plume, and, thus, the resulting atomic and molecular population. With the advent of ultra-short (nano- and femto-second) laser sources, LA plasmas have been re-introduced as a lab-scale scheme for studying nuclear fireball chemistry as well as for non-

destructive and remote analysis of materials (e.g., laser-induced breakdown spectroscopy - LIBS). However, a fundamental exploration of shock dynamics, multiphase physics, and reactive chemistry has not been fully elucidated for LA plasmas which is necessary for improving the analytical capability of LA-based techniques, particularly for in situ analysis. While computational fluid dynamic (CFD) simulations have been employed for studying LA plasma evolution in the past, these simulations exclude the coupling of shock dynamics, transport phenomena, and plasma microchemistry. A novel Multiphysics simulation is presented and validated against experimental results using pure aluminum and titanium ablation targets under ambient atmospheric conditions. This talk will focus on radiological material detection and characterization through emission, absorption, and fluorescence spectroscopy of atoms and molecules in laser-produced plasmas. A foundational framework for studying reactive chemistry in LA plasma plumes and their interaction with the ambient environment will be presented. Perspectives on application to more complex species such as uranium and other actinides of interest to nuclear fireballs.

21RAM10: Applications of SERS I

Chair: Courtney Morder
On-site Chair: Courtney Morder

(RAM-10.1) **Plasmonic Nanoprobes for in vivo and Direct Sensing of Nucleic Acid Targets in Plants**
Vanessa K. Cupil-Garcia[1], Pietro Strobbia[2], Hsin-neng Wang[1], Jianhong Hu[1], Kenneth M. Kemner[3], Tai-ping Sun[1], Tuan Vo-Dinh[1]; *[1]Duke University, [2]University of Cincinnati, [3]Argonne National Laboratory*

In fifteen words or less, explain the significance of this contribution (Novel Aspect).: We have developed nanosensors for microRNA detection in plants in combination with SERS techniques.

Abstract Text: Plant biotechnology and biofuel research is crucial in addressing increasing global demands for energy. Further understanding of biomass producing associated metabolic pathways in plants can be used to exploit and increase the production of biomass for energy purposes. In vivo detection of biomarkers associated with plant growth for bioenergy has proved to be limited due to complex sample preparation required by traditional methods. In addition, genetic transformation and biomolecule monitoring inside plant cells is regulated by diameter and size exclusion limits of the plant cell wall (5 - 20 nm). Currently limited methods exist for enabling direct entry into plant cells. Moreover, these methods, such as biolistic particle delivery and electroporation use mechanical force that causes damages to the plant tissue. Nanoparticles pose as promising candidates to characterize intercellular and intracellular plant biomarkers and pathways. Recently the Vo-Dinh group has designed a platform to detect nucleic acid targets in biological systems called “inverse Molecular Sentinel” (iMS) which utilize surface-enhanced Raman scattering (SERS). These nanomaterials have been shown to detect energy relevant microRNAs directly in plants. Imaging technologies such as confocal imaging, X-ray fluorescence imaging, and transmission electron microscopy have been utilized to determine the compartmentalization and location of the SERS iMS biosensors inside tobacco plants.

(RAM-10.2) **Surface-enhanced Raman Spectroscopy, a Sensitive and Label-free Technique for Drug Discovery: Ligand and RNA Specific Binding**
Lamyaa Almehmadi[1], Vibhav Valsangkar[2], Ken Halvorsen[2], Qiang Zhang[2], Jia Sheng[2], Igor K. Lednev[3]; *[1]University at Albany, State University of New York, [2]University At Albany, State University of New York, [3]University at Albany SUNY*

In fifteen words or less, explain the significance of this contribution (Novel Aspect).: Using SERS to detect and characterize the binding between ligands and RNA at ultralow concentrations

Abstract Text: Surface-enhanced Raman spectroscopy (SERS) is an emerging tool for detecting molecular

interactions. The detection and characterization of molecular binding events is a key step in the drug discovery process. Current methods used for drug discovery would profit from SERS's high sensitivity and label-free capability, thus limiting laborious steps and resource-consuming needs. Previously, we have developed a SERS platform with single-molecule sensitivity to detect a protein-linker adduct at a single molecule level. This platform has also shown the possibility of differentiating the proteins' spectral contribution from the linker's via visual inspection and statistical analysis. Therefore, we extended the application of this platform to detect a binding of a peptide ligand to a targeted RNA repeats at a nanomolar concentration. The selected ligands are potential drug molecules that interact with a disease-related RNA repeats. The binding trends found using SERS detection correlated with the binding affinity of different ligands. Furthermore, the bound ligands were also differentiated from each other and from the RNA based on the analysis of the collected SER spectra. These differentiations were possible via visual inspection and statistical analysis.

(RAM-10.3) **Surface Enhanced Raman Spectroscopy of Bacterial Metabolites for Bacterial Growth Monitoring and Diagnosis of Viral Infection**

Wei Wang[1], Peter J. Vikesland[1]; [1]*Virginia Tech*

In fifteen words or less, explain the significance of this contribution (Novel Aspect).: The SERS results suggest an alternative pathway to examine how environmental stimuli affect bacterial growth

Abstract Text: Bacterial metabolites reflect bacterial metabolic activity. In this study, we report the use of surface enhanced Raman spectroscopy (SERS) for detection of both volatile and non-volatile metabolites and the application of this approach for bacterial growth quantification and diagnosis of viral infection. The time dependent SERS signal of the volatile metabolite dimethyl disulfide in the headspace above bacteria growing on an agar plate was detected and quantified. In addition, SERS signals arising from the plate reflected nutrient consumption and production of non-volatile metabolites. The measurement of metabolite accumulation can be used for bacterial quantification and show comparable quantitative performance to classic culture based optical density measurements. In the presence of bacteriophage virus, bacterial metabolism is suppressed, and the relative decrease in SERS intensity reflects the initial virus concentration. we detect viral infection with a prediction accuracy of 93% with the help of multivariate analysis. Our SERS based approach for metabolite production monitoring provides new insight towards viral infection diagnosis.

(RAM-10.4) **Shifted Excitation Raman Difference Spectroscopy for Remote Detection of Plant miRNA biomarkers Under Field Conditions**

Ren Abelard A. Odion[1], Pietro Strobbia[2], Bridget Crawford[3], Rodolfo Zentella[1], Martin Maiwald[4], Bernd Sumpf[4], Tai-ping Sun[1], Tuan Vo-Dinh[1]; [1]*Duke University,* [2]*University of Cincinnati,* [3]*Northrup Gruman,* [4]*Ferdinand-Braun-Institut*

In fifteen words or less, explain the significance of this contribution (Novel Aspect).: SERDS and SERS allow for a novel method of monitoring gene activity in the field

Abstract Text: The detection of micro-RNAs (miRNAs) is crucial in understanding the developmental process of key genes involved in the biomass production of plant biofuels. Current methods for understanding these pathways rely on slow methods such as polymerase chain reaction (PCR) to amplify a tediously purified sample of miRNA from plants. To this end, we have developed a combined plasmonic biosensing method based on a Surface Enhanced Raman Spectroscopy (SERS) platform called the inverse Molecular Sentinel (iMS) to directly detect miRNA such as miR858a to understand ligin production and increased biomass. This biosensor is then coupled with the Shifted Excitation Raman Difference Spectroscopy (SERDS) technique to remotely

detect these targets in the field, even in the presence of harsh background illumination. The application of such technology for monitoring plant gene expression in the field may potentially revolutionize agriculture technology using nanotechnology-based monitoring for plant health, pollution, and pathogen detection.

(RAM-10.5) **Determining Viral Titer By Surface Enhanced Raman Spectroscopy**

Courtney Morder[1], Karin M. Balss[2], Zachary Schultz[1]; [1]*The Ohio State University,* [2]*Janssen Supply Group, LLC*

In fifteen words or less, explain the significance of this contribution (Novel Aspect).: A rapid method of determining viral titer of lentiviruses using SERS was developed.

Abstract Text: Lentiviruses have been shown to be useful in gene therapy due to their ability to deliver genetic information to reprogram cells. To prevent unintended pandemics, the viruses used for therapy are rendered replication incompetent, so that each virus particle can only infect a single cell. Therefore, it is necessary to know the effective titer of the virus to determine the dose and expectations for successful cell therapy. Current methods to determine titer involve infecting a known number of cells and then performing analysis, such as PCR, to determine successful reprogramming. The time and sample preparation required for cell culture means these methods can take anywhere from days to weeks. To provide a more straightforward and rapid viral titer, we have explored the use of surface enhanced Raman spectroscopy (SERS). SERS utilizes plasmonic metallic nanostructures to amplify the Raman signal of the virus particles in the media used for virus production. Two different lentiviruses, one containing a vector encoding for green fluorescent protein (GFP) and one without, were analyzed at various concentrations. The virus particles were injected over a commercial SERS substrate and the SERS signal was recorded. Due to the complexity of the virus structures, and thus the signals obtained, multivariate curve resolution (MCR) was used to differentiate the spectra within each sample. The MCR model shows a linear relationship between scores and viral concentration. From the SERS response, the viral titer of GFP encoding lentivirus particles was determined.

21SPECIAL03: Remote Teaching Chemistry

Chair: Christopher Harrison
Co-Chair: Charles Lucy
On-site Chair: Christopher Harrison

(SPEC-03.1) **Paper Microfluidics as a Safe and Flexible Method for Delivering Active Learning Laboratory Experiments At-Home or In-Person**

Kimberley Frederick[1], Marya Lieberman[2], Rachel Roller[2], Andrea Van Wyck[3], Vincent Remcho[4], Renee Cole[3]; [1]*Skidmore College,* [2]*University of Notre Dame,* [3]*University of Iowa,* [4]*Oregon State University*

Studies of laboratory pedagogy have long been advocating for exercises that challenge students to develop authentic scientific skills and practices including planning investigations, analyzing and interpreting data and constructing explanations and designing solutions. The MICRO project has developed paper microfluidic-based laboratory experiments that are safe and flexible yet inexpensive enough to be developed as active learning experiments. MICRO experiments introduce many of the same techniques as traditional sophomore-level analytical labs including dilutions, standards, graphical analysis, titrations, colorimetry, and potentionmetr and also encourage development of scientific practices. We will present information on the ever growing number of MICRO labs and the available open-source support materials. To date, over 25 different institutions have successfully implemented these labs for students both at home and in person. We will also present feedback on how the experience is helping them rethink their laboratory courses.

(SPEC-03.2) **A Resource for Remote and Virtual Learning in Analytical Chemistry – ASDLIB Remote Labs**

Tom Spudich[1]; [1]*Maryville University*

The development and implementation of remote labs has been essential for a variety of reasons. First, most of Higher Education had some modified form of lab in some format to include functioning off-campus at all course levels during the spring of 2020, fall of 2020 and spring of 2021 semesters. During the summer of 2020, a subset group of ASDLIB met to discuss and develop resources for labs that can be taught off-campus/at home for high school, general chemistry, quantitative analysis and instrumental analysis courses. The group constructed labs and simulations that include finding the pressure inside an unopened carbonated beverage, a penny statistics lab, creating and using a 3D printed photometer for quantitative determination of dyes, gravimetric acid-base titrations and an Excel-based HPLC simulator. Other resources highlighted here from others include a signal-to-noise ratio exercise using virtual instruments created using LabView, MICROLab titrations, and an interactive web application highlighting NMR Fourier transform calculations. All of these resources, to include some supplementary material from the authors, can be found at remotelabs.asdlib.org and are freely accessible under a Creative Commons license.

(SPEC-03.3) **The Distributed Pharmaceutical Analysis Lab (DPAL): Enabling Undergraduates in Analytical Chemistry Courses to Find Fake Medicines**

Marya Lieberman[1], Kathleen Hayes[1], Sarah Bliese[2]; [1]*University of Notre Dame,* [2]*Medicines For All Institute*

Substandard and falsified (SF) pharmaceuticals continue to be a problem in many countries that lack stringent regulatory oversight. In 2014, we developed an approach that enables undergraduate chemistry students to help solve this problem. The Distributed Pharmaceutical Analysis Lab (DPAL) is a consortium of 29 institutions led by analytical chemists at the University of Notre Dame. DPAL provides detailed guidance for analyzing real medicines in the context of instrumental analysis, analytical chemistry, or undergraduate research courses. Sample of pharmaceuticals are collected in Kenya, Liberia, Ethiopia, Malawi, and Bangladesh by covert shoppers; a portion of each sample is reserved for the local regulatory authority, and a portion is shipped to Notre Dame. Each institution chooses one or more pharmacopeial monographs, then follows a set of checklists and spreadsheets to evaluate the analytical metrics of their HPLC assay. Once an institution has demonstrated system suitability using reference standards and samples of expired or degraded dosage forms, DPAL ships samples of the pharmaceuticals for assay. Samples that fail assay undergo further analysis to confirm the result and gain insight into why the sample might have failed. Since 2014, DPAL participants have analyzed over a thousand samples and 168 SF samples have been reported to regulatory authorities and the WHO Rapid Alert system. This presentation will focus on the practical aspects of implementing DPAL in undergraduate analytical chemistry programs.

(SPEC-03.4) **Priming Remote Learning of Instrumental Analytical Chemistry Using a Custom and Free Micro-Textbook**

Russ Algar[1]; [1]*University of British Columbia*

The pandemic turned teaching and learning on its head. There was tuition for undergraduate students at UBC, yet no lecture halls, no labs, no learning spaces, and no time spent on campus. Many people were also struggling with lost wages. Under these circumstances, how could I ask my students to spend more money on a textbook? Especially when they would read only a fraction of the content and use it in only one course? I didn't. Instead, I wrote a custom and free micro-textbook for the students in my instrumental analytical chemistry course. This textbook had approximately 25 pages of written content designed as pre-reading for online lectures, plus post-reading and post-lecture question sets. It covered 22 sub-topics across 9 sets of instrumental methods, ranging from optical methods to voltammetry to chromatography to mass spectrometry. This presentation will share the design, motivation, and rationale behind my micro-textbook for instrumental

analytical chemistry. I will also share the compromises and challenges in its writing, how it integrated with my online lectures, and student feedback received on the micro-textbook. Audience feedback will be used to guide revisions to the textbook and to evaluate potential release as an open-access resource beyond UBC.

(SPEC-03.5) **Open Discussion**
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21SPSJ01: NIR Spectroscopy

Chair: Christian W. Huck
On-site Chair: Richard Crocombe

(SPSJ-01.2) **Modern tools of NIR spectroscopy in natural products analysis**

Christian W. W. Huck[1], Justyna Grabska[1], Krzysztof B. Bec[1]; [1]*University of Innsbruck*

Near-infrared (NIR) spectroscopy is a powerful tool for qualitative and quantitative analysis of natural products. As a rapid and high-throughput analytical method, it offers high chemical specificity, and no/minimal sample preparation making it a superior alternative to the conventional methods of analysis with significant practical values. These advantages are particularly exposed in the field of natural products. In contrast to synthetic medicines, natural products feature chemical diversity that can vary depending on the medicinal plant cultivation conditions, geographical origin or harvest time. The content of bioactive compounds and their derivatives, and thus, the quality parameters of the natural medicine need to be controlled with respect to a number of conditions to ensure suitability of the medicine. Various processes, e.g. drying of the plant material, need to be closely monitored as well. NIR spectroscopy was proven to be a potent analytical tool in such scenario. The last decade marked rapid advances in the field of spectroscopic instrumentation and methods of analysis have appeared. Accelerating trend in the miniaturization of NIR spectrometers brings remarkable increase in the flexibility of analysis by offering on-site analysis - a decisive leap in the natural product industry. However, attention needs to be paid to the various factors affecting their performance in different scenarios. New technology is being implemented, e.g. miniaturized FT-NIR instruments equipped with large-mirror Michelson interferometers. This is accompanied with the use of advanced multi-variate analysis (MVA); e.g. non-linear regression methods (e.g. Gaussian process regression) and artificial neural networks (ANN). Quantum chemical calculation of NIR spectra, as well as two-dimensional correlation spectroscopic (2D-COS) analysis enables to elucidate the spectral regions where the most relevant information on the active compounds can be measured by a particular miniaturized spectrometer. This enables intelligent design of the analysis towards rapidness, accuracy and reliability. This presentation highlights the major novelties introduced to NIR spectroscopy in recent decade with focus on the natural product analysis, which is a challenging but increasingly important field of application that serves as a benchmark for new fundamental developments in the miniaturized instrumentation and spectra-analytical methods of NIR spectroscopy.

(SPSJ-01.3) **A quantitative evaluation for the number of amide bonds in peptides using near-infrared spectroscopy**

Mika Ishigaki[1], Atsushi Ito, Risa Hara, Shun-ichi Miyazaki, Kodai Murayama, Keisuke Yoshikiyo[1], Tatsuyuki Yamamoto[1], Yukihiro Ozaki[2]; [1]*Shimane University,* [2]*Kwansei Gakuin University*

Special peptide drugs have recently attracted attention as a next generation breakthrough pharmaceutical, and a microflow reactor is a promising candidate for their synthesis technology. To establish an industrial production line with the method with a microflow reactor, process analytical technology (PAT) for monitoring chemical reactions over time and in multiple ways is essential. Near-infrared (NIR) spectroscopy is one of the most compelling techniques for PAT. In the present study, we searched for key absorption bands that change with the increase in the number of amide bonds formed by dehydration condensation between amino acids and estimated the quantitative accuracy of the number of amide bonds using the key bands. Since the elongation of peptide

chain can be monitored by evaluating the increasing number of amide bonds with dehydration occurring between amino acids, the key bands whose absorption intensities increased with the elongation of the chain length, such as glycine, diglycine, triglycine, and tetraglycine, were searched. The results revealed that the combination of the amide A and II/III modes became key bands, and partial least squares regression (PLSR) results gave correlation coefficients higher than 0.99. The present results not only have demonstrated the usefulness of NIR spectroscopy as a PAT tool for studying peptide synthesis in micro flow reactors but also have provided basic knowledge for analyzing amide bonds in the NIR spectra of protein, polyamino acids, polypeptides, and polyamides.

(SPSJ-01.1) **It is not like we thought. New insights into NIR spectral features of water and hydrated molecules as unveiled by quantum chemical calculations.**

Krzysztof B. Bec[1], Justyna Grabska[2], Christian W. W. Huck[2]; [1]*Leopold-Franzens-Universität,* [2]*University of Innsbruck*

Infrared (IR) spectrum of water has righteously attracted keen interest, and numerous studies oriented at the water bands have been reported. The near-infrared (NIR) spectrum of water is at least equally interesting, yet comparatively it was not investigated with similar effort. Notwithstanding, NIR spectroscopy delivered important physical insights into the structure of water and its interactions. Furthermore, water content is often encountered circumstance in analytical applications of NIR spectroscopy. Either present in the matrix and obscuring the signal from the targeted analyte or being the analyzed content itself, as moisture is an essential quality parameter for variety of samples. Physicochemical studies of water structure by IR spectroscopy often used quantum chemical calculations to yield essential insights. However, direct modeling of NIR spectra has long remained troublesome. The interpretation of NIR spectra of water was often performed in correlation with the patterns observed in IR region. However, this approach could easily lead to a pitfall. Recent advances in theoretical methods suitable for calculation of NIR spectra opened the pathway to modeling NIR spectra of various molecules. In this presentation it will be shown that NIR bands of water cover less straightforward structures than those anticipated from the deconvolution of the corresponding IR bands. The commonly assumed scenario, in which all the band components contributing to the observed NIR lineshape of water have been assigned with single, symmetric profiles, which directly correspond to variously associated water, needs to be reconsidered. The picture unveiled by anharmonic calculations shows that the contributions to NIR bands of water should be assigned with multi-component profiles. Effectively, the deconvoluted NIR spectrum of water demonstrates more complex structure than commonly assumed.

(SPSJ-01.4) **Adding value to speciality foods – the unreasonable roles of NIR spectroscopy**

Daniel Cozzolino[1], Yasmina Sultanbawa, Heather Smyth; [1]*University of Queensland*

Agri-food supply and value chain markets have become increasingly complex due to the changes in consumers demands, the development of complex food standards associated with food safety and quality, advances in technology (e.g. big data, machine learning), and changes in the food industry structure. The utilization of rapid analytical methods based in vibrational spectroscopy (e.g. infrared, Raman) have provided with tools to analyse and monitor not only composition but other issues such as authenticity, adulteration, fraud, mislabelling, traceability and provenance. The incorporation of near infrared (NIR) spectroscopy combined with data analytics (e.g. chemometrics), are determining a paradigm shift in the way that speciality food ingredients and foods are both evaluated and monitored. Examples on the utilisation of NIR spectroscopy combined with data analytics addressing a wide range of issues in these “speciality foods” value chains will be discussed.

21ATOM01: Medical & Pharma

Chair: Uwe Karst

(ATOM-01.1) **The use of a Time of Flight ICP-MS for Life Science Applications**

Phil Shaw[1], Lukas Schlatt[1]; [1]*Nu Instruments*

ICP-MS is an important part of many analytical techniques used in life sciences like laser ablation imaging and nanoparticle analysis. Scanning detection systems are most often utilized when conducting ICP-MS analyses and while they can deliver important results, there are major downsides especially when considering the speed of acquisitions and the ability to only detect one isotope at a time. A time-of-flight ICP-MS can solve many of these issues and is therefore an important part of modern analytical techniques to increase productivity and generate more detailed results. In this presentation, data recorded using the Vitesse time-of-flight ICP-MS will be shown and the important advantages over similar techniques will be demonstrated. Examples using laser ablation imaging will show the important advantages in the speed and pixel-to-pixel resolution. Furthermore, the possibility to distinguish multielement nanoparticles in a single solution by recording full elemental information for each particle will be demonstrated.

(ATOM-01.2) **A Metallomics Approach Towards Improved Diagnosis and Treatment of Wilson's Disease**

Heidi Goenaga-Infante[1], Heidi Goenaga Infante[2], Estela Del Castillo Busto[2], Stanislav Strekopytov[1], Christian Ward Dietrich[3], Kharmen Billimoria[4], Tim Morley[5]; [1]*LGC Limited,* [2]*LGC National Measurement Laboratory,* [3]*LGC National Measuremet Laboratory,* [4]*LGC,* [5]*Orphalan*

Wilson's disease (WD) is a genetic disorder of Copper (Cu) metabolism characterised by the accumulation of this metal in various body tissues. Diagnosis of WD is primarily based on multiple clinical manifestations (e.g. Kayser-Fleisher ring), abnormal measures of Cu metabolism and genetic testing using a diagnostic scoring system. Nowadays WD is still incurable and must be treated for life, however it can be controlled with medication using Cu-chelators (to increase Cu excretion) or Zn salts (to reduce Cu uptake), and eventually liver transplantation in cases of acute liver failure. An early diagnosis and treatment of WD is crucial to prevent the progression of the disease that could lead to irreversible hepatic, neurological and psychiatric damages. These highlight the need for reliable multi-modal platforms to determine non ceruloplasmin copper (NCC) as indicator of de-coppering as well as to monitor effect of drug chelation treatment. This lecture will demonstrate the potential of novel Metallomic approaches based on the combination of plasma Cu protein speciation and tissue imaging to monitor NCC and effects of drug chelation treatment, respectively. Cu-species in human serum (Cu-Albumin and Cu-Ceruloplasmin) were quantified by anion-exchange HPLC-ICP-MS using a relative peak area quantification strategy combined with total Cu determination. The speciation approach was validated against Cu-protein quantification using species-specific isotope dilution analysis. It was applied to both distinguish between healthy and WD patients as well as to provide insights into the potential bias of the classical EDTA/ultrafiltration method for the determination of CuEXC. The high throughput of this method makes it attractive for Cu-protein screening in a clinical trial where a large number of samples have to be analysed within their stability window. Finally, quantitative imaging analysis of liver, kidney, spleen and brain tissues from a pre-clinical WD model submitted to treatment with a molybdenum based Cu-chelating drug using fast laser ablation (LA) coupled to ICP-Time-of-flight-MS provided new insights into the Cu/Mo distribution across tissues. Such information was found essential to understand observed effects of drug treatment in relation to Cu chelation/release as well as drug upload and accumulation.

(ATOM-01.3) **Quantification of biomarkers at the individual cell level by single-cell ICP-MS**

Mario Corte-Rodriguez[1], Alejandro Fernandez-Asensio, Bettmer Jörg, María Montes-Bayón[1]; [1]*University of Oviedo*

Single-cell (SC) analysis is currently gaining importance due to the growing interest on studying the differences at the single-cell level or, in other words, the cell-to-cell heterogeneity. Such differences are nowadays believed to play a crucial role in different aspects, such as disease development and progression or drug response prediction, and they can not be studied by traditional bulk techniques because they need to lyse and/or digest a big number of cells, enabling the access to only average values and hiding the single-cell heterogeneity within

the big majority of cells. Elemental single cell analysis, as opposed to flow cytometry, makes use of inductively coupled plasma mass spectrometry (ICP-MS) to quantify metals in individual cells. Metals detected by SC-ICP-MS may be naturally present in the cells or metallodrugs taken by them. But metal-labelled antibodies can also be used as tags to detect and, in some cases, quantify surface or intracellular molecular biomarkers. Being breast cancer one of the main diseases in women, the search for biomarkers that allow for early detection, prognosis and drug response prediction is an important field of research. In this regard, the Human Epidermal Growth Factor Receptor-2 (HER2) is one of the most specific prognostic and predictive biomarkers of breast cancer and it is usually analyzed by immunohistochemistry allowing classification of breast cancers into HER2-positive or -negative groups, which determines the treatment to be applied by clinicians. However, the tight relationship between breast tumor growth and its high iron needs, opens the door to the study of other potential markers. One of them is the Transferrin Receptor 1 (TfR1), whose overexpression has been related to a poorer outcome for the patients. This presentation will address the possibilities that SC-ICP-MS opens for the quantification of HER2 and TfR1 as potential breast cancer biomarkers in single cells by using metal-labelled antibodies in breast cancer cell cultures. The levels of expression of these markers will be correlated to the malignancy of the cell lines.

(ATOM-01.4) **One-year Gadolinium Retention in Healthy Rats after Repeated Injections of Macrocyclic and Linear Contrast Agents**

Sabrina Funke[1], Uwe Karst[2], Cécile Factor, Marlène Rasschaert, Philippe Robert, Michael Sperling[2];
[1]University of Muenster, Institute for Inorganic and Analytical Chemistry, [2]Institute of Inorganic and Analytical Chemistry, University of Münster

Gadolinium-based MRI contrast agents (GBCAs) are used since the 1980s and were considered safe to use with restrictions in patients with kidney insufficiency. Recently, they have come into discussion after multiple studies have shown Gd accumulation in numerous organs of healthy subjects. While no clinical effects have been identified yet, the retention of gadolinium has evoked safety concerns surrounding the application of GBCAs. In this study, healthy rats were treated with either a macrocyclic or linear GBCA and sacrificed one or twelve months after the last injection to study the gadolinium retention within the organism. For this project, a 266 nm laser ablation system (LA) coupled to inductively coupled plasma-mass spectrometry (ICP MS) was applied to perform high-throughput quantitative elemental bioimaging of gadolinium and other endogenous elements. The used setup enabled fast and stable analysis while also providing high sensitivity to compensate for the little sample volumes supplied by high-resolution LA. The investigation of rats treated with different types of GBCAs under the same conditions, enabled the direct comparison of their long-term in-body behavior up to one year after injection. In brain, the application of linear contrast agents resulted in rather permanent gadolinium depositions in iron-rich regions of up to 4.5 µg/g after 12 months, which supports the common theory of transmetallation. While these depositions were still prominently found twelve months after injection, macrocyclics showed a strongly different behavior. Here, gadolinium hotspots were found in the choroid plexus at earlier stages after injection, which states decisive information surrounding the discussion on potential pathways of gadolinium into the brain. Moreover, comparing the quantitative results of LA-ICP-MS analysis for different time points after injection, enabled monitoring the gadolinium washout over time. Gadolinium was selectively quantified in regions of interest, showing that gadolinium concentrations are strongly dependent on the targeted area. In brain, for a linear GBCA twelve months after injection, no major washout over time was found in the deep cerebellar nuclei, while cortical tissue of the same rat showed a Gd reduction of 80 %. Generally, it was found a distinctly higher gadolinium washout for macrocyclics compared to linear contrast agents.

21AWD04: ANACHEM Award Symposium Honoring Mark Meyerhoff

Chair: Mark Meyerhoff
On-site Chair: Mark Meyerhoff

(AWD-04.1) **Noninvasive Glucose Monitoring: Challenge of the Background**

Mark A. Arnold[1]; [1]*University of Iowa*

Near infrared (NIR) spectroscopy is a well-known and accepted method for quantitative measurements in a wide range of sample types. Attractive features include the ability to quantify multiple analytes within untreated samples without chemical reagents. Measurements are nondestructive and can be performed in real-time. These attributes make NIR spectroscopy attractive for monitoring chemical processes. Real-time monitors for clinical processes typically require measurements in aqueous samples, which presents a challenge for NIR spectroscopy because of the absorption spectrum of water. Three NIR spectra windows are available for measurements in water, including the combination band (5000-4000 cm-1, 2.0-2.5 μm), the first overtone band (6500-5500 cm-1, 1.54-1.82 μm) and the short wavelength band (14,286-7300 cm-1, 0.7-1.37 μm). The combination band provides the highest selectivity and sensitivity for analytical measurements owing to sharper and stronger absorption bands. Regardless of the spectral range, understanding variations in the spectral background is critical for successful analytical measurements, including the noninvasive measurements of glucose in people with diabetes. For such measurements, incident NIR radiation passes through a vascular region of the body and a multivariate calibration model is used to extract the concentration of glucose from the resulting spectrum. Ideally, this concentration information originates from the net analyte signal (NAS) for glucose, where the NAS corresponds to the component of the glucose spectrum that is orthogonal to the features associated with the spectral background of the sample. This presentation focuses on the issue of background spectral variance for NIR spectroscopy performed over the combination band with two aqueous sample types. First, the importance of properly characterizing the background variance is illustrated by NAS calibration models derived from NIR spectra collected for a set of 50 temperature-controlled ternary mixtures of glucose, urea, and β hydroxybutyrate. Second, the impact of background spectral variance is demonstrated from an analysis of noninvasive NIR spectra collected from people under fasting conditions. In this second case, the noninvasive spectra are collected from a fold of skin on the back of the hand and represent interstitial fluid located within the dermal layer. The presented findings highlight the challenge of measuring glucose noninvasively in human subjects.

(AWD-04.2) **Ultrasensitive Protein Assays for Clinical Applications**

David R. Walt[1], David R. Walt[1]; [1]*Harvard*

We have developed microwell arrays as a platform for both fundamental discovery and bioanalytical measurements. The microwells can be used as miniature reaction chambers to measure the concentrations of proteins more than a thousand times lower than traditional assays. This ultrasensitivity provides the ability to measure molecules in biological samples, such as blood, at levels that cannot be detected using conventional methods. The technology has been applied to diagnostics including cancer, neurodegenerative diseases, and infectious diseases such as tuberculosis and COVID-19.

(AWD-04.3) **Real-Time Detection of Erythrocyte-Camouflaged Microsensors for Therapeutic Drug Monitoring**

Heather Clark[1], Wenjun Di[1], Xuefei Tan[1], Isen Andrew Calderon[1], Fernando Ivich[1], Ashlyn Neal Reilly[1], Mark Niedre[1]; [1]*Northeastern University*

Setting dosage levels for therapeutic drugs rely on population averages, in contrast to an individualized response to the drug. Precision medicine has focused on the personalized needs of patients to achieve high therapeutic efficacy while minimizing side effects. Successful implementation of precision medicine would benefit from frequent monitoring of both the drug of interest and the indicators of toxicity. Drug therapies that have narrow therapeutic windows could benefit from tailor-fitted treatment regimens. For example, lithium therapy for Bipolar Disorder has a narrow therapeutic window ([Li+] = 0.6-1.5 mM) with a low toxicity tolerance (1.5 mM), risking kidney damage and seizures. Monitoring [Na+] in the bloodstream is the most useful indicator for

toxicity during lithium therapy, traditionally requiring frequent blood draws to monitor therapeutic dosing. In this work, we developed a sensor platform that facilitates non-invasive and real-time monitoring of [Na+] in the bloodstream. Taking advantage of the well-studied fluorescent optode-based sensing mechanism, we used polymer-free formulations to facilitate quantification of target analytes (Na+ and Li+) within the physiologically relevant concentrations, each formulation responding independently and selectively towards the target analytes. For sodium detection specifically, we encapsulated our Na+-responsive optode microparticles with red blood cell membranes (fRBCs) to impart red blood cell mimicry camouflaging and utilized Diffuse In Vivo Flow Cytometry (DiFC) to facilitate non-invasive fluorescence detection of fRBCs circulating in the bloodstream of mouse models. The fRBC sensors have linear response within 1-400 mM Na+ and sensitivity of 30% / log unit, showing reversible response and response stability over 7 days in 10% serum. Furthermore, we observed at least six-fold increase in the elimination half-life of fRBCs in comparison to PEGylated counterparts, enabling long term detection of the sensors for up to two weeks in circulation. Recent advancements in our work have focused on improving the reproducibility and control in the fabrication of the fRBC microsensors and on enabling dual-color detection using DiFC for ratiometric quantification. Integrating these different technologies together advances the development of our sensor platform towards continuous monitoring for lithium therapy with potential for translation towards other therapeutic drug monitoring applications for precision medicine.

(AWD-04.4) **Detection of Clinical and Environmental Targets via Microfluidic Droptrodes**

Ryan V. Bailey[1], Shannon Quevedo, Nicholas Glenn, Mark E. Meyerhoff[1]; [1]*University of Michigan*

This presentation will describe a droplet microfluidic approach to the quantification of ions using chemistry analogous to ion-selective electrodes/opt(r)odes, but offering many advantages in terms of sample/reagent consumption, continuous regeneration of the sensing interface, and optical detection in the oil, rather than aqueous phase, which eliminates background signal from biological matrices. We have termed this type of sensor as an ion-selective droptrode. We demonstrate the general concept and show generalizability towards multiple ions using well-known ionophores. We also show the applicability to polyions, which have many charges per analyte molecule, and also probe microfluidic flow parameters that can influence analytical detection metrics.

(AWD-04.5) **Manufacturing of Miniaturized Sensors for Clinical Applications**

Sohrab Masnouri[1]; [1]*Instrumentation Laboratory (IL)*

Advancements in electrochemical sensing technologies and manufacturing have allowed for fabrication of disposable miniaturized sensors with low cost and ease-of-use. Thin film on silicon and thick film on ceramics are prevailing methods of fabrication. These substrates allow for creation of conductive, dielectric and resistive patterns for planar sensor fabrication. However, inadequate adhesion of polymeric sensing films to these substrates is a major limitation in constructing reliable sensors for long term use. Several methodologies are adopted to overcome the adhesion issue, such as bonding polymeric encapsulants on the substrate or employing a compression gasket over the sensor membrane. The drawback is the added design and fabrication complexities. Another design complexity is from having individual reference and counter electrodes for each amperometric sensor with a requirement for electrical isolation among sensors. Assuring high reliability at the point of use is another challenge due to prevailing quality control procedures based on random sampling from production lots, which may not be adequate to meet quality demands. We have addressed the design, fabrication and quality challenges by developing a simple sensor configuration adaptable for measuring a variety of analytes. Thermoplastic materials as sensor substrate allows for solvent bonding with the polymeric sensor membranes. The molded plastic forms fluidic channels for sensor packaging. Through-whole metallic contacts under each sensor form electrical contacts with the instrument. A key design feature is uniform configuration across all sensors through application of common external reference and common counter electrode, negating the need for electrical isolation across sensors. Application of an open liquid junction electrode composed of

Ag/Ag+ also simplifies the main reference configuration. when a sample is pumped into the sensor chamber, fresh silver ion solution flows into the reference chamber and encounters the sample solution near exit section of the sensor chamber. Sensor reliability is assured by wet testing of all manufactured sensors in automated computer-controlled testing systems in the production line. The wet testing is made possible because of superior adhesion of the polymeric membranes to the plastic substrate which allows for multiple hydration and drying cycles without any deterioration in the sensor performance.

21BIM04: Nanotheranostics: Diagnosis and Treatment of Disease using Nanomaterials

Chair: Samuel Mabbott
On-site Chair: Samuel Mabbott

(BIM-04.1) **Deep Raman enabled search-(SERS)-ing of cancer tumour through 7 cm of animal tissue.**
Priyanka Dey[1], Alexandra Vaideanu[2], Sara Mosca[3], Andreas Schatzlein[2], Pavel Matousek[4], Nick Stone[5];
[1]Teesside University, [2]University College London, [3]CLF, RAL, STFC, [4]STFC Rutherford Appleton Laboratory, [5]Biomedical Physics, School of Physics and Astronomy, Uni of Exeter, UK

Surgical tissue biopsies are still the gold standard for cancer diagnosis, which can be painful and often inconclusive. On the other hand, non-surgical diagnosis techniques are less painful and enable rapid treatments to be applied. They also offer the possibility of combining diagnosis and therapy - a step towards personalized medicine. Nevertheless, this requires custom-designed nano-imaging agents. When these are injected into the bloodstream, they can accumulate at targeted cancer sites. Specialized medical devices are then employed to track these agents and detect the location and type of cancer, aiding non-surgical cancer diagnosis. Optical near-infrared (NIR) Raman spectroscopy offers unparalleled sensitivity for diagnosis and when appropriate optical systems are used, the significant tissue penetration of NIR photons allows for collection of scattered Raman photons from depths of many mm. However, for specific detection of deep lesions, strong and distinct signals are required to distinguish the tumour from the surrounding soft tissue. Attempts using Raman labelled plasmonic nanostructures benefitting from surface enhanced Raman scattering (SERS) with specialised subsurface detection methods like deep Raman spectroscopy (DRS) techniques are promising. This presentation will discuss the detection of gold nanostructures in a cancer mouse model through layers of heterogeneous animal tissue of thickness 76 mm. This was experimentally achieved by employing SERS nanostructures (biphenylthiol labelled non-resonant 60 nm spherical gold nanoparticles) were intratumorally (at the tumour base) injected in a 4T1 mammary carcinoma tumour-bearing mouse (tumour in the flank). The mouse was then wrapped in several porcine tissue layers resulting in the tumour being embedded in a heterogeneous animal tissue comprising of protein, fat, bone and blood.. The measurement was carried out ex vivo by employing a compact but flexible 830 nm in-house built DRS set-up in transmission mode. Nanoparticle concentration, multiple modes of SEDRS set-up (including SORS), and various depths were thoroughly investigated, which will be discussed in the presentation. Therefore, with further improvements in detection depths, Raman cancer diagnosis has the scope to become a clinical reality.

(BIM-04.2) **Engineering the tumor microenvironment to improve immunotherapy**
Isaac Adjei[1]; *[1] Texas A&M University*

Advanced-stage cancer that has metastasized is responsible for 90% of cancer-associated deaths. Even with aggressive treatments, the median survival time of patients after being diagnosed with metastasized disease is a few months. Immunotherapy is a powerful clinical tool for treating cancers that have shown promise in treating primary solid and liquid tumors. However, less than 30% of patients with advanced breast or lung cancer respond to immunotherapy, and complete response is rare. The tumor microenvironment (TME) exerts an immunosuppressive microenvironment mediated by hypoxia that cytotoxic immune cells such as natural killer cells and T cells actively avoid. Our goal is to make the TME immune permissive to improve the efficacy of immunotherapies. We are particularly interested in enhancing the potency of adoptive natural killer cell

immunotherapy. Natural killer cells recognize and obliterate malignant cells without the need for prior priming. We have developed nanoparticles that produce oxygen in the presence of the ubiquitous hydrogen peroxide in the TME. We have developed strategies that allow us to control the particles' rate of oxygen production, resulting in sustained oxygen production. Using these NPs, we have shown that sustained oxygen production in tumors decreases the expression of decreased hypoxia-induced factor 1 alpha and a corresponding decrease in the immunosuppressive metabolites adenosine and kynurenine. Critically, relieving hypoxia increases NK cell recognition of cancer cells and promotes their killing. Our studies demonstrate that modifying the TME to make it immunosuppressive is a viable strategy to improve the efficacy of immunotherapies.

(BIM-04.3) **SESORS for Pre-Clinical Cancer Imaging**

Fay Nicolson[1], Bohdan Andreiuk, Bridget O'Donnell[2], Andrew Whitley[3], Scott Rudder, Quang-De Nguyen, Kevin Haigis; *[1]Dana-Farber Cancer Institute and Harvard Medical School, [2]HORIBA Scientific, [3]HORIBA*

The ability to image tumors at depth with high selectivity and specificity remains a significant challenge in the field of biomedical optical imaging. Owing to their unique “fingerprint” like spectra, “surface-enhanced Raman scattering” (SERS) nanoparticles can be employed as image contrast agents and in addition, when functionalized with biomolecules such as antibodies, can specifically target cells in vivo. However, while the detection of SERS contrast agents is extremely sensitive and specific, traditional Raman imaging approaches are limited in their ability to probe through tissue depths of more than a few millimeters. Here, we combine the use of "spatially offset Raman spectroscopy" (SORS) with that of SERS in a technique known as "surface enhanced spatially offset Raman spectroscopy" (SESORS) to image deep-seated tumors in vivo. We will discuss optimization of SORS instrumentation and sampling methods, and subsequent application to pre-clinical SESORS cancer imaging. In addition, SESORS will be compared to other molecular imaging strategies such as magnetic resonance imaging, bioluminescence imaging, and histopathology.

(BIM-04.4) **Multifunctional Theranostic Nanoprobe for Sensitive Brain Tumor Imaging and Photoimmunotherapy**

Yang Liu[1], Austin Carpenter[2], Christopher Pirozzi[1], Pakawat Chongsathidkiet[1], Hai Yan[1], Peter Fecci[1], Tuan Vo-Dinh[1]; *[1]Duke University, [2]Georgetown University*

In fifteen words or less, explain the significance of this contribution (Novel Aspect).: We have developed a novel multifunctional nanoprobe for sensitive brain cancer imaging and potent photoimmunotherapy.

Abstract Text: Despite decades of efforts, non-invasive sensitive detection of small malignant brain tumors (e.g. glioblastoma (GBM) with a median survival of 12-15 months) still remains challenging. Early detection of sub-millimeter brain tumor can potentially improve patients’ prognosis. Here we have developed a dual-modality 124I-labeled gold nanostar (124I-GNS) probe for sensitive GBM detection using positron emission tomography (PET) and two-photon photoluminescence (TPL) microscopy. Our brain tumor detection method with the developed 124I-GNS nanoprobe using PET scan exhibits three important features: (i) extremely high detection sensitivity (0.5 pM limit of detection for 124I loaded on GNS) (ii) specific brain tumor targeting (T/N up to 7.8) via the enhanced permeability and retention (EPR) effect, and (iii) high spatial resolution. We have demonstrated the capability to detect a brain tumor as small as 0.5 mm in diameter using an intracranial brain tumor animal model, which is superior to any currently available non-invasive imaging modality. Furthermore, we have developed a novel photoimmunotherapy, named Synergistic Immuno Photothermal Nanotherapy (SYMPHONY) by combining GNS-mediated photothermal ablation with checkpoint inhibitor immunotherapy. Experiment results demonstrated that SYMPHONY therapy could trigger memorized anti-cancer immune responses to treat cancer metastasis and prevent cancer recurrence. As a result, our biocompatible GNS nanoprobe has great promise for future preclinical and translational applications aimed to improve brain cancer

patients' outcomes.

(BIM-04.5) **Nanoscale IR Analysis of Ligand-functionalized Individual Nanoparticles for Targeted Therapeutics & Diagnostics**

Derek B. Nowak[1], Junghoon Jahng[2]; [1]*Molecular Vista Inc,* [2]*Korea Research Institute of Standards and Science (KRISS)*

In fifteen words or less, explain the significance of this contribution (Novel Aspect).: Understanding the surface chemistry of functionalized nanoparticles is of critical importance for targeted therapeutics

Abstract Text: Ligand-functionalized nano-particles (NPs) are attractive candidates for use as carriers in various biomedical applications such as target-specific diagnostics/therapeutics and drug delivery. While it is important to precisely determine the chemicals on the surface of NPs since the surface functionality of the NPs influences their cytotoxicity and cellular uptake efficiency, characterization of these NPs is not straightforward; tools with sufficient spatial resolution such as transmission electron microscope (TEM) may not highlight the monolayer of organic chemicals while bulk techniques such as FTIR cannot provide any chemical details on individual particles. In this talk, we will introduce infrared (IR) photo-induced force microscopy (PiFM)1, which is used to acquire nanoscale mid-IR hyperspectral images with a 10 nm spatial resolution of a monolayer ligand-functionalized single gold nanoparticle under ambient and environmental conditions. We also demonstrate the diagnosis of nanoscale chemical contaminants that arise from a particle functionalization process but are undetectable in conventional ensemble-averaged imaging technique. In another example, PiFM demonstrates that the hydrophobic−hydrophobic interactions between alkyl substituents of two surface chemicals facilitate nucleation of one component into ordered structures at the particle surface. IR PiFM provides a new way to directly detect heterogeneous nano-chemicals at the single-component level, which is necessary to evaluate nanomaterial safety in biomedical applications.

21IR03: Nanoscale IR II

Chair: Francesco Simone Ruggeri
On-site Chair: Luisa Profeta

(IR-03.1) **Vibrational exciton nano-imaging: molecular ruler probing coupling and disorder on the molecular scale**

Markus B. Raschke[1]; [1]*University of Colorado*

Properties and functions of molecular materials often emerge from intermolecular interactions and associated nanoscale structure and morphology. However, defects and disorder give rise to confinement and many-body localization of the associated wavefunction, disturbing the performance of, e.g., molecular electronic or photonic materials. However, conventional microscopy and even nanoscopy lacks spatio-spectral sensitivity to the low-energy and molecular length scales of intermolecular interactions, carrier-phonon coupling, and polaron formation, thus leaving a missing link between material structure and observed heterogeneity in the electronic or photonic response. We address these outstanding problems in several novel combinations of spatio-spectral and spatio-temporal infrared nano-imaging. Through probing vibrational exciton formation as a molecular ruler, we resolve the evolution of defects in organic electronic materials and nano-domains in self-assembled monolayer on molecular length scales [1]. In the extension to probing both electronic and lattice degrees of freedom, in organic-inorganic perovskites we image the elementary processes of heterogeneous cation-lattice coupling that control the photovoltaic response [2]. We further demonstrate through Purcell-enhanced and strongly coupled light matter interaction the emergence of new hybrid light-matter state and their control for nano-metrology and -sensing [3]. As a perspective we show how electronic, vibrational, and polaron quantum

state nano-spectroscopy in the low energy landscape of molecular matter provides for functional imaging as a new tool to guide the molecular device fabrication with improved performance. [1] E. A. Muller, T. P. Gray, Z. Zhou, X. Cheng, O. Khatib, H. A. Bechtel, and M. B. Raschke, "Vibrational exciton nanoimaging of phases and domains in porphyrin nanocrystals", PNAS 117, 7030 (2020). [2] J. Nishida, A. H. Alfaifi, T. G. Gray, S. E. Shaheen, and M. B. Raschke, "Heterogeneous cation-lattice interaction and dynamics in triple-cation perovskites revealed by infrared nanoscopy", ACS Energy Letters. 5, 1636 (2020). [3] B. Metzger, E. Muller, J. Nishida, B. Pollard, M. Hentschel, and M. B. Raschke, "Purcell- enhanced spontaneous emission of molecular vibrations" Phys. Rev. Lett. 123, 153001 (2019).

(IR-03.2) **Nanoscale Label-free Imaging and Spectroscopy of Interfacial Protein Self-Assembly**

Derek B. Nowak[1], Padraic O'Reilly[1], Tanner Fink[2], Caleb Wigham[2], Helen Zha[2]; *[1]Molecular Vista Inc, [2]Dept. of Chem. & Bio. Eng., Rensselaer Polytechnic Institute*

In fifteen words or less, explain the significance of this contribution (Novel Aspect).: Label-free visualization of self assembled proteins using IR spectroscopy to understand the seeding dynamics

Abstract Text: Nanoscale real space imaging of biological and biomaterial surface is not straightforward even with advances in microscopy techniques. Photo-induced Force Microscopy (PiFM)1 combines infrared (IR) absorption spectroscopy and atomic force microscopy (AFM) via illumination of the tip-sample junction with tunable IR laser light and mechanical detection of forces acting on the tip in response to absorption of light by the sample. By mapping the IR absorption of the sample as a function of IR wavelength and position, ~5 nm spatial resolution is achieved in displaying the locations of heterogeneous materials on the surface of a sample. For protein molecules, amide I and II bands are readily accessible via tunable quantum cascade laser and provide ways to interrogate the molecule's local chemical environment. PiFM can provide both high resolution spectral imaging at a fixed wavenumber and full PiF-IR spectrum (analogue to FTIR spectrum) with a spectral resolution of ~1 cm-1 and spatial resolution of sub-10 nm. IR PiFM capability will be demonstrated on self-assembly of silk fibroin on TiO2 substrate, which represents the native oxide layer on titanium medical implates. With PiF-IR, the contributions of secondary protein structures to amide I band are clearly visible without having to differentiate the spectrum as is the case with bulk FTIR spectra. Nanoscale images of the secondary structures of silk fibroin at different self-assembly conditions will be presented.

(IR-03.3) **Advancement of Peak Force Infrared Microscopy into Fluid Phase and Two-dimensional Spectroscopy**

Xiaoji Xu[1], Haomin Wang; *[1]Lehigh University*

The peak force infrared (PFIR) microscopy is one of the emerging AFM-based nano-infrared with excellent spatial resolution. In this presentation, I will present the recent development of the PFIR microscopy into the liquid phase to address the need for in situ non-destructive chemical imaging. We equip the liquid-phase peak force infrared (LiPFIR) microscope with the capability of controlling fluid compositions during the measurement so as to initiate physical transformation and chemical reactions. LiPFIR microscopy is capable of tracking the polymer surface reorganization in fluids and detect the product of click chemical reaction in the aqueous phase. We also measure the hyperbolic phonon polaritons of hexagonal boron nitride submerged in water to reveal its dispersion relations in the fluid phase as a biological application of LiPFIR, the budding site of yeast cell wall particles is imaged in water. The advantage of the LiPFIR microscopy will facilitate investigations of chemical compositions and transformations at the liquid/solid interface. In addition, I will present the progress of the PFIR microscopy into two-dimensional (2D) spectroscopy. As a demonstration of feasibility, we have constructed a frequency domain pump/probe PFIR microscope to measure vibrational excited state absorption and ground-state depletion. The spatial resolution of the mapping of excited state

absorption with the 2D PFIR is much better than Abbe's diffraction limit and consistent with typical nano-IR imaging methods.

(IR-03.4) **Nanoscale Structural Characterization of a-Synuclein Oligomers Grown in the Presence of Phospholipids**

Dmitry Kurouski[1], Dmitry Kurouski[1], Tianyi Dou; [1]*Texas A&M University*

Parkinson disease (PD) is a severe neurological disorder that affects more than a million people in the U.S. alone. A hallmark of PD is the formation of intracellular α-synuclein (α-Syn) protein aggregates called Lewy bodies (LBs). Although this protein does not have a particular localization in the central neural system, α-Syn aggregates are primarily found in certain areas of midbrain, hypothalamus and thalamus. Microscopic analysis of LBs reveals fragments of lipid-rich membranes, organelles and vesicles. These and other pieces of experimental evidence suggest α-Syn aggregation can be triggered by lipids. In this talk, I will show the advantage of atomic force microscope Infrared (AFM-IR) spectroscopy in structural characterization of individual α-Syn oligomers grown in the presence of two different phospholipids vesicles. AFM-IR is a modern optical nanoscopy technique that has single-molecule sensitivity and sub-diffraction spatial resolution. Our results show that α-Syn oligomers grown in the presence of phosphatidylcholine have distinctly different structure than oligomers grown in the presence on phosphatidylserine. We infer that this occurs because of specific charges adopted by lipids, which in turn governs protein aggregation. We also found that protein to phospholipid ratio makes a substantial impact on the structure of α-Syn oligomers. These findings demonstrate that α-Syn is far more complex than expected from the perspective of structural organization of oligomeric species.

(IR-03.5) **Nano is not just smaller – Multimodal chemical imaging at nanoscale spatial resolution**

Catarina Santos[1], Ufuk Yilmaz[1], Bernhard Lendl[1], Georg Ramer[1]; [1]*TU Wien*

In fifteen words or less, explain the significance of this contribution (Novel Aspect).: Making established chemometric techniques compatible with nanoscale spatial resolution mid-infrared hyper spectral imaging

Abstract Text: First invented a bit over a decade ago [1], nanoscale spatial resolution chemical imaging techniques based on reading out mid-infrared (MIR) absorption with a scanning probe tip have now matured into a continuously growing field with applications ranging from sub-cellular imaging, to single molecule spectroscopy and MIR spectroscopy mainstays like polymer analysis. While initial demonstrations of the technique in a new field typically restrict themselves to few single point spectra and images as select wavelengths, there is now a growing number of works applying techniques established for macroscopic and microscopic MIR hyper spectral imaging for nanoscale spatial resolution MIR spectral maps [2,3] . This, however, brings with it several challenges: 1. It is virtually impossible to position an scanning probe tip at nanoscale precision for the duration of a whole hyper spectral image 2. Calibration of supervised methods is hard as creating calibration samples of known composition and homogeneity is non-trivial 3. Few chemically selective techniques achieve nanoscale resolution precluding us also to use such data for calibration 4. Our signal is "just" proportional to absorption: proportionality constants are not only instrument and sample specific but also depend on the location of the tip and the shape of the sample. We present protocols and an open tool kit that enable us to not only reliably generate hyper spectral images in presence of sample drifts but also offer the possibility of training supervised algorithms on nanoscale resolution data. We apply this approach to image intracellular protein distribution in microorganisms and demonstrate a multi-step hyper spectral imaging procedure to acquire chemical images of industrially relevant polymers at nanometre spatial resolution.
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21LIBS03: LIBS Analytical Applications I

Chair: Christian Goueguel
On-site Chair: François Doucet

(LIBS-03.1) **High-Resolution Elemental Bioimaging Employing Hyphenated Mass Spectrometry**

Mikhail Belov[1], Christoph Wehe, Lothar Rottmann; [1]*Thermo Fisher Scientific*

Laser Ablation – Inductively Coupled Plasma – Mass Spectrometry (LA-ICP-MS) has been increasingly employed in a variety of applications, including biomedical, environmental and geological studies. In this work, we report on a novel elemental imaging platform based on an OrbitrapTM mass analyzer, which provides unsurpassed mass resolution and mass accuracy along with panoramic mass spectral data. The instrument is compatible with both liquid sample and laser ablation ionization techniques. The UV laser at 213 nm has been employed for laser ablation of tissue samples. Upon extraction from plasma, atomized ion species were directed to an analytical quadrupole for optional ion preselection and then either diverted to a secondary electron multiplier (SEM) or further transmitted to the higher-energy collision cell of the Orbitrap mass spectrometer. The performance of the novel platform was initially characterized with acid aqueous solutions containing a variety of analytes (up to 35 elements in the m/z range of 20 to 238) in the concentration range of several ppm down to several ppt. The signal response was found to be linear in the concentration range over 4 orders of magnitude, exhibiting a limit of detection of 2-4 ppt. The instrument was shown to exhibit sub-ppm mass measurement accuracy at a mass resolution exceeding 1,000,000. The closely spaced isobaric species, e.g. strontium (87Sr) and rubidium (87Rb), were baseline resolved. Laser ablation imaging experiments were performed with a number of biological tissue samples. Laser ablation events have been synchronized with ion trapping in the higher-energy collision cell (HCD) that resulted in higher sensitivity analysis at scan rates of up to 10 Hz. Importantly, data were acquired as panoramic mass spectra that enabled concurrent real-time monitoring of all species emanating from each ablated pixel at once. Spatial distributions of different metals, including iron, copper, zinc, strontium etc. in e.g. mouse embryo samples at a spatial resolution of 1-10 µm have been reconstructed and registered against optical images. Our data indicate that the developed approach for high-speed, high-spatial and mass resolution, as well as high-mass-accuracy multi-elemental imaging is poised to become an important analytical tool for practical biomedical applications.

(LIBS-03.2) **Laser-Induced Breakdown Spectroscopy (LIBS) for Liquids**

Chet R. Bhatt[1], Daniel Hartzler, Dustin L. McIntyre[2]; [1]*Leidos - National Energy Technology Laboratory,* [2]*US National Energy Technology Laboratory*

This presentation will focus on the investigation of LIBS for subterranean measurements, monitoring of EPA-concerned species in flue gas desulphurization (FGD) and acid mine drainage (AMD) waste waters, and alternative source characterization for critical elements. While subsurface-level geological storage sites are being considered as a potential permanent host for sequestered CO2, there are some risks associated with it. Therefore, there is a need for robust and sensitive early detection methods for ensuring that there is no containment failure, either by ingress of the CO2 or by changes in pressure induced by the injection. LIBS is a good fit for detecting specific (proxy) elements that signal leakage of the CO2. To demonstrate the concept, dissolution of select carbonates in underwater high-pressure CO2 environment was investigated by LIBS. Behavior of plasma emission and carbonate dissolution with elevated CO2 pressure was studied, and the amount of metals released from the carbonates were calculated as a function of CO2 pressure. Hence, LIBS appears to be suitable for real-time monitoring of the subsurface concentrations of proxy elements, which can provide an indirect detection system for CO2 leakage from geological carbon storage sites. To detect and

measure toxic species in wastewaters, single pulse and double pulse LIBS experiments were performed on liquid samples containing As, Hg, S, and Se as test analytes. Detection of these elements using a submerged laser plasma proved difficult. Therefore, the laser was focused on the surface of a liquid jet so that plasma expansion and emission occur in air; hence, the plasma emission efficiency increases. Underwater LIBS was also evaluated for subsurface aqueous source characterization for rare earth elements. Apart from performing the measurements in the laboratory with a bench top set up, a miniaturized LIBS probe has been developed for field testing. It consists of a passive Q-switch microchip laser that is affixed to a custom-machined frame. The entire assembly is less than 20 cm in length and 5 cm in diameter, with all the optics enclosed inside a pressure-resistant enclosure capable of being submerged in up to 30 m of water.

(LIBS-03.3) **LIBS and Laser Ablation-Spark Discharge-OES: Chemical Imaging and Element Analysis**
Stefan Grünberger[1], Simon Eschlböck-Fuchs[2], Josef Hofstadler[2], Andreas Pissenberger[2], Hubert Duchaczek[2], Stefan Trautner[1], Johannes D. Pedarnig[1]; *[1]Johannes Kepler University Linz, Institute of Applied Physics, [2]voestalpine Stahl GmbH*

In fifteen words or less, explain the significance of this contribution (Novel Aspect).: Promising element analysis method with additional spectral lines in comparison to conventional spark-OES and LIBS

Abstract Text: We compare two Optical Emission Spectroscopy (OES) techniques by chemical imaging and element analysis; Laser-Induced Breakdown Spectroscopy (LIBS) and Laser Ablation-Spark Discharge-OES (LA-SD-OES). The method LA-SD-OES combines LIBS with electric spark discharge (spark-OES), the last is routinely used in the steel industry. A high voltage (2.5 kV) discharge is triggered by a low energy laser pulse applied to the sample. Differences and advantages of the individual methods to the combination are reported. The spark discharge is guided to the laser spot on the sample surface. Hence, a good spatial resolution with the electric spark is achieved. The optical emission exhibits a higher intensity ratio of singly ionized to neutral spectral lines in LA-SD-OES as compared to LIBS. Moreover, doubly ionized spectral lines not visible with LIBS under usual conditions are observed [1]. The appearance of such lines indicates that the mechanisms of plasma excitation are different in LA-SD-OES and LIBS. A higher sensitivity for minor elements in steel is obtained with LA-SD-OES when singly ionized lines are detected [2]. The poor localization in spark-OES is overcome and spectrochemical imaging with good spatial resolution can be performed with LA-SD-OES.
Acknowledgements Financial support by the Austrian Research Promotion Agency FFG is gratefully acknowledged (K-project PSSP 871974). References [1] Stefan Grünberger, et al, Chemical imaging with Laser Ablation - Spark Discharge - Optical Emission Spectroscopy (LA-SD-OES) and Laser-Induced Breakdown Spectroscopy (LIBS), Optics and Laser Technology 123 (2020) 105944. https://doi.org/10.1016/j.optlastec.2019.105944 [2] Stefan Grünberger, et al, Analysis of minor elements in steel and chemical imaging of micro-patterned polymer by Laser ablation-spark discharge-optical emission spectroscopy (LA-SD-OES) and Laser-induced breakdown spectroscopy (LIBS), Spectrochimica Acta Part B 169 (2020) 105884. https://doi.org/10.1016/j.sab.2020.105884

(LIBS-03.4) **Forensic Discrimination of Copper Metal by Laser Induced Breakdown Spectroscopy (LIBS)**
Chase Notari[1], Brooke W. Kammrath[2]; *[1]University of New Haven, [2]Henry C. Lee College of Criminal Justice and Forensic Sciences, University of New Haven*

In fifteen words or less, explain the significance of this contribution (Novel Aspect).: To evaluate LIBS for its ability to discriminate the copper jackets of different bullets.

Abstract Text: Copper metal has great potential as forensic evidence due to its presence in a range of cases from thefts of copper wiring and pipes, the use of copper wiring in IEDs, and its common function as bullet

jackets. Excellent discrimination of copper metal has been demonstrated through trace element profiles collected using solution-based ICP-MS. Although ICP-MS has many advantages for elemental analysis, including its low detection limits, high accuracy and excellent precision, alternative methods that are faster, require less (or no) sample preparation, and require smaller sample sizes are being investigated for a range of forensic samples (e.g., glass, polymers, paint, tape, geological materials, etc.). LIBS is an analytical technique that has gained prominence as a valuable tool for elemental profiling, and continues to grow in acceptance in numerous industries. LIBS is an advantageous method due to the fact that it is rapid, requires no sample preparation, is able to simultaneously provide information on multiple elements at once, and is less expensive than other instruments used for elemental analysis. LIBS has proven value for the analysis of glass, paint, soil, ink, and other samples of forensic interest. The purpose of this research was to evaluate LIBS to determine if it has the ability to perform comparative analysis of copper, specifically the jacketed metal on different bullets. This study first explored the detection capabilities of LIBS, determined an appropriate element menu, and outlined the optimal parameters for LIBS such as laser pulse energy, spot size, pattern size, gate delay, number of pulses per spot, and repetition rate using a copper density block. These optimal parameters were then applied to the analysis of the copper used in jacketed bullets, and the discrimination ability was explored using multivariate statistical methods. The ability of LIBS to perform comparative elemental analysis on copper-jacketed bullets has the potential to provide a novel method for forensic scientists to use in comparing ballistic evidence. The results of this research can be extended to other sources of copper, such as pipes and wiring, thus expanding the utility of LIBS instrumentation in forensic laboratories to alternative evidence items.

21RAM07: Industrial Raman

Chair: Ian Lewis
On-site Chair: Tim Prusnick

(RAM-07.1) **Residence time distribution in the feed frame of a tablet press for different process parameters using Raman spectroscopy**

Shaun J. Fraser[1], Andres Roman-Ospino[2], James Scicolone[2], Yukteshwar Baranwal[2]; *[1]Tornado Spectral Systems, [2]Rutgers*

In fifteen words or less, explain the significance of this contribution (Novel Aspect).: Robust feed frame blend composition prediction as low as 1.5% w/w collected over 10 days

Abstract Text: Residence time distribution has become an essential parameter to characterize pharmaceutical manufacturing equipment in continuous manufacturing. This technology has been implemented in several production lines worldwide, and characterization under potential process parameters still needs to be evaluated and applied for a predictive control strategy. Over the last few years, the feed frame of the tablet press has been demonstrated to be a feasible sampling point for blend uniformity. In this work, a Raman probe was integrated to detect low amounts of a tracer, changing the throughput, feed frame paddles speed, and the height of the powder bed in the chute connecting the blender with the tablet press. Three levels for each process parameter were considered, and the tracer was predicted utilizing a calibration model developed that accounted for the physical variables for four levels of composition. Raman spectra of the twenty-one combinations of manufacturing conditions were measured using the Tornado HyperFlux Pro Plus™ Raman system and its High-Throughput Virtual Slit™ to provide a 10x to 30x increase in the signal-to-noise ratio over other conventional spectrometers. The Partial Least Squares calibration model with three latent variables demonstrated an excellent performance predicting blend compositions as low as 1.5 % w/w in the feed frame. Results indicate the robustness of the Tornado Raman system executing an extensive evaluation of a continuous manufacturing line sustaining the instrument calibration and performance over the ten days of data acquisition.

(RAM-07.2) **Rapid Screening of Alcohol-Based Hand Sanitizers Using Spatially Offset Raman Spectroscopy**

Huzeyfe Yilmaz[1], Nirzari Gupta[1], Jason D. Rodriguez[1]; [1]*US Food and Drug Administration*

In fifteen words or less, explain the significance of this contribution (Novel Aspect).: This work utilizes spatially-offset Raman and nonlinear machine learning to demonstrate container-universal quantification.

Abstract Text: Rapid screening of medical countermeasures is essential for ensuring a safe and high-quality supply chain in the case of a public health threat. During the COVID-19 pandemic, hygiene supplies such as hand sanitizers have been utilized to prevent transmission from surfaces. To ensure that hand sanitizer products are not adulterated, highly contaminated or sub-potent, spectroscopic screening can be employed. Here, semi-quantitative and quantitative spectroscopic methods were developed for through-container screening of hand sanitizers. Based on spatially offset Raman spectroscopy (SORS) and Pearson and first derivative correlations, semi-quantitative assessments were made to identify out-of-specification or contaminated hand sanitizer products. Pass/fail tests were performed on over 100 products, seven of which were identified as out of specification using the semi-quantitative SORS method and prioritized for additional testing. In addition, a container-universal method was developed to quantify active ingredients ethanol and isopropanol, as well as common contaminants/adulterants methanol and 1-propanol in hand sanitizer products. The quantitative SORS method was assisted with nonlinear machine learning algorithms (support vector regression-SVR) to enable through-container quantification. More than 100 commercial and in-house products in various types of containers were identified and quantified using SORS-SVR. Alcohols in hand sanitizer formulations were quantified with high accuracy (R^2>0.99) using SVR and more than 95% of the substandard test samples were identified accurately.

(RAM-07.3) **Portable Raman Spectroscopy for Screening of Plasticizers in Food Contact Production Line Tubing**

Josh Moskowitz[1], Katherine Carlos[2], Luke Lindahl-Ackerman[2], kristen Reese[2], Eric Crump[2], Timothy Begley[2], Betsy Jean Yakes[2]; [1]*University of Maryland, Joint Institute for Food Safety and Applied Nutrition, 2134 Patapsco Building, College Park, MD 20742,* [2]*U.S. Food and Drug Administration, Center for Food Safety and Applied Nutrition, 5001 Campus Drive, College Park, MD 20740*

In fifteen words or less, explain the significance of this contribution (Novel Aspect).: Portable identification of ortho-phthalate plasticizers in food contact materials can help to ensure food safety.

Abstract Text: Plasticizers are used to alter physical properties such as softness and flexibility in a wide range of materials and may be present in everyday items such as personal care products, packaging, toys, paints, and adhesives. They are also used to increase the flexibility of milk extraction and production line tubing, liners, and gaskets. It has been shown that the ortho-phthalates class of plasticizers are fat-soluble, opening potential for their leaching and dissolution in fatty food products such as milk, which has led to further investigations on the use of these in food contact materials. Portable detection of ortho-phthalates in food contact materials is important, as it alleviates the need for high cost and time-consuming laboratory-based testing, while allowing for rapid, onsite detection of plasticizers. In this work we evaluate two commercially available portable Raman spectrometers for their ability to accurately classify the ortho-phthalate group of plasticizers in polyvinyl chloride (PVC) food contact production line tubing. Further, we aim to identify individual plasticizer compounds in the PVC tubing from a panel of multiple components. This is accomplished through both library matching and chemometric modelling of the Raman spectra. These library and chemometric methods function via the comparison of Raman spectra between unknown food contact tubing samples and a panel of previously scanned, known tubing samples. In both known and unknown tubing, the samples are also analyzed via GC-MS and DART-MS to confirm the plasticizer identity and evaluate the accuracy of the Raman spectroscopy

methods. This work may provide information regarding the prevalence of various plasticizers currently used in food contact, dairy production line tubing. The results from the spectrometers investigated in this work show that the technologies hold potential as a valuable plasticizer screening tool for FDA and for the food industry.

(RAM-07.4) **Paving the way for Raman spectroscopic analysis of heterogeneous foods**

Nils K. Afseth[1], Petter V. Andersen[1], Olga Monago-Maraña[2], Sileshi G. Wubshet[1], Jens P. Wold[1]; *[1]Nofima, [2]National Distance Education University*

In fifteen words or less, explain the significance of this contribution (Novel Aspect).: Novel use of Raman spectroscopy for assessment of quality features of heterogeneous foods

Abstract Text: The unique potential of Raman spectroscopy in food analysis is related to the ability to capture subtle chemical distinctions in foods in a non-destructive way. However, food matrices are generally heterogeneous, and the lack of appropriate tools for addressing sample heterogeneity has been one of the main challenges preventing the widespread use of this technique in food analysis. One of the milestones in representative Raman analysis has been the development of wide area Raman probes. Here, a defocused laser beam is used to illuminate a larger area within the sample, and multiple collection fibers covering the same sample area are used for photon collection. The technique has found a range of applications within pharmaceutical, biomedical and chemical analysis, but surprisingly, wide area Raman has not been frequently used in food analysis. We have shown how wide area Raman can be used to provide reliable quantitative information from samples that traditionally have been regarded as challenging in a Raman setting, from whole salmon and meat to chicken carcasses and salmon by-products. Recently, we have shown that quantification of collagen contents in meat samples can be achieved. The collagen contents could be quantified independent of the overall protein contents, which is a true validation of the approach. In another recent study we have used Raman for quantification of individual sugars in apples. In this study, good quantitative models for individual sugars in both intact and peeled apples could be obtained. Finally, we have shown that Raman also can be used to assess bulk composition of very heterogeneous foods, and the obtained quantitative models were clearly comparable to the respective models obtained using near-infrared spectroscopy. All of this could be achieved by benefiting from the key features of wide area Raman probes, including sufficient depth penetration and surface coverage. Also, with appropriate sampling, focusing issues will have minor impact on the resulting spectra. All in all, reliable Raman sampling could be expected to have great impact in the field of Raman food analysis in the years to come and thus pave the way for designated at-line and in-line Raman solutions.

(RAM-07.5) **Analysis of Biofuel Blends using Long-Wavelength Raman Concatenation**

Greg W. Charache[1], jun zhao[2], Kristen Frano[2], Christopher Kautz[2], Bharath Molakala[1], Igor Nosov[1], Aaron Cohn[1], Nancy Morris[1], Jack Zhou[2]; *[1]Innovative Photonic Solutions, [2]B&W Tek, LLC, A Metrohm Group Company*

In fifteen words or less, explain the significance of this contribution (Novel Aspect).: A new dual-wavelength (860/1064 nm) Raman Spectrometer was developed to mitigate sample fluorescence

Abstract Text: Long-wavelength (860 nm / 1064 nm) Raman concatenation is utilized to analyze and discriminate various biofuel blends in order to eliminate sample fluorescence and enhance quantitative analysis capability. In general, many Raman measurements suffer from fluorescence, which forces the use of longer excitation wavelength (lower photon energy) lasers to prevent the fluorescence signal overwhelming the Raman signal. The first longer wavelength laser is selected to avoid fluorescence in the sample and to probe the "fingerprint" region of the spectrum. For the case of a 1064 nm wavelength-stabilized multimode excitation

laser, 0-2000 cm-1 corresponds to a single-grating-spectrometer detection wavelength of 1064 nm to 1350 nm. The second shorter wavelength laser is selected to probe the "stretch" region of the spectrum using the same spectrometer detection wavelengths. In this example, an 860 nm wavelength-stabilized multimode excitation laser corresponds to a 2000-4000 cm-1 Raman shift. The Raman probe filters are selected to allow excitation and collection at both wavelengths with a throughput > 85%. In operation, each portion of the Raman spectra is collected sequentially and then the composite spectrum is "concatenated" or stitched together. Biodiesel fuels represent an alternative energy source that could potentially replace or supplement fossil fuels. It is shown that the biodiesel content of these fuels can be discriminated using this rapid, non-contact analysis technique. As pointed out previously, the C-H stretch bonds (2,800 – 3,000 cm-1) in these biofuels represent the best region of the spectrum for quantitative analysis for sample discrimination for this application; while use of Raman concatenation in the stretch region has demonstrated ~ 10X improvement in signal-to-noise ratio.

(RAM-07.6) **Understanding the Anomalous Surface Enhanced Raman Scattering Signature of Tryptophan Arising from Plasmon Driven Electron Transfer**

Chelsea Zoltowski[1], Zachary Schultz[1]; [1]*The Ohio State University*

In fifteen words or less, explain the significance of this contribution (Novel Aspect).: A plasmon driven electron transfer process can explain distinct changes often observed in SERS signals.

Abstract Text: Surface enhanced Raman spectroscopy (SERS) has become a popular analytical technique for the detection and quantification of biological samples such as proteins, due to its molecular specificity, non-destructive nature, and minimal water background. SERS probes the same vibrational modes as Raman scattering, which are specific to the molecular structure of an analyte. Although Raman scattering often occurs with a lower probability, the addition of noble metal nanostructures to the analyte allows SERS to enhance this signal. This enhancement is the result of two contributing mechanisms. Much of the enhancement has been well established to result from the increase in the local electric field when there is excitation of the localized surface plasmon resonance (LSPR) of the metal nanostructure. This is known as the electromagnetic enhancement mechanism (EM). The chemical enhancement mechanism (CM), on the other hand, results from an interaction created between the analyte of interest and the metal nanostructure, typically as a charge transfer complex. Yet, distinct changes in the SERS spectrum compared to the normal Raman spectrum are not currently fully understood. We have shown the essential amino acid tryptophan (Trp) exhibits a unique SERS signature vastly different from the normal Raman signal that cannot be fully explained by any current enhancement mechanism alone. Instead, it appears that this signal is the result of a resonantly enhanced radical anion species. This radical anion forms from transfer of an electron from the gold nanoparticle, excited by the LSPR, to the Trp species creating a stable radical species that has a characteristic electronic excitation near our laser excitation, creating an additional resonance enhancement. The combination of density functional theory (DFT) calculations and experiments support the formation of a stable anion radical Trp species. We have also investigated the pH dependence of this electron transfer process. Understanding of this mechanism will allow for specific peak assignments of the selectively enhanced Raman bands, which can be utilized for measurements of environmental parameters to which plasmon induced electron transfer is sensitive as well as improved spectroscopic assignments of proteins containing Trp residues.

21RAM12: Applications of SERS II

Chair: Derek Guenther
On-site Chair: Derek Guenther

(RAM-12.1) **SERS Detection of Bacterial Biomarkers Using Paper Based Nanosensor**

Waleed Hassanain[1], Julia Spoors[2], Christopher Johnson[2], Karen Faulds[1], Neil Keegan[2], Duncan Graham[1];
[1]*University of Strathclyde,* [2]*Newcastle University*

In fifteen words or less, explain the significance of this contribution (Novel Aspect).: Novel diagnostic test for clostridium difficile bacterial infection using duplex SERS-based lateral flow assay

Abstract Text: Clostridium difficile (C.diff) bacterial infection is one of the most contagious diseases associated with high morbidity and mortality rates in hospitalised patients. Despite the availability of therapies, treatment failure and recurrence are common. Accurate diagnosis can slow its spread by determining the most effective treatment. Currently, there are some commercially available lateral flow tests that have been introduced to the diagnostic market for the simultaneous testing of glutamate dehydrogenase enzyme and toxins A/B, as C.diff biomarkers. Although these tests allow the rapid clinical diagnosis of C.diff infection (CDI), they still have limitations, in particular, the selectivity, sensitivity and quantification capacity for the results. In 2018, the surface layer protein A (SlpA) biomarker was first reported by our group as a new and unique species-specific identification tool for CDI that presents in all C.diff strains sequenced to-date. We have also reported the successful generation of SlpA antibodies, that displayed negligible cross-reactivity to other closely related species. Building on this success, herein we present a novel proof-of-concept duplex surface enhanced Raman scattering-based lateral flow assay (SERS-based LFA) for CDI diagnosis via the ultra-sensitive quantitative detection of its biomarkers, SlpA and toxin B, down to 0.01 pg/μL within 20 minutes. The simultaneous novel detection of toxin B and SlpA instead of glutamate dehydrogenase can indicate the presence of CDI and its toxigenicity without any cross-reactivity from other species, which is a potential risk with glutamate dehydrogenase assays. Therefore, this novel SERS-based LFA platform can overcome the drawbacks of the conventional lateral flow tests for CDI, in terms of sensitivity, selectivity and quantification capability. Additionally, the integration of a handheld Raman spectrometer with this duplex lateral flow test, paves the way to move the C.diff diagnostic test from localised laboratories to points of care application. Accordingly, we believe this novel duplex test can be used in the future as an alternative diagnostic test for CDI after large scale population wide-testing study.

(RAM-12.2) **Enhancing Spectral Repeatability and Limits of Detection Using Liquid Phase Nanoparticles for Quantitative Raman**

Derek A. Guenther[1]; [1]*Ocean Insight*

In fifteen words or less, explain the significance of this contribution (Novel Aspect).: High repeatability of liquid-phase SERS allows for greater statistical confidence and lower limits of detection.

Abstract Text: As the global pandemic has fueled the need for new rapid detection methods, much of that focus has been on optical techniques for their speed and portability. One detection method that has seen much growth and commercial adoption over the last decade is SERS, or Surface Enhanced Raman Spectroscopy. While these consumable substrates are offered in a variety of flavors and form factors from many suppliers, one inherent limitation to all is their repeatability within and between production batches. A separate approach is presented here which leaves the enhancing nanoparticles in aqueous suspension, and allows the analyte interaction and Raman enhancement to occur in the liquid phase. These LSERS (Liquid SERS) form factors can be provided in gold, silver, and other metals, and offer remarkable repeatability both from the background signal and the subsequent analyte emission. By introducing a low-concentration analyte to a small vial of 2mL nanoparticle suspension, meaningful Raman emissions are quickly seen down to the part-per-billion level or below. In this study the common antifungal dye and histological stain crystal violet is investigated as dissolved in water at a range of concentrations from 100ppm to 1ppm. This was introduced to 2mL of gold nanoparticles (≈40nm) in aqueous suspension, and interrogated using a 785nm Raman laser and HDX-RAMAN spectrometer from Ocean Insight. In addition to high repeatability of background and analyte scans, other benefits include

better quantification due to more uniform distribution, and the ability to run at maximum laser power without risk of burning the sample. These combined benefits allow more statistically significant Raman acquisitions at the lowest analyte concentrations, and can be further enhanced with the use of dilute acid or salt solution to promote nanoparticle aggregation at the time of measurement. We present these findings as an improvement to the solid SERS substrates that have become common in the world of Raman applications.

(RAM-12.3) **Fabrication of low-cost SERS sensors and development of signal processing techniques to improve the detection of nitrite**

Robert B. Chevalier[1], Brian S. Sheetz[1], James T. Hagan[1], Jason R. Dwyer[1]; [1]*University of Rhode Island*

In fifteen words or less, explain the significance of this contribution (Novel Aspect).: Straightforward fabrication and signal processing techniques to improve the detection of nitrite in field measurements

Abstract Text: Nitrites are a well-known contaminant found in industrial processes, wastewater treatments, and agricultural runoffs. In seawater, natural and anthropogenic activities can lead to a change in nitrite levels which can provide important information when assessing coastal ecosystems. Working in the Ocean State, the detection and monitoring of nitrites has become a central goal for our lab group. Presently, nitrite test kits using the colorimetric Griess reaction and Flow Injection Analysis (FIA) systems are available for the detection of nitrites in the field and on a lab benchtop. We are exploring the use of a buoy-deployable method, capable of SERS sensing for real-time nitrite monitoring in marine environments. Along with SERS' high sensitivity capabilities, technological advances have made portable spectrometers possible, making it a viable option for field measurements. We will present several advances from our work focusing on the fabrication of solid-state and solution-based SERS sensors, sensor and signal processing, and surface functionalization of SERS sensors for the selective detection of nitrite.

(RAM-12.4) **Direct and Water-mediated Adsorption of Stabilizers on SERS-active Colloidal Bimetallic Plasmonic Nanomaterials: Insight on Citrate-AuAg Interactions from DFT Calculations**

Chiara Deriu[1], Alexander N. Morozov[1], Alexander M. Mebel[1]; [1]*Florida International University*

In fifteen words or less, explain the significance of this contribution (Novel Aspect).: DFT calculations of citrate-AuAg complexes highlight significance of water in the adsorption process.

Abstract Text: The amplification of the signal observed in Surface Enhanced Raman Spectroscopy (SERS) is the result of a near field effect sustained by plasmonic nanostructures. For this reason, the intensity of a SERS spectrum strongly depends on the distance at which the analyte is able to reside on the plasmonic surface, with direct adsorption to the surface being the optimal condition. Consequently, when developing colloidal nanostructures for SERS use, it is of paramount importance to consider their capping system (i.e., stabilizers), and to tailor it to the desired application. In previous studies, a library of differently capped AuAg (18:1) colloidal nanostar formulations was developed, with the two-fold aim of producing a set of SERS substrates tailored to forensic analysis, and of investigating the nature of the capping process itself. Findings demonstrated the nanoparticle metals are alloyed and neutral, and stabilizers cap the nanostructures via specific adsorption. The present study utilizes citrate as the model stabilizer and investigates the mechanistic aspects of its interaction with the bimetallic surface by Density Functional Theory (DFT) calculations. The bimetallic nanostars were modeled as a Au19Ag neutral cluster, while citrate was modeled in its doubly deprotonated form according to the pH of the colloid, and surrounded by a first solvation shell composed of 12 water molecules and one sodium cation. A population of stable cluster-citrate structures was obtained, and energies were refined at the B3LYP/LANL2TZ(f) (Au, Ag) and cc-pVTZ (H, C, O, Na) level of theory. Solvation was accounted for both explicitly (vide supra) and by applying the continuum solvent model SMD. Our results indicate that both

direct binding and binding by proxy through charge-transfer complex formation with water are thermodynamically favorable. Water participation in citrate adsorption is supported by the positive cooperative adsorption behavior observed experimentally for this colloid, as well as by the comparison between experimental IR and DFT-simulated spectra. Indeed, the analysis of vibrational modes suggests possible presence of water within a crystal in dried nanostar residues. All ΔG of adsorption in solution indicate a weak chemisorptive process, leading to the hypothesis that citrate could be displaced by analytes during SERS measurements.

21SPSJ03: DUV Spectroscopy

Chair: Igor Lednev
On-site Chair: Igor Lednev

(SPSJ-03.1) **Deep UV Raman for biomedical applications**

Juergen Popp[1], Anja Silge, Petra Rösch[2]; [1]*Leibniz Institute of Photonic Technology,* [2]*Institute of Physical Chemistry and Abbe Center of Photonics (IPC), Friedrich-Schiller-University Jena, Helmholtzweg 4, 07743 Jena, Germany; Leibniz Institute of Photonic Technology (Leibniz-IPHT), Albert-Einstein-Straße 9, 07745 Jena, Germany; Research Campus Infectognostics e.v., Philosophenweg 7, 07743 Jena, Germany*

Raman spectroscopy has become one of the most researched analytical methods in the last decade. Raman spectra depict molecular vibrations that are characteristic of the molecular system under investigation and can be seen as a kind of "molecular fingerprint". The major challenge in the application of Raman spectroscopy to investigate biomedical samples is the possible occurrence of an interfering fluorescence background and the low signal yields compared to, for example, fluorescence spectroscopy. Here, the utilization of electronically resonant excitation wavelengths in the UV region can be beneficial to overcome these limitations. The application of Raman excitation wavelengths in the deep UV marks a promising approach exhibiting several advantages: (I) higher scattering efficiency compared to VIS-IR Raman excitation wavelengths, (II) electronic resonance effects increasing the intrinsically weak Raman signal thus improving the S/N ratio of the detected Raman signals, (III) spectral separation of Raman and fluorescence signals. Within this presentation we report about some of our recent results highlighting the unique potential of deep UV Raman for biophotonic applications. We will show that UV-Raman spectroscopy in combination with innovative chemometrics allows for fast identification of fungal spores or clinically relevant Candida species and can be a potential alternative to currently used time-consuming cultivation procedures. The bacteria-induced mushroom diseases were investigated as a general model for infection processes. In situ molecular analysis of host-microbe interactions could be advanced by UV Raman spectroscopy. The resonance enhancement enables a unique chemical contrast at the bacterial-fungal interface and allowed the detection and identification of antifungal agents and toxins. In addition, UV resonance Raman spectroscopy (UVRR) on sensitive and resistant bacteria of one species can be used to get a fast pre-screening of potentially harmful strains. The application of different stable isotopes in various physiological or medical relevant components in combination with 2D correlation can be utilized to gain more information about metabolic pathways. Here UVRR spectroscopy can be used as complimentary information to the excitation in the visible ore infrared spectral region. Furthermore, we will introduce UVRR as an analytical tool for ultrasensitive analysis of antibiotics.

(SPSJ-03.2) **Pushing DUV Raman microscopy to its limits: toward nanoscale resolution**

Atsushi Taguchi[1]; [1]*Hokkaido University*

Deep UV (DUV) light has high energy and short wavelength, offering a unique opportunity to study electronic and vibrational properties of electronically resonant materials with high spatial resolution. However, the spatial resolution of 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 DUV wavelength region. Breaking the technical barrier

of spatial resolution in DUV imaging system will bring the DUV microscopy to the next level that can be used as a highly sensitive analytical tool of nanomaterials, biomolecules, and semiconductors with extremely high spatial resolution. Here, I discuss recent progress of DUV Raman microscopy in view of spatial resolution. A classical and straightforward approach to gain high resolution is to increase numerical aperture (NA) of DUV objective lens. We have recently proposed 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 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 plasmonic nano-tip that acts as nano-antenna to confine DUV light. We have demonstrated plasmonic-tip-enhancement effect at a wavelength of 266 nm by observing resonance Raman scattering of adenine nanocrystal near aluminum tip [2,3]. References: [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-03.3) **Stability of nucleic acids in Deep Eutectic Solvents as revealed by Synchrotron UV Resonance Raman spectroscopy**

Barbara Rossi[1], Mariagrazia Tortora[2], Jacopo Vigna, Ines Mancini, Andrea Mele, Alessandro Gessini[1], Claudio Masciovecchio[1]; [1]*Elettra-Sincrotrone Trieste,* [2]*AREA SCIENCE PARK, Elettra-Sincrotrone Trieste*

The predicted capability of hydrated deep eutectic solvents (DES) for stabilizing and preserving for a long time the native structure of DNA is getting growing attention for biotechnological and biomedical applications in the near future. This property of DES as novel co-solvent in alternative to water medium could be particular relevant for the DNA storage and handling, thus favoring the further expanding of the use of DNA in nanotechnology. The deep understanding of the delicate balance between stabilizing and destabilizing effects of the DES-mediated interactions with the structure of DNA is crucial for developing new-generation DES to be used as solvents for biomolecules. In this work, we report a fundamental investigation on the effect exerted by choline-based DES on the thermal structural stability of large nucleic acid molecules. The sensitivity and selectivity of synchrotron radiation UV Resonance Raman (SR-UVRR) spectroscopy is exploited for detecting heat-induced structural transitions of DNA localized on specific base-tracts. Our study unveils the establishment of preferential H-bonds interactions between specific DES moieties and the guanine and adenine bases in the DNA groove that lead to a more effective stacking between of these bases even at high temperature values. The results of SR-UVRR investigation contribute to a more comprehensive view of the DES-mediated interactions with specific tracts of DNA sequence that could facilitate the designing of effective stabilizing DES for their exploitation in biomedical and life science field.

(SPSJ-03.4) **Recent Advances in Instrumentation for Deep UV Resonance Raman Explosive Detection**

Sergei V. Bykov[1], Ryan Roppel, Sanford A. Asher[1]; [1]*University of Pittsburgh*

We report on the recent development of state-of-the-art deep UV Raman instrumentation for the detection of energetic molecules. One of the major barriers for the widespread adoption of UVRR spectroscopy is the lack of inexpensive, compact, lightweight, energy efficient and easy-to-use deep ultraviolet (DUV) lasers. In collaboration with UVISIR Inc., we co developed second-generation, miniaturized, diode-pumped, solid-state Nd:YVO4 and Nd:GdVO4 lasers that generate 10-100 mW of 213 and 228 nm quasi-continuous wave DUV light as ns pulses at a tunable kHz repetition rate. We have also recently obtained a continuously wavelength-tunable, continuous wave, deep UV laser. Spectra-Physics and Sirah Lasertechnik pioneered the commercialization of this unique “ring-cavity” laser. This is the first CW DUV wavelength tunable (206 – 250+ nm) laser that avoids the non-linear optical responses that may affect pulsed laser UVRR measurements. We developed a new state-of-the-art, high-efficiency standoff DUV Raman spectrometer. This spectrometer uses a custom DUV telescope with a 200 mm primary mirror. This telescope is equipped with an electronically driven focus operating between 3 m to infinity. The UV Raman spectrograph utilizes high-efficiency custom DUV

optics. We utilized a novel spectrometer and unique laser light sources for trace detection of explosive materials at stand-off distances > 3 m.

(SPSJ-03.5) **DUV Raman spectroscopy for biomedical diagnostics**

Igor K. Lednev[1], Nicole Ralbovsky[2]; [1]*University at Albany SUNY,* [2]*Merck*

Deep ultraviolet resonance Raman spectroscopy (DUVRS) offers significant advantages over visible and near-infrared Raman spectroscopy for biological applications, including cancer identification. Cancer is the second-leading cause of death in the United States. Early diagnostics plays a crucial role in providing the best chances for an afflicted individual to seek successful treatment opportunities. Current methods for diagnosing various forms of cancer are both expensive and invasive. As such, the objective of this study was to explore the feasibility of DUVRS for discrimination of cancerous tissues and cancer cells from normal samples. Cancerous brain tissues from nonobese diabetic/severe combined immunodeficiency (NOD-SCID) model mice injected with 435-tdT cells (human adenocarcinoma breast cancer cells) at known locations and adjacent normal brain tissues as well as normal and cancer (adenocarcinoma PC-3) prostate cells were studied using DUVRS. The obtained Raman spectra of the healthy and cancerous samples are compared in order to identify biochemical differences between them. The obtained spectra reflect biochemical differences which occur between the healthy and malignant samples in both brain and prostate cancers. DUVRS provides distinctive resonance signatures of major biochemical components, including proteins and nucleic acids, and it does not suffer from fluorescence interference, nor does it require high laser power levels for excitation. These advantages allow for clear and effective spectral discrimination between samples. The safety issues of using ultraviolet light for human applications will be discussed analyzed.

21ATOM02: Single Cell and Nanoparticle ICP-MS

Chair: Heidi Goenaga-Infante
Co-Chair: John Olesik
On-site Chair: John Olesik

(ATOM-02.1) **Challenges Measuring the Number, Composition and Size of Nanoparticles and Microparticles using single particle ICP-MS and ICP-ToFMS**

John Olesik[1], Madeleine Lomax-Vogt[1], Gabrielli Paolo[1], Ryan Sullivan[2], Garret Bland[2], Luke Monroe[2]; [1]*Ohio State University,* [2]*Carnegie Mellon University*

Single particle ICP-MS (spICP-MS) can, under the right circumstances, accurately measure the number, composition, and size of nanoparticles and nanoparticles suspended in solutions. Matrix effects can affect the signal/femtogram in calibration solutions differently than in nanoparticle suspensions resulting in errors in the calibration of signal/fg. Complete vaporization of microparticles depends on their size and composition. In order to accurately determine the particle size, the density of each particle must be known or estimated. The calculated particle size is based on a spherical particle, even if the particle is not spherical. spICP-Quadrupole MS or spICP-Sector Field MS can provide element selective nanoparticle and microparticle analysis which may be sufficient for engineered nanoparticles but can only measure one (or maybe two) user selected elements per particle. A large particle containing small fraction of a particular element will produce the small signal as a smaller, pure particle. ICP-ToFMS can provide a complete ICP-mass spectrum for each particle. However, detection limits (the smallest number of femtograms of an element in a particle) may depend on the dissolved element concentrations. Therefore, if one element (Si, e.g.) has a high dissolved element concentration it's nanoparticle detection limit may be much larger than another element in the same particle that produces a similar signal/fg but has a lower dissolved concentration in the sample solution. That can complicate identification of some particles. Examples of these challenges will be presented, and their impact discussed. Despite these potential problems, spICP-ToFMS, is uniquely capable of measuring large numbers of suspended

nanoparticles and microparticles in a few minutes that provide a statistically representative sample of the population of particles in the suspension.

(ATOM-02.2) **The application of single cell ICP-MS in microbiology: a world to explore**

María Montes-Bayón[1], Roberto Álvarez-Fernández García[1], Paula Garcia-Cancela, Mario Corte-Rodriguez[1], Jörg Bettmer[1]; [1]*University of Oviedo*

Single-cell-ICP-MS analysis has been used for the monitoring of metal/metalloids incorporated in different types of unicellular systems of different origin including human, bacterial, yeast or even algae. In each one of these applications, parameters like cell size, morphology or stability, determined by the composition and structure of their cellular envelope, have an influence on their analysis, facilitating or hindering their transport into the plasma, where cellular integrity must be preserved. For instance, yeast cells, being eukaryotic unicellular microorganisms that belong to the fungi kingdom, have a lipid bilayer membrane and a second envelope, the cell wall. Such cell wall preserves morphology and stabilizes the internal osmotic conditions of the cell. This protects the cell against physical damage by providing significant mechanical resistance. Similarly, bacteria which are prokaryotic unicellular microorganisms that comprise a very heterogeneous domain of different morphologies, contain a cytoplasmatic membrane, cell wall and, in some cases, a second outer membrane providing them of mechanical resistance and morphological robustness. Both models are ideal for evaluating the performance of SC-ICP-MS. [1] In this presentation, the potential of SC-ICP-MS in microbiology will be illustrated by taking two significant examples: the presence of Se and Se-nanoparticles in Saccharomyces cerevisiae and the formation of endogenous Cu-nanoparticles in Streptomyces coelicolor. In both cases, the use of SC-ICP-MS as a biotechnological tool to evaluate the incorporation level of Se/Cu in the corresponding microorganisms, used with different purposes, as well as the evaluation on the formation of Se or Cu nanoparticles is included. [1]. M. Corte-Rodríguez, R. Alvarez-Fernández, P. García-Cancela, M. Montes-Bayón, J. Bettmer. Trends in Analytical Chemistry 132 (2020) 116042

(ATOM-02.3) **Automated Single-Particle-ICP-TOFMS for Quantification and Classification of Nanoparticles in Environmental Samples**

Alexander Gundlach-Graham[1], Richard Lancaster[1], Stasia Harycki[1], Sarah E. Szakas[1]; [1]*Iowa State University*

Single-particle Inductively Coupled Plasma Time-of-Flight Mass Spectrometry (sp-ICP-TOFMS) is rapidly becoming an established method for the analysis of mixtures of metal-containing (NPs) at low number concentrations ($< 10^6$ particles/mL). TOFMS has emerged as a method of choice because it provides fast and quasi-simultaneous complete mass spectral measurements. Full spectrum detection enables many types of NPs to be measured concurrently from a sample, which dramatically improves throughput. In addition, with TOFMS, we can measure multiple isotopes and multiple elements from individual NPs, which is essential for the discrimination and assignment of particle types that share major-element compositions. Altogether, sp-ICP-TOFMS presents a tool for rapid acquisition of large, multi-variant, sp-ICP-MS data sets. This presentation is about what we do with such multiplexed data. A major goal in sp-ICP-TOFMS work is to be able to catalog particle types—both natural and anthropogenic particles—and then to use our understandings of particle composition to separate and quantify anthropogenic NP pollution in real-world samples. I will discuss our work toward developing an automated sp-ICP-TOFMS system, which includes data acquisition, quantification, and interpretation. I will highlight recent work we've done toward improving algorithms to find particle-derived signals based on Monte Carlo modeling of TOF detection responses. After accurate particle finding and mass quantification, NP signals need to be assigned to classes within overall particle populations and identified as anthropogenic or natural in origin. In this regard, I will discuss our work toward building an inventory of elemental signatures found in particles from natural samples, and toward defining uncertainty metrics for NP class assignments. This critical analysis of NP classification by sp-ICP-TOFMS is the next step toward broader application of the approach for screening and quantification of anthropogenic NP inputs.

(ATOM-02.4) **Building A Robust Nanomaterial Measurement Infrastructure: Successes and Challenges**

Karen E. Murphy[1], Antonio R. Montoro Bustos[1], Monique Johnson[1]; [1]*NIST*

Accurate measurements at the nanoscale are critically needed to refine manufacturing processes, evaluate efficacy and ensure the responsible use of engineered nanomaterials. The ability to control and harness phenomena that occur on a dimensional scale below 100 nm has led to exponential growth in nanomedicine applications and steady growth in the areas of nanoelectronics, novel industrial materials and the use of nanomaterials in food packaging and nutrient delivery. Accurate and informative measurements are the outcome of a robust measurement infrastructure composed of transferable measurement methods, protocols, reference materials, documentary standards and interlaboratory studies. This talk will review the progress that has been made in the last decade and the challenges that remain, particularly with respect to reference material production. Our group has engaged in rigorous validation of single particle inductively coupled plasma mass spectrometry (spICP-MS) for the measurement of particle size and number concentration with efforts to establish traceability to the SI via comparative measurements with established methods, including TEM, SAXS and in particular, HR SEM. Contributions of the measured transport efficiency, calibration strategies and spICP-MS data acquisition artifacts to measurement trueness and precision are examined theoretically and experimentally. In addition, our group has engaged in studies using the nematode Caenorhabditis elegans as model organism for nanomaterial risk assessment. Both conventional ICP-MS and spICP-MS are used to quantify uptake of AuNP exposed nematodes with respect to numbers and size dependency of ingested particles. The use of orthogonal methods, including the capability of dynamic SIMS for imaging depth profiling of the ingested AuNPs will be presented.

(ATOM-02.5) **Novel Methods for the Characterization of Upconversion Nanoparticles by Single-Particle ICP-MS**

Sarah Meyer[1], Raquel Gonzales de Vega[1], Xiaoxue Xu[2], Ziqing Du[2], Philip Andrew Doble[2], David Clases[2]; [1]*University of Technology Sydney,* [2]*University of Technology Sydney*

Functional nanoparticles (NPs) with dimensions between 1 and 100 nm have a tremendous potential and versatile applications unlocking new possibilities in various scientific branches such as medicine, chemistry, biology, and physics. Upconversion nanoparticles (UCNPs) are manufactured by doping lanthanide ions into a NaYF4 host structure and have unique optical, electronic, and magnetic characteristics. The UCNP's optical properties are tunable and can be tailored for their application by controlling dimensions, structuring and stoichiometries. Methods to characterize the elemental composition, size, and interactions of UCNPs are necessary to fully understand, control and predict their properties. This presentation introduces novel methods based on single-particle inductively coupled plasma-mass spectrometry (SP ICP-MS) to characterize UCNPs regarding size, stoichiometry, and particle-particle interactions. The low ion transmission in ICP-MS is limiting the detection of small NPs as signals become indistinguishable from the background and noise. Specifically, analyzing NPs like UCNPs consisting of various elements and isotopes is problematic as most mass filters can only analyze one m/z at a time while all other m/z are eliminated. In this work, the ion transmission was optimized and improved by application of two strategies. For method development, gold NPs were initially investigated as model system before adapting the method for the analysis of UCNPs. The first strategy changed the ion lenses' parameters to optimize ion extraction and transport, whilst the second operated the quadrupole with an increased mass bandwidth, improving signal-to-noise ratios which significantly decreased size-detection limits for all NP-dispersions investigated. This allowed for the first time the detailed characterization of UCNPs. As a proof of principle, three UCNP dispersions containing particles with different dimensions and stoichiometries were investigated with the developed methods. To understand NP interactions within dispersions, a Poisson-based model was developed and described the aggregation of UCNPs. The developed methods are applicable for the investigation of any kind of NP dispersion but will specifically improve the analysis of heterogeneously structured NPs consisting of various isotopes and elements by providing detailed descriptions regarding size distributions and stoichiometries.

21AWD08: Coblentz Society Clara Craver Award Symposium Honoring Zachary Schultz

Chair: Zachary Schultz
On-site Chair: Zachary Schultz

(AWD-08.1) **Surface-Area Enhanced Raman Spectroscopy for Label-Free DNA Biosensing in Porous Silica Supports**

Joel M. Harris[1], Grant J. Myres[1], Eric Peterson[1]; [1]*University of Utah*

Investigating the chemistry of DNA biosensing at solid/liquid interfaces remains a challenge because most surface-sensitive techniques are unable to provide quantitative and structural insight into base composition, length, or structure. Surface-enhancement of Raman scattering (SERS) from DNA at plasmonic-metal substrates has been used to overcome the sensitivity challenges of these measurements; unfortunately, SERS detection is not generally quantitative due to the dependence of scattering on the proximity and orientation of molecules with respect to the plasmonic surface. The decay of the electric-field enhancement at nanometer distances from the surface also limits the length of DNA strands that can be investigated. In this work, we introduce an experimental methodology whereby confocal-Raman microscopy is used to characterize DNA immobilized at solid/liquid interfaces in porous silica particles. By focusing the femtoliter confocal-probe volume within a single porous particle, signal enhancement arises from the ~1,500-times greater surface area compared to a planar substrate. Because the porous support is a purely dielectric material and the internal surfaces are oriented randomly within the probe volume, the scattering signal is independent of both orientation and proximity of the oligonucleotide to the silica surface. With this technique, we characterize a 19-mer capture strand and determine its hybridization efficiency with 9-mer and 16-mer target sequences from the scattering of a structurally-insensitive phosphate mode. Quantification of base content allows discrimination of hybridization of target strands having equivalent length but with different recognition sequences. A duplex having a single-nucleotide polymorphism (SNP) can be distinguished from hybridization by a fully-complementary strand based on differences in both base content and duplex conformation. Future applications of this methodology are being directed toward characterization of immobilized DNA aptamers and their association with target proteins.

(AWD-08.2) **Monitoring molecular interactions at gold nanostar surfaces using surface enhanced Raman spectroscopy**

Laura Fabris[1], Hao Wang[2], Kaleigh Ryan[3], Kevin Christian[3], Jinisha Chheda[3], Ajita Nair[3]; [1]*Rutgers, the State University of New Jersey,* [2]*Duke University,* [3]*Rutgers University*

Surface enhanced Raman spectroscopy is well known for its sensitivity and selectivity, which make it widely applicable in sensing and diagnostics. When implemented as direct SERS, whereby the spectral fingerprint of the analyte, rather than that of a reporter molecule, is measured, a wealth of information can be extracted from the SERS spectra concerning the interaction between analyte and metallic surface at the molecular level. This information is important not only because it allows one to depart from the use of reporters, but mostly because it enables measuring conformational transitions in complex macromolecules. These interactions can however be extremely complex, as they depend on the crystallographic properties of the metallic surface, on the presence and identity of ions and surfactants, and on the properties of the molecule. In my talk, I will discuss how we approach the understanding of analyte-nanoparticle interactions using SERS, leveraging gold nanostars for their well-known field enhancing properties, but also comparing them to nanospheres, as their morphology and surface properties are less complex to decouple. I will report our studies on traditional Raman reporter molecules, on small molecular drugs as fentanyl, and on complex macromolecules such as DNA, integrating the experimental SERS results with molecular dynamics simulations and machine learning-driven data analysis.

(AWD-08.3) **New Bioorthogonal Probes for use in Bioanalysis using SRS**

Duncan Graham[1], William J. Tipping[1], Liam Wilson[2], Nicholas Tomkinson[2], Karen Faulds[1]; [1]*University of Strathclyde,* [2]*The University of Strathclyde*

Stimulated Raman scattering (SRS) is a version of Raman scattering which offers significant advances in terms of speed for imaging of biological systems and also in sensitivity over spontaneous Raman scattering. In this presentation data will be presented to show the design of new bioorthogonal probes which produce a unique vibration that can be imaged rapidly using SRS within biological cells. The vibration preferred is that of the alkyne and various examples will be given where modified alkynes have been used as probes within cells to report on changes in for example pH and also provide accurate uptake and localisation data. There will also be further discussion on the measurement of lipid production and changes in lipid production using SRS in a variety of different cell lines and how this produces a complementary set of data to that of spontaneous Raman mapping and imaging. There are advantages in using SRS with spontaneous Raman and the selected examples will provide the evidence for these advantages.

(AWD-08.4) **Multi-parameter single nanoparticle imaging**

Kallie Willets[1]; [1]*Temple University*

This talk will introduce a new strategy for single nanoparticle imaging that encodes super-temporal and sub-diffraction-limited structural information, allowing for rapid analysis of time-dependent processes on single nanoparticles. We will discuss the principles of the technique, then highlight several applications in which the enhanced kinetic/structural information provides a richer understanding of plasmonic nanoparticles than traditional optical imaging approaches.

21BIM06: Studying Biomolecular Activity and Localization Using Optical Spectroscopy

Chair: Linda Kidder
On-site Chair: Linda Kidder

(BIM-06.1) **Assessment of cavity shave margins in breast-conserving surgery using infrared spectroscopic imaging**

Anirudh Mittal[1], Shachi Mittal[1], Anna Higham[2], Rohit Bhargava[1]; [1]*University of Illinois at Urbana-Champaign,* [2]*Mills Breast Cancer Institute, Carle Foundation Hospital*

In fifteen words or less, explain the significance of this contribution (Novel Aspect).: IR imaging addresses the need to accurately identify margin status enabling better patient care

Abstract Text: As an increasing number of women choose breast conservation surgery (BCS) as their treatment modality, it is essential to achieve an oncologically acceptable resection while preserving the cosmesis of the breast. The cavity shave margin (CSM) technique can be used to decrease the margin re-excision rate. Currently, the shave margins are manually evaluated using standard histological procedures, a process that is time-consuming and subject to user-dependent errors. This adversely affects the patient's care and can lead to higher healthcare costs. Infrared (IR) spectroscopic imaging coupled with artificial intelligence (AI) algorithms has the potential to address this as it provides the molecular data to identify cellular changes in cancer, and AI algorithms can provide a label-free and rapid readout of tumor cells around the margins. The underlying principle for the proposed research is that the absorption of IR light is a chemical signature of tissue identity and physiology. By exploring the subtle correlations of peaks within the spectra, AI methods will be applied to each pixel for histopathology without dyes or stains. In this study, we developed a cascaded approach to segment the cavity shave margins, first into epithelium, stroma & fat, and then into malignant and benign epithelium. The final classified image is then generated by combining the results from both stages. This study aims to provide accurate diagnostic information regarding the tumor margin to the surgeon in a much shorter time frame. This imaging platform, coupled with the trained AI models, can help achieve better patient clinical and cosmetic outcomes and significantly reduce healthcare costs.

(BIM-06.3) **Raman Microscopy Investigation of C-H Dipolar Coupling Reveals Segregation of Deuterated and Protonated Phospholipids in Mixed Bilayers**

Jay P. Kitt[1], Joel M. Harris[1]; [1]*University of Utah*

In fifteen words or less, explain the significance of this contribution (Novel Aspect).: Raman spectroscopy shows lipid segregation in mixed-deuterated-proteated phospholipid vesicles questioning assumption of "non-perturbing" deuterated phospholipids.

Abstract Text: Phospholipids with perdeuterated acyl chains are commonly used as “non-perturbing” components of phospholipid membranes where frequencies of vibrational modes from deuterated phospholipids are shifted from those of their protonated counterparts allowing resolution of individual phospholipids in mixed phospholipid systems. However, recent calorimetric investigations of phospholipid melting transitions in mixed deuterated and protonated vesicles has shown that deuterated lipids exhibit lower melting transitions, that incorporation of deuterated phospholipids reduces cooperative melting of adjacent acyl-chains leading to phase-separation during the transition, and that mixed-lipid melting transitions occur at lower temperatures than in equivalent protonated membranes. In this work, we investigate phase segregation in individual mixed protonated and deuterated (DPPC & DPPC-D62) phospholipid vesicles using optical-trapping confocal Raman microscopy. Raman spectra of trapped vesicles were collected as a function of membrane composition across a range of deuterated lipid fractions. Examining the spectra, we observe vibrational modes in the C-H and C-D stretching regions of the spectrum indicative of C-H dipolar coupling between adjacent acyl-chains, which differ only in relative intensity as the ratio of deuterated to protonated lipid is varied. This result indicates segregation of the lipids and formation of microdomains of relatively pure deuterated and protonated lipids allowing short-range intermolecular dipolar coupling to persist within the mixed lipid system. To further characterize the mixed-lipid bilayer, the size of the lipid microdomains was computed from crystal-field splitting of the C-H and C-D bending modes, where a linear relationship between deuterated-lipid fraction and microdomain size is observed but where microdomains persist throughout the range of compositions. Finally, spectra of DSPC-DMPC-D54 mixed-lipid vesicles were monitored as a function of temperature. Here, two distinct melting transitions are observed and the melting-transition-temperature of each lipid is shifted from that of a pure sample. C-H and C-D dipolar coupling is observed in each lipid until completion of the phase transition suggesting that dipolar coupling between adjacent phospholipids may contribute to phase segregation during the melting transition. These results question the assumption that deuterated phospholipids are non-perturbing and suggest careful consideration of the use of deuterated phospholipids in membrane research, particularly for investigation of lipid-rafts and phospholipid melting transitions.

(BIM-06.4) **Small-Molecule Association with Duplex-DNA at Solid/Liquid Interfaces Characterized with Confocal-Raman Microscopy**

Grant J. Myres[1], Emily C. Heider[2], Joel M. Harris[1]; [1]*University of Utah,* [2]*Utah Valley University*

In fifteen words or less, explain the significance of this contribution (Novel Aspect).: Application of quantitative-Raman microscopy for the surface analysis of small-molecule association reactions to interfacial dsDNA.

Abstract Text: Development and application of small-molecule therapeutics that target duplex-DNA remains an attractive intervention for controlling gene expression. Although assays like SPR can characterize molecular association affinities to surface-immobilized DNA, these techniques are unable to provide insight into conformational changes and interactions that are informative of intermolecular association. Here we describe a label-free, quantitative, and structurally informative approach for characterizing small-molecule association with duplex-DNA immobilized at porous silica particles with confocal-Raman microscopy. By immobilizing DNA at porous silica, we maintain desirable molecular packing densities (<50 nmol/m2) while simultaneously

achieving within-particle DNA concentrations (~3 mM) necessary to detect interfacial DNA by in-situ single-particle characterization with confocal-Raman microscopy. Using this methodology, we study the surface association of the minor-groove binding small-molecules 4,6'-diamidino-2-phenylindole (DAPI) and the therapeutic peptide netropsin to immobilized duplex-DNA containing an AT binding region. We test our assay's sensitivity and report detectable and specific association interactions at sub-nanomolar solution concentrations, while also detecting a concentration-dependent association response. Following complexation of netropsin, the amide I band was observed to blue-shift ~14 cm-1 which is consistent with the amide-coordinating hydrogen bonds with the DNA as previously proposed in the literature. Additionally, the Raman spectral features of DAPI following complexation with DNA at porous silica were observed to be qualitatively consistent with previous in-vitro Raman microscopy analysis of DAPI stained cells. This work presents a major advancement in quantitative spectroscopic characterization of small-molecule interactions with duplex-DNA at solid/liquid interfaces.

(BIM-06.5) **Analysis of Live HeLa Cells using Optical-Photothermal Imaging**

Chalapathi Charan Gajjela[1], Xiaoliang Li[1], Xiaonan Shan[1], Rohith Reddy[2]; *[1]Department of Electrical and Computer Engineering, University of Houston, [2]University of Houston*

In fifteen words or less, explain the significance of this contribution (Novel Aspect).: Observe biochemical profiles within a live cell using Optical photothermal infrared imaging

Abstract Text: Mid-infrared spectroscopic imaging is used in a broad range of applications, from archeology to material characterization to cancer grading. However, it has been limited to dried samples due to strong infrared absorption by water. Moreover, it suffers from low spatial resolution due to long mid-infrared wavelengths. Optical photothermal infrared imaging (O-PTIR) overcomes some of these limitations and is shown to provide an order of magnitude improvement in spatial resolution. We have utilized this improved spatial resolution for the improved analysis of ovarian cancer tissue. Ovarian cancer results in alteration of both the morphology and biochemical properties of tissue. Histopathologic analysis of the morphology of epithelial cells in chemically stained cancer tissue has been the golden standard for cancer grading and clinical diagnosis. We demonstrate the difference in resolution using TMA of ovarian cancer cores at different stages of cancer between FTIR and O-PTIR. This improvement in resolution is used via machine learning to classify epithelium and stroma using only five-band images of the entire TMA with approximately 94% accuracy. We also demonstrate live-cell imaging using O-PTIR on HeLa cell cultures (cervical cancer cells). Cells primarily contain water which has strong absorption in mid-IR wavelengths, which made conventional FTIR imaging challenging. We can overcome limitations using O-PTIR. We use a visible green laser for data acquisition, which is not absorbed by water or the culture medium present around the cells. We show biochemical profiles and present the nucleic acid distribution in cells, observe lipids distribution in the cell membrane, and study glucose transport. This helps us understand changes in metabolic processes between a healthy living cell and a cancerous cell.

21FORENS02: Food Forensics

Chair: Betsy Jean Yakes
Co-Chair: Luis Rodriguez-Saona
On-site Chair: Luis Rodriguez-Saona

(FORENS-02.1) **Rapid Detection of Undeclared Pharmaceuticals in Dietary Supplements using Surface Enhanced Raman Scattering (SERS) with Handheld Raman Spectrometers.**

Martin Kimani[1], Adam Lanzarotta[2], Skyler Smith, PhD, JaCinta Batson[2]; *[1]U.S. FDA/ORA, [2]United States Food Drug Administration*

The US Food and Drug Administration's (FDA) Forensic Chemistry Center (FCC) has developed surface enhanced Raman spectroscopy (SERS) methods for the detection of opioids, sildenafil and benzodiazepines in finished dosage pharmaceuticals using handheld Raman spectrometers. We are currently interested in the detection of adulterants such as sibutramine, phentermine, phenolphthalein, amphetamine, fluoxetine, phenytoin, steroids and PDE5 inhibitors previously observed in suspect dietary supplements. To support FDA Consumer Safety Officers and Office of Criminal Investigations Special Agents working at international mail facilities and express courier hubs, rapid and field-friendly methods to screen and detect these undeclared prescription ingredients is needed. FCC has employed conventional Raman spectroscopy for this application due to its ability to identify active pharmaceutical ingredients in dietary supplements and pharmaceutical products. Unfortunately, sensitivity is the most significant limitation of Raman handheld devices, which often precludes their use for detecting low concentration APIs in finished products when used under normal operating conditions. The purpose of this study is to overcome this sensitivity limitation by use of SERS to screen and detect low-dose adulterants ranging from anorexic drugs, stimulants, diuretics, antidepressants, life-style drugs and steroids observed in suspect dietary supplements. The use of SERS is also impeded by the lack of readily available commercial spectral libraries needed to perform the necessary identification. This is due, in a large part, to the uniqueness of the commercially available SERS substrates, each of which can produce different spectra for the same compound. To overcome these limitations, we have developed methods to collect SERS signatures of several active pharmaceutical ingredient standards using Ag and Au colloids and handheld Raman devices utilizing 785 and 1064 nm laser excitations. These methods were validated for identifying adulterants in dietary supplements based on comparison to results generated using LC/MS. SERS validation studies of a few representative standards revealed the minimum concentration (Cmin) of vardenafil in a 10% methanol in water/colloid solution was 5 µg/mL; sibutramine 1 µg/mL; and phentermine HCl 10 µg/mL. The use of SERS for rapid chemical identification at remote sampling sites provides rugged, simple and practical methods applicable to point of entry sampling and screening.

(FORENS-02.3) **Optimization of Optical Sampling Design for In-line Monitoring of Heterogeneous Foods by NIR Interaction Spectroscopy**

Jens P. Wold[1], Marion O'Farrell, Jon Tschudi; [1]*Nofima*

Near-infrared spectroscopy (NIRS) is widely used for in-line analysis of fat, water and protein in foods. Previously, we have developed tailor-made, in-line solutions, where NIRS and multispectral imaging have been combined to handle rather complex samples: e.g. fat in salmon fillets, meat content in brown crabs, blood in white fish and protein in chicken fillets. These sensor systems have been optimised in terms of optical sampling, signal quality and unknown variation identification. However, NIRS has the potential to be used on even more complex samples, such as individually measuring whole, intact potatoes and whole fish. Such samples are extremely heterogeneous and are covered by an outer layer, e.g. peel or skin, making it necessary to have optical sampling depths of 10-15 mm to achieve good results. Currently, we are developing NIRS for such applications and studying the system performance in industrial process lines. The goal is to advance our instrumentation and how it is applied in the food industry. We present the results obtained by a novel NIRS prototype designed to probe deep into complex food samples at high speed. The instrument is based on non-contact interaction measurements, with approximately 20 cm between the instrument and samples. It measures rapidly, up to 50 measurements per second, and enables several interaction distances to be recorded for each measurement. An increased interaction distance generally leads to deeper optical sampling because the signal from deeper regions in the samples is able to dominate the measured signal. Examples of this will be shown for different food products. Since these small differences in optical measurement geometry influence the depth of sampling, selecting the correct distance can have a positive impact on the accuracy of the calibration models for heterogeneous food products. Examples will be shown for in-line determination of dry matter in potatoes and fat in whole salmon, where an optimal choice of interaction distance can improve accuracy by 30% compared to a less optimal distance. The results illustrate that optimization of the optical measurement geometry is crucial to succeed with NIRS-based inline measurements of complex samples in process lines.

(FORENS-02.4) **Determining Food Freshness with Low-cost Electrical Sensing of Water-soluble Gases**
Michael Kasimatis[1], Giandrin Barandun, Max Grell, Firat Guder; [1]*BlakBear*

Our research focuses on real-time digital information at commercially viable prices, concerning the freshness of a product via disposable electrical sensors integrated into meat and fish packaging. We use an ultra-low-cost paper-based electrical gas sensor ('PEGS') that gathers real-time data on the freshness of food by non-destructively monitoring total volatile basic nitrogen (TVB-N) contents in the headspace of packed foods. PEGS function in the high relative humidity environments of packed meat and fish and detect TVB-N gases dissolving in the porous sensor structure.
The main components of TVB-N, ammonia and trimethylamine, result from enzymatic protein breakdown in foods. These gases are highly water-soluble and show a strong correlation to the total viable count (TVC) in protein-rich foods. The porous, hygroscopic structure in PEGS attracts water and allows water-soluble gases to dissolve, dissociate and change the electrical characteristics of the sensor.
Our sensors aim to extend shelf-life of high value food by giving real-time digital information on product freshness along the food supply chain. The increased visibility of spoilage processes will help to optimize handling and storage of perishable products to improve the industry's bottom-line and minimize environmental impacts from waste or rotting in the supply chain.

(FORENS-02.5) **Portable Optical and Spectroscopic Techniques for Food and Water Contaminants**
Wei-Chuan Shih[1]; [1]*University of Houston*

The ability to rapidly detect and quantify food and water contaminants on site is critical for many applications. However, many existing approaches require the transportation of collected samples back to a laboratory for analysis, which can delay response, create logistic hurdles, and increase cost. In this talk, I will discuss two examples of bringing analytical measurements to the field for on-site analysis by portable optical imaging and spectroscopic techniques. First, I will present a technique of quantifying small molecule malachite green (MG) based on surface-enhanced Raman spectroscopy (SERS) implemented on nanoporous gold array (NPGA) substrate. Using G-quadruplex functionalized NPGA, MG SERS fingerprints can be readily detected at the nM level. An extension of this technique allows us to quantify telomerase activities. Next, I will present a technique we call nano-colorimetry for quantifying lead and mercury in drinking water at ppb level by DotLens smartphone microscopy. These new technologies have the potential for many on-site applications.

21IR04: Evanescent Wave Sensing: New Developments and Applications

Chair: Benedikt Schwarz
On-site Chair: Richard Crocombe

(IR-04.1) **Mesoporous Silica, Titania and Zirconia for Improved Selectivity and Sensitivity in Evanescent Wave IR Spectroscopy**
Benedikt Schwarz[1], Dominik Wacht, Felix Frank, Jakob Hayden[2], Stephan Freitag[1], Nuria Benéitez, Andreas Schwaighofer[1], Bettina Baumgartner[3]; [1]*TU Wien,* [2]*IRsweep AG,* [3]*Osaka Prefecture University*

We report on synthesis, characterization and application of mesoporous sensing layers on internal reflection elements (Si and Ge) and their use in evanescent wave IR spectroscopy. Surface-modified mesoporous oxides were obtained by the sol-gel soft templating process using surfactants to adjust pore size, porosity and layer thickness. Well defined mesoporous structures with engineered surface chemistries could be prepared. For ATR experiments reflection elements (20*10 mm) were cut from Si and Ge wavers whose facets were grinded to an angle 45° to facilitate coupling of IR radiation from an FTIR spectrometer. Advantages of mesoporous titania and zirconia films over mesoporous silica films are their better chemical stability also at alkaline pH as well as increased transparency below 1200cm-1. Evanescent wave sensors employing mesoporous materials allow significantly faster analyte enrichment compared to polymer coated ATR elements, fibers or waveguides as in this case the enrichment process is governed by the process of adsorption and not by slow analyte diffusion into

polymers. Applications will be shown for the detection of hydrophobic analytes from aqueous [1] and gas [2] phaseas well as determination of nitrate [3] and phosphate in water reaching ppm sensitivities. [1] B. Baumgartner, J. Hayden, A. Schwaighofer, B. Lendl "In Situ IR Spectroscopy of Mesoporous Silica Films for Monitoring Adsorption Processes and Trace Analysis" ACS Applied Nano Materials 1 (2018) 7083-7091 [2] B. Baumgartner, J. Hayden, B. Lendl „"Mesoporous silica films for sensing volatile organic compounds using attenuated total reflection spectroscopy" Sensors and Actuators, B: Chemical 302 (2020) 127194 [3] B. Baumgartner, S. Freitag, C. Gasser, B. Lendl „A pocket-sized 3D-printed attenuated total reflection-infrared filtometer combined with functionalized silica films for nitrate sensing in water" Sensors and Actuators, B: Chemical 310 (2020) 127847

(IR-04.4) **Mid-IR plasmonics for next generation liquid sensing**

Borislav Hinkov[1], Mauro David[1], Florian Pilat[1], Laurin Lux[1], Patricia Lustoza da Souza[2], Andreas Schwaighofer[1], Benedikt Schwarz[1], Hermann Detz[1], Aaron Maxwell Andrews[1], Bernhard Lendl[1], Gottfried Strasser[1]; *[1]TU Wien, [2]PUC-Rio*

The peculiar mid-IR spectral range hosts the well-known molecular fingerprint-region, i.e. the fundamental roto-vibrational absorptions of many molecules. It is therefore of particular interest in various fields of research including environmental sensing, medical diagnostics and process-monitoring. All those applications share the need for small, compact and robust portable highly sensitive detection systems. This is where quantum cascade (QC) technology, i.e. quantum cascade lasers (QCLs) and detectors (QCDs), play an enabling role in recent years for mid-IR sensors. In this context, the very recent proof-of-concept of QCLDs, being able to emit and detect identical-wavelength mid-IR light from one QC active region, is regarded as breakthrough-technology. It allows the realization of ultra-compact monolithic sensors where QCL and QCD are separated by 10s-100s of micrometer only, optimal for liquid sensing and in stark contrast to established ATR-FTIR-based systems addressing only a few micrometers of effective optical path lengths. To exploit this configuration towards monolithic lab-on-a-chip geometries, it is highly beneficial to connect emitter and detector by a section that: a.) guides the light efficiently and simultaneously b.) supports a strong interaction between optical mode and surrounding medium. Following these criteria in particular, mid-IR dielectric loaded surface plasmon polariton waveguides (DLSPPW) are excellent candidates. The additional dielectric layer efficiently confines the mode to the dielectric-metal structure, while still efficiently guiding it on-chip and up to several hundreds of micrometers or even millimeters. Another big advantage arises from the fact, that far more than 90% of the mode can be guided in the surrounding medium, i.e. the analyte. In this paper we discuss theoretically and experimentally the concept of mid-IR DLSPPWs, including their design (effective-index-method) and realization (state-of-the-art and beyond semiconductor fabrication techniques) and how they can be further improved towards optimized propagation-lengths and -losses as well as adjustable mode confinement (different novel materials, geometries and concepts). This helps to improve spectroscopic measurements in liquids including surface protection and functionalization, on-chip mode guiding and beam-combining schemes for e.g. heterodyne or interferometric measurements. We will in particular show recent protein-measurements of bovine serum albumin (BSA) around 1550–1650 cm-1 employing such an above described lab-on-a-chip sensor.

(IR-04.5) **Development of a new Sensor Concept based on Attenuated Total Reflection Infrared Spectroscopy via Quantum Cascade Laser**

Andrea Teuber[1], Boris Mizaikoff[2]; *[1]Ulm University / Institute of Analytical and Bioanalytical Chemistry, [2]Institute of Analytical and Bioanalytical Chemistry, Ulm University and Hahn-Schickard, Institute for Microanalysis Systems, Ulm, Germany*

In fifteen words or less, explain the significance of this contribution (Novel Aspect).: Innovative sensor design based on IR-ATR spectroscopy combined with hollow waveguide technology for several applications.

Abstract Text: The investigation of several liquid and solid compounds in a variety of different scenarios, e.g. environmental or medical monitoring, via Mid-Infrared (MIR) sensors based on attenuated total reflection (ATR) provide robust and sensitive chemical sensing platforms. Using such sensors combined with the advantages of laser spectroscopy in the MIR range, portable field-deployable measurement systems, which can be furthermore miniaturized, lead to a real-world condition measurement, if the sensor is sufficiently robust, without demanding for complex and time-consuming sampling, transport, storage, and sample preparation procedures. Herein, we present a novel IR-ATR sensing concept based on an innovative and exceedingly robust combination of an ATR waveguide, which serves as an evanescent field transducer, with a hollow waveguide-based direct optical coupling concept. The advantages of such coupling concept are the optical alignment via substrate-integrated hollow waveguide (iHWG) light guiding structures, the temperature stability, and the innerness against shock and vibration. Furthermore, these characteristics and the versatility of the optical concept leads to a suitable approach for a wide range of in-field sensing scenarios including also clinical monitoring settings. As a proof-of-principle, this developed IR-ATR sensor design will be compared with a well-established and conventional horizontal ATR assembly, studying several liquid phase samples.

21LIBS01: Deep Dive in LIBS Principle

Chair: Alessandro De Giacomo
On-site Chair: Jonathan Merten

(LIBS-01.1) **Influence of the Atmosphere on Laser-Ablation Atomic Absorption Measurements- Findings and Potential for Artifacts**

Jonathan A. Merten[1], Anna G. Anders[1], Erin Nicholas[1], Aaron Hopson[1], Jackie Brees[1], Shawnda Ethridge[1]; [1]*Arkansas State University*

In fifteen words or less, explain the significance of this contribution (Novel Aspect).: Absorption measurements in LIPs are complex and the potential for artifacts varies with plasma morphology

Abstract Text: Atomic absorption spectroscopy provides a unique perspective on the evolution of the laser-induced plasma (LIP). It is under development for isotope ratio measurements and as a tool for plasma characterization. Among the measurement paradigms currently in use, our lab uses a derivative of continuum-source atomic absorption spectroscopy and acquires a full absorption spectrum with each sample ablation. Because LIPs are a uniquely challenging reservoir for atomic absorption measurements, we discuss the limitations of the measurement in terms of the Lambert-Beer Law. In turn, the potential for departures from the Lambert-Beer Law and resulting artifacts are discussed as a function of cover gas composition and pressure. Finally, we present long-delay absorption measurements of the varying morphologies and compositions of titanium plasmas under different cover gas conditions. These data should further our understanding the ablation process and ultimate fate of the ablated material. They also have implications for the development of laser-ablation fluorescence measurements.

(LIBS-01.2) **Sensing nanoparticles-protein corona with Nanoparticle Enhanced Laser Induced Breakdown Spectroscopy**

Alessandro De Giacomo[1], Marcella Dell'Aglio[2], Zita Salajkova[3], Antonia Mallardi, Gerardo Palazzo, Nicola Cioffi; [1]*University of Bari,* [2]*CNR-NANOTEC,* [3]*Central European Institute of Technology*

In the last decade, the use of plasmonic systems based on NPs have been revolutionizing the analytical spectroscopy, opening the way to extremely high sensitivity with extremely low Limits of detection. One of the most recent application of plasmonic system to analytical chemistry is the Nanoparticle enhanced ablation, that has been exploited mainly for improving the performance of Laser Induced Breakdown Spectroscopy (LIBS)

and lately also for Laser Ablation Inductively Coupled Plasma Mass Spectrometry (LA-ICP-MS). NPs enhanced laser ablation (NELA) is based on the interaction of the plasmonic system of metallic NPs with the ablated matter during the laser pulse irradiation with ns-laser pulse. Although several aspects still need to be elucidated, differently to the SERS, in the case of NELA, the high energy laser pulse is used for inducing a plasma, initially at density very close to the sample/target material. The concentration of the electromagnetic field of the laser pulse between the NPs, coupling with the high density ablated matter allows to transfer the laser energy to the sample in a much more efficient way with respect to conventional laser ablation, allowing the enhancement of the analytical signal up to tow orders of magnitude. In this paper a different investigation of this phenomenon is proposed. Instead of focusing our attention to the improvement of the analytical signal, the effect of the protein-NPs interaction, is investigated with the observation of how the protein affects the electromagnetic field enhancement of the NPs system during the ablation process. In other words the dependence of the signal enhancement on protein-NPs interaction, during NP enhanced Laser Induced Breakdown Spectroscopy (NELIBS) of a Titanium sample, is investigated for sensing the structural characteristics of the colloidal solution of AuNP with protein.

(LIBS-01.3) **Use of a Novel Microstrip Resonator for Microwave-Assisted Laser-Induced Breakdown Spectroscopy and Laser Ablation Molecular Isotopic Spectrometry**

Kelsey L. Williams[1], Steven J. Ray[1]; [1]*The State University of New York at Buffalo*

In fifteen words or less, explain the significance of this contribution (Novel Aspect).: Application of a novel microstrip resonator for microwave enhancement in laser-induced breakdown spectroscopy.

Abstract Text: Laser-induced breakdown spectroscopy (LIBS) and laser ablation molecular isotopic spectrometry (LAMIS) are techniques that allow for the optical detection of a sample's atomic and isotopic composition, respectively. In both techniques, a laser is focused on the surface of a sample resulting in formation of a laser-induced plasma, from which atomic emission is measured after a 1 – 10 μs delay. If detection is further delayed to observe emission from molecular diatomic molecules formed from the sample and atmospheric gases, the LAMIS technique can be used to measure isotopic information based on the shift in emission wavelength observed for different isotopologues. Microwave-assisted LIBS has previously been shown to increase S/N of atomic emission measurements in LIBS. Here we evaluate the efficacy of a novel microwave waveguide for microwave-enhanced LIBS, and investigate the effect of intense microwaves on the LAMIS measurement for the first time. The novel microstrip resonator is created as a copper circuit atop a thin dielectric, and effectively focuses power from a microwave radiation source into an approximately 1-mm diameter open area, creating an intense, localized field. The sample is placed beneath the thin microstrip, and the laser focused into the interaction volume, permitting optimum interaction of the high-field microwaves with the laser plasma. Design considerations, optimal experimental conditions, and analytical performance of microwave-assisted LIBS and microwave-assisted LAMIS will be examined.

(LIBS-01.4) **Laser Induced Gas Breakdown in Reactive Mixtures**

Igor B. Gornushkin[1], Piotr Sennikov[2], Roman Kornev[2]; [1]*BAM Federal Institute for Materials Research and Testing,* [2]*G.G. Devyatykh Institute of Chemistry of High-Purity Substances of RAS*

In fifteen words or less, explain the significance of this contribution (Novel Aspect).: Laser breakdown in reactive gas mixtures is promising for plasma vapor deposition of materials

Abstract Text: Laser Induced Gas Breakdown in Reactive Mixtures Igor B. Gornushkin1, Piotr G. Sennikov2 and Roman A. Kornev2 1BAM Federal Institute for Materials Research and Testing, Berlin, Germany 2 Institute of Chemistry of High-Purity Substances of RAS, Nizhny Novgorod, Russia Plasma-chemical approach is used for synthesis of various gaseous, liquid, and solid substances since 1960th. Nowadays, the method of

plasma enhanced chemical vapor deposition (PECVD) is used for production of thin films, protective coatings, carbon-based nanostructures, high purity isotopic materials, biomaterials, and other products. Plasma for PECVD is typically created in various electrical discharges, e.g. DC and AC glow discharges or discharges operated at audio (10-20 kHz), radio (13.56 MHz), and microwave (2.45 GHz) frequencies. Plasma induced by a laser, a laser induced plasma (LIP), is rarely used to deposit materials from the gas phase as in PECVD. This work is aimed at reviving interest to this latter technology and showing its efficiency and potential. We run several pilot experiments. First, LIP is excited in BCl3 or BF3 plus H2 or CH4 to evaluate the efficiency of deposition of solid boron and boron carbide, the materials, which are largely used for refractory coatings. Second, we investigate a possibility of synthesis of fluorochlorosilanes SiFxCl4-x (x = 1, 2, 3) by LIP induced in SiF4 + SiCl4 gas mixtures. Using fluorochlorosilanes with different combinations of F and Cl in the SiFxCly molecule may add flexibility in processes of silicon deposition and etching. Third, LIP is excited in reactive mixture MoF6+H2+BF3 or on a Mo target ablated into H2/BF3 atmosphere. The goal is obtaining superhard molybdenum borides MoB, Mo2B, or MoB2. The gases used and solid deposits are analyzed by optical emission spectroscopy (OES), IR and mass spectrometry (MS). We also model the plasma and perform static equilibrium chemistry calculations to see if the desired reaction products are thermodynamically favorable. Dynamic calculations of the expanding plasma plume are performed using a hydrodynamic code [1] combined with the open source chemical software [2]. 1. S.V. Shabanov, I.B. Gornushkin, Chemistry in laser-induced plasma at local thermodynamic equilibrium, Appl. Phys. A 124 (2018) 716 2. https://cearun.grc.nasa.gov

(LIBS-01.5) **Femtosecond Single-Pulse and Orthogonal Double-Pulse Laser-Induced Breakdown Spectroscopy (LIBS): Femtogram Mass Detection and Chemical Imaging with Micrometer Spatial Resolution**

Nikolaos Giannakaris[1], Anna Haider[1], Christoph Ahamer[1], Stefan Grünberger[2], Stefan Trautner[2], Johannes D. Pedarnig[2]; *[1]Johannes Kepler University Linz, [2]Johannes Kepler University Linz, Institute of Applied Physics*

In fifteen words or less, explain the significance of this contribution (Novel Aspect).: We detect sample mass of 100 fg and perform LIBS imaging with high spatial resolution.

Abstract Text: Femtosecond laser-induced breakdown spectroscopy (fs-LIBS) is employed to detect tiny amounts of mass ablated from macroscopic specimens and to measure chemical images of micro-structured samples with high spatial resolution. Frequency-doubled fs-pulses (length 400 fs, wavelength 520 nm) are tightly focused with a Schwarzschild microscope objective to ablate the sample surface. The optical emission of laser-induced plasma (LIP) is collected by the objective and measured with an Echelle spectrometer equipped with an intensified CCD camera. A second fs-laser pulse (1040 nm) in orthogonal beam arrangement is reheating the LIP. The optimization of experimental setup and measurement parameters enables us to record single-pulse fs-LIBS spectra of 5 nm thin metal layers with an ablated mass per pulse of 100 fg for Cu and 370 fg for Ag films. The orthogonal double-pulse fs-LIBS enhances the recorded emission line intensities (2-3×) and improves the contrast of chemical images in comparison to single-pulse measurements. The size of ablation craters (diameters as small as 1.5 μm) is not increased by the second laser pulse. The combination of minimally invasive sampling by a tightly focused low-energy fs-pulse and of strong enhancement of plasma emission by an orthogonal high-energy fs-pulse appears promising for future LIBS chemical imaging with high spatial resolution and with high spectrochemical sensitivity.

21PMA04: Structure Elucidation of Chiral and Biological Molecules

(PMA-04.2) **Using Molecular Rotational Resonance Spectroscopy to Tackle Challenging Structure Elucidation Problems of Chiral Molecules**

Reilly E. Sonstrom[1]; *[1]Brightspec*

This work presents the use of molecular rotational resonance (MRR) spectroscopy to perform the structure analysis of chiral molecules with a focus on isomers that are difficult to analyze using existing techniques (NMR, MS). MRR has many attractive features over other analytical techniques. The technique, enabled by advances in high speed digital electronics, has high spectral resolution and a large dynamic range that allows for simultaneous detection of multiple species. Additionally, the high sensitivity and spectral resolution allows analysis of crude reaction mixtures with no additional purification needed. Finally, rotational spectra can be identified with a high degree of accuracy by comparison between their experimental and theoretical moments of inertia, enabling "library free" identification. MRR spectra are extremely sensitive to changes in mass distribution, making it well suited to analyze the structurally similar molecules such as diastereomers, regioisomers, and isotopic isomers. Additionally, enantiomers, which have identical moments of inertia, can be resolved using a technique called chiral tagging allowing for determination of enantiomeric excess and absolute configuration by MRR. This talk will present examples where MRR provides accurate structural identification and quantification in industrially relevant samples.

(PMA-04.5) **Raman optical activity for drug discovery: Structural characterization of artemisinin derivatives in solution**

Jonathan Bogaerts[1], Filip Desmet, Roy Aerts, Patrick Bultinck, Wouter Herrebout, Christian Johannessen[1]; [1]*University of Antwerp*

The natural compound artemisinin, which is currently employed in malaria treatment, is showing great potential as a candidate for next generation cancer treatment. One of the biggest challenges in drugs discovery research is the stereochemical characterization and the identification of conformational preferences in solution to have a better understanding of the molecular mechanism and the structure-activity relationship. Considering the limitations and side effects caused by conventional cancer treatments, new techniques for the structural characterization of drugs are strongly required, in order to obtain an accurate, site directed therapy. The application of Raman opical acivity (ROA) to the structural characterization of natural products has increased significantly in the past years. ROA is based on inelastic (Raman) scattering of circular polarised light. When the experiment is combined with DFT calculations, ROA provides an unparalleled sensitivity towards solution phase conformation and stereochemistry of chiral molecules. Furthermore, ROA has emerged as a very strong tool in the structural analysis of proteins making this method an ideal candidate for studying the structure of natural and synthetic lead compounds with potential pharmacological activity. In this contribution, the capability of ROA in assigning the correct stereochemistry of artemisinin, dihydroartemisinin and artesunate combined with quantum chemical calculations will be discussed. Furthermore, the in particular sensitivity of ROA observed epimers towards epimers will be shown, highlighting the full potential of ROA spectroscopy to assign the the absolute configuration of this important class of compounds.

21PMA04: Structure Elucidation of Chiral and Biological Molecules

Chair: Rina Dukor
Co-Chair: Christian Johannessen
On-site Chair: Rina Dukor

(PMA-04.4) **Vibrational circular dichroism measurements using IR microscopes: opportunities and challenges**

Rohit Bhargava[1], Yamuna Phal[1], Kevin Yeh[2], Ruo-Jing Ho; [1]*University of Illinois at Urbana-Champaign,* [2]*University of Illinois Urbana-Champaign*

Vibrational circular dichroism (VCD) measurements are possible today with a sophisticated understanding of theory, instrumentation and modeling to quantify chirality, a vital biological property of molecular species directly relevant to life processes. However, VCD measurements of small sample volumes, such as in an imaging microscope, have only now become feasible. We first describe the technological advancements in IR

microscopy that set the stage for VCD microscopy. Our microscopy platform consists of a laser scanning infrared microscope that provides exceptional signal to noise ratio measurements. This design is based on theory of IR image formation, an understanding of detection limits and the effects of including polarization control. Next, we describe the hardware for IR microscopy and the use of a photoelastic modulator (PEM) integrated into the setup to demonstrate a point scanning VCD instrument capable of acquiring spectra rapidly across all fingerprint region IR wavelengths and micron scale pixels. Next, we demonstrate examples of VCD data recorded from the setup for a variety of homogeneous and heterogeneous samples. Finally, we describe extant challenges and potential confounding variables in the measurement of VCD using IR microscopes.

21PMA04: Structure Elucidation of Chiral and Biological Molecules

(PMA-04.1) **Stereochemistry at the Interface: Novel Analytical Approaches in Support of Organic Synthesis**

Leo A. Joyce[1]; [1]*Arrowhead Pharmaceuticals, Inc.*

Over the last several decades, a multitude of innovative synthetic methods have been developed and reported both by academic and industrial research groups. Determining success of these transformations generally requires extensive analytical characterization, though this task is rarely straightforward. Industrial settings often require close collaboration between synthetic chemists and analytical chemists, ensuring that the right analytical techniques are being used to assess these new transformations. Frequently this requires a change from the established paradigm and requires either repurposing existing technology or developing novel platforms. This presentation will demonstrate the power of this collaboration by giving examples where analytical measurements played a key role in understanding the development in real time. Examples presented will cover a wide swath of synthetic landscape, with an emphasis on stereochemical understanding, and highlight how the synergy between analytical and organic chemistry can lead to a deeper understand of organic synthesis.

(PMA-04.3) **Implementing a Vibrational Circular Dichroism Analysis Platform for Chiral Small Molecules**

Christopher A. LeClair[1], Kamaria Butler[2], Nishita Rao[2]; [1]*Division of Preclinical Innovation, NCATS, NIH,* [2]*Analytical Chemistry Core, Division of Preclinical Innovation, NCATS, NIH*

Small molecule therapeutics are the primary modality for treatment of targeted diseases with chirality being increasingly incorporated into structure design as a means to improve selectivity. The determination of stereochemistry within these compounds is critical as structure is highly involved in a drug's activity. Nuclear magnetic resonance (NMR) spectroscopy and x-ray crystallography (XRC) are traditional methods for determining molecular structure and configuration. However, these techniques have drawbacks that can make the determination of absolute stereochemistry unachievable for certain compounds. Therefore, the development and application of alternate analytical techniques to expeditiously acquire quantitative markers for structural deconvolution of small molecules is essential. Vibrational circular dichroism (VCD) spectroscopy measures the differential response of a chiral molecule to left and right circularly polarized infrared light with every chiral molecule exhibiting a unique spectrum. Using conformational analysis software and ab initio quantum chemistry calculations, the VCD spectra for all possible diastereomeric configurations of a chiral molecule can be simulated. Comparison of the experimental VCD spectrum to the predicted spectra allows for assignment of absolute configuration by use of a confidence level algorithm. Additionally, VCD is an accepted technique for absolute stereochemistry designations as part of new drug applications to the FDA. The Analytical Chemistry Core (ACC) within the Division of Preclinical Innovation (DPI) at NCATS has been developing a VCD spectroscopy platform to expand our chiral analysis capabilities in the determination of absolute stereochemistry. We have acquired a BioTools ChiralIR-2X VCD spectrometer to perform the experiments while the computational analysis will use Schrödinger software in conjunction with Compute/Compare VOA. By having this analytical technique available as an internal resource, we are providing an alternative to

contracting out external analysis saving overall time and money. Preliminary application of VCD has already been highly beneficial to several medicinal chemistry projects. As such, the ACC is creating an automated conformational analysis program utilizing acquired VCD experimental data and leveraging the NCATS high-performance computing (HPC) cluster to perform structural calculations. This is of great importance to DPI Medicinal Chemistry programs as larger numbers of chiral molecules are being synthesized to impart greater target selectivity during structure optimization.

21RAM11: Raman Sensing

Chair: Phillip Wilcox
On-site Chair: Phillip Wilcox

(RAM-11.1) **Optimization Of A Cheap And Efficient Raman Sensor For Point Of Care Analysis**

Benjamin Charron[1], Jean-François Masson[2]; *[1]University of Montreal, [2]Université de Montréal*

In fifteen words or less, explain the significance of this contribution (Novel Aspect).: Raman sensor with large enhancement produced easily, cheaply and rapidly.

Abstract Text: Due to its ability to directly probe water containing samples as well as yielding specific signal, Raman spectroscopy is rapidly expanding to various fields. This technique shows great potential for directly probing the environment, especially the oceans from which we do not know much. Although Raman measurements can easily be done with a proper microscope, such instruments are expansive and can't be used in a point of care fashion. For a near real-time analysis in a point of care fashion, the Raman signal must be enhanced by a proper sensor. Such sensors can be fabricated by various methods including nanosphere lithography and E-beam lithography. Although these types of sensors are efficient, the methods used for their fabrication have drawbacks like high cost or long fabrication time for small sensors. Here we report the optimization of a Raman sensor fabricated using shrinking polymer as a support material. Metal is deposited on the polymer sheet, followed by heating to shrink the support. Doing so causes the metal film to wrinkle and become an efficient Raman sensor. Other means of signal enhancement such as nanoparticles, various metals, and oxidation were implemented and evaluated in these sensors, individually and all together. This process allows fabrication of multiple sensors of a few centimeters at once with minimal instrumental requirements. All sensors were evaluated with Raman reporters in water as well as in air with both a 633nm and a 785 nm laser.

(RAM-11.2) **Detection of Organophosphate Pesticides Residues in Edible Oils with Surface Enhanced Raman Spectroscopy**

Adam J. Hopkins[1], Wei Yu[2]; *[1]Metrohm USA, [2]Metrohm Raman*

In fifteen words or less, explain the significance of this contribution (Novel Aspect).: Demonstrate a reproducible, field deployable SERS assay that complements laboratory trace testing

Abstract Text: Organophosphate pesticides are widely used in olive cultivation for the control of insect pests. Due to the high solubility of these pesticides in oils and the minimal processing of virgin oils, these harmful chemicals may accumulate in the end product. Detection of pesticide residues typically require the use of analytical techniques such as GC-MS and HPLC, but technical complexity and cost requirements limits their usage to the confines of well-equipped laboratories. In this study, we demonstrate the reproducible use of SERS with a dedicated analysis platform, Misa, in detecting Fenthion in extra virgin olive oil (EVOO). Fenthion is an organophosphate that has been classified as a restricted-use insecticide by the US EPA. However, the use of this pesticide in olive orchards in Mediterranean countries results in EVOO that occasionally exceed the maximum residue limits (1mg/kg) established for virgin olive oil. Misa easily achieves sensitive trace detection of Fenthion in spiked EVOO after a simple organic solvent extraction in less than 15 minutes. The field deployable

assay is suitable for non-expert users and is based on the acquisition of SERS-specific spectra for Fenthion in the extract using Misa with a gold colloid solution. Misa features simple one-touch operation and guided workflows for many common pesticides and contaminants in a variety of food matrices, making it a valuable tool for the rapid detection and identification of trace food contaminants.

(RAM-11.3) **Rapid Microplastic Characterization Using a Raman Touch Probe**

Bridget O'Donnell[1]; [1]*HORIBA Scientific*

In fifteen words or less, explain the significance of this contribution (Novel Aspect).: Touch probe based Raman spectroscopy for rapid, inexpensive characterization of microplastics with possible field deployment

Abstract Text: Microplastic pollution is a ubiquitous problem caused by the ever-growing commercial production of plastics and their long lifetimes in the environment. Raman spectroscopy has been identified as a powerful technique to understand the nature of microplastics in the environment, with demonstrated chemical specificity of both polymers and common additives including pigments. However, confocal Raman microscopes are costly and require extensive user training. In addition, field deployment is difficult given precise optical alignment requirements and system size. Handheld systems, on the other hand, can suffer from low sensitivity and lack powerful software capable of advanced data processing, analysis, and database searching. In this study, a benchtop macroscopic Raman system equipped with touch probe is assessed as a compact and rugged option for microplastic characterization of particle sizes down to 100 μm. Assignments are compared with results generated using a confocal Raman microscope to verify chemical identification and assess accuracy.

(RAM-11.4) **Virocell identification via Raman microspectroscopy**

Indra Monsees[1], Victoria Turzynski[1], Sarah P. Esser[1], André Rodrigues Soares[1], Lara Timmermann[1], Michael Kloster[2], Bánk Beszteri[2], Alexander J. Probst[1]; [1]*Group for Aquatic Microbial Ecology, Environmental Microbiology and Biotechnology, University Duisburg-Essen,* [2]*Phycology Group, Department of Biology, University Duisburg-Essen*

In fifteen words or less, explain the significance of this contribution (Novel Aspect).: Shifts in Raman spectra can be used to differentiate virocells from non-infected cells

Abstract Text: Raman microspectroscopy allows the study of growth dynamics and heterogeneity of prokaryotic cells[1] as well as the characterization of viral particles[2]. Consequently, we hypothesized that Raman microspectroscopy is sensitive enough to characterize the biochemical changes of individual cells during infection with lytic viruses resulting in so-called virocells. This work was set out with the aim of identifying Raman marker shifts of viral infections in bacterial pure cultures using unsupervised machine learning algorithms to define a spectral marker using univariate statistics. To achieve this, two virus host systems (Pseudomonas sp./phage phi 6. and Bacillus subtilis/phage phi 29) were incubated and samples for Raman spectroscopy with and without phage addition were fixed and measured using a Renishaw inVia™ confocal Raman Microscope. The acquired spectra were analyzed using a Principal Coordinates Analysis (PCoA) and Orthogonal Partial Least Squares (OPLS). We identified significant differences in the spectra of single cells and cells after amendment of lytic phage and defined a general ratio of wavenumbers that contributed the greatest differences in the recorded spectra as an indicator for virocells. Our data suggests that Raman microspectroscopy is a robust tool for chemically discerning gram-positive and gram-negative virocells undergoing infection with lytic DNA or RNA viruses. We propose that Raman spectroscopy in combination with other microscopy techniques has a high potential for providing key information to detect virocells in environmental samples, based on a change in the ratio of the nucleic acid and protein band intensities. 1. Huang,

W.E., et al. (2004) Analytical Chemistry 76(15): p. 4452-4458. 2. Li, T., et al. (1993) Journal of Molecular Biology 230(2): p. 461-472.

(RAM-11.5) **Classification of Unknown Fentanyl Analogs using Raman Spectroscopy**

Phillip G. Wilcox[1], Jason A. Guicheteau[1]; [1]*US Army DEVCOM CBC*

In fifteen words or less, explain the significance of this contribution (Novel Aspect).: Ability to classify unknown fentanyl analogs using spectral barcode technique

Abstract Text: Fentanyl is a powerful synthetic opioid that has fueled the ongoing opioid crisis. The chemical structure of fentanyl consists of four main components: an aniline ring, a piperidine ring, an N-propionyl group, and a phenethyl group. The replacement or substitution of any of these functional groups creates fentanyl analogs of varying toxicity. The US Drug Enforcement Agency explicitly schedules 35 different fentanyl compounds but has a catch-all for any "fentanyl-related substances, their isomers, esters, ethers, salts and salts of isomers, esters and ethers." This broad definition poses a challenge for Raman detection instruments which work by comparing collected spectra to an internal library. As new analogs emerge and gain prominence, it is not always feasible, cost-effective, or timely to acquire a sample of the new compound, add a new element to the library, and disseminate the new library to operators in the field. Instead, it is preferable to have a screening method capable of classifying unknown fentanyl analogs while minimizing false alarms on other benign unknowns. In this effort we will demonstrate a method to perform classifying spectra as fentanyl or non-fentanyl using spectral barcoding.

September 28, 2021

21AES01: AES Lifetime Achievement Award Symposium Honoring Juan Santiago

Chair: Alexandra Ros
Co-Chair: Juan Santiago
On-site Chair: Alexandra Ros

(AES-01.1) **Microfluidics and CRISPR for detection of the RNA of SARS-CoV-2**

Juan G. Santiago[1], Juan G. Santiago[1], Ashwin Ramachandran[1], Diego Huyke[1], Jared Nesvet, Eesha Sharma, Malaya Sahoo[1], ChunHong Huang, Niaz Banaei, Benjamin Pinsky[1]; [1]*Stanford University*

The COVID-19 pandemic has made clear the need for point of care (POC) systems for rapid and sensitive nucleic acid-based detection directly from raw patient samples, including nasopharyngeal swab and blood. We present an update on our bioassay efforts including studies of fundamental CRISPR kinetics and development of microfluidic devices for detection of RNA of SARS-CoV-2, the virus responsible for COVID-19. We performed a theoretical and experimental study of fundamental CRISPR reaction kinetics. Despite their immense importance, we discovered that all but one of all CRISPR enzyme kinetics studies to date show data that grossly violate basic rules of mass conservation and rate laws, and hence likely report incorrect estimates of sensitivity. To demonstrate this, we performed measurements of CRISPR kinetics and developed experimentally validated models. We applied our findings to explore ultimate limits of detection and speed of CRISPR-based molecular diagnostic assays, including preamplification-free approaches. Our work prompted published errata in two seminal papers in the field. We also report on our ongoing development microfluidics devices for automated detection of SARS-CoV-2 using on-chip isotachophoresis (ITP). ITP is an electrokinetic technique that can selectively purify species, mix and preconcentrate target species by more than 1,000 fold, and accelerate biochemical reactions, including enzymatic reactions. We previously demonstrated a 40 min assay that uses on-chip ITP but this assay included manual steps for sample transfer. We are currently developing microfluidic devices which avoids these manual steps and uses electric field control and ITP to

integrate the following steps: Purification of nucleic acids, reverse transcription, LAMP amplification, CRISPR reaction, and fluorescence detection. Our reconfigurable assay and platform have potential to achieve rapid and sensitive detection of infectious diseases at the POC and/or low-resource settings.

(AES-01.2) **Isotachophoresis for staining and modifying intact cells**

Micahel Breadmore[1], Monica Alves, Sui Ching Phung, Shne Powell, Yi Heng Nai, Rosanne M. Guijt[2];
[1]*University of Tasmania,* [2]*Deakin University, Geelong, Australia*

The use of isotachophoresis to concentrate molecules into a small volume has been shown as a powerful approach to increase reaction kinetics of nucleic acids. Here, we extend this concept to introduce nucleic acid sequences into intact live cells. By concentrating both cells and small fluorescently labelled nucleotides (20-30 nt) matching the rRNA sequence of the cell by isotachophoresis, we perform rapid and selective staining for quantitation down to 6 x 104 cells/mL within 30 min. This process is not limited to small molecules, and can be used to introduce larger plasmid DNA (2.5 – 10 kb) for transfection of bacteria and transformation of eukaryotic cells. We demonstrate the rapid (30 min) modification of E. Coli and Jurkat cells by isotachophoresis without the length and process of making them compotent and lengthy overnight incubations with efficiencies and rates superior to those obtained with chemical transformation and electroporation.

(AES-01.3) **Microfluidic Particle Handling Using Combined Electrokinetic and Hydrodynamic Effects**

Elisabeth (Sabeth) Verpoorte[1]; [1]*University of Groningen*

The advent of microfluidics in the late 1980's has proven to be an extraordinarily important development for scientists in diverse fields stretching far beyond the analytical chemistry domain. The unique properties of flow at the micrometer scale, and the ability to be able to control these flows so very precisely in micromachined channels, have offered a myriad of experimental possibilities. This is especially true in processes involving electric fields to drive electrokinetic sample processing, as has been demonstrated by the groundbreaking contributions of Prof. Juan Santiago. We turned our attention a number of years ago to the precise manipulation of micrometer-sized particles in microfluidic channels, using a phenomenon we termed flow-induced electrokinetic trapping, or FIET. Underlying this process is the generation of bidirectional flow in a narrow channel by opposing electro-osmotic flow and pressure-driven flow. In straight, narrow channels that open up on both ends, this combination leads to recirculating flow patterns, in which it becomes possible to trap and concentrate micrometer-sized particles. Particle charge (zeta potential) determines the electric field-pressure balance required to trap a particular particle type. Thus, scanning the electric field applied makes separation of a mixture of particles having different charge possible. We also discovered that in fact the bidirectional flow pattern can be exploited to perform a form of hydrodynamic chromatography for separation of particles with different sizes. The fact that the two separation mechanisms co-exist in the same microsystem allows for particle samples to be separated orthogonally, opening a route to a wide range of possibilities for particle separation. Highlights of our work with this system will be presented in this session, showing work with polymer microspheres, lambda-DNA, and cells.

(AES-01.4) **Ion concentration shock waves: From microfluidics to porous media**

Martin Z. Bazant[1]; [1]*Massachusetts Institute of Technology*

Juan Santiago has made pioneering contributions to the field of microfluidic electrokinetics, including a variety of new methods of electrophoretic separations and electrokinetic mixing that leverage nonlinearities in ion transport. This talk will focus on the legacy of his seminal work with Ali Mani and Tom Zangle (2009) on ion concentration shock waves in micro/nanofluidic devices. It has since been shown that analogous deionization shocks, sustained by surface conduction and electro-osmotic convection, can also arise in any charged porous medium during the passage of over-limiting current (faster than diffusion). This has opened a new field of "shock electrochemistry", in which macroscopic ion transport and electrochemical reactions are mediated by

deionization shocks in porous media under exotic conditions of over-limiting current (faster than diffusion). For example, electrodeposition can be stabilized by shocks in charged porous media, leading to stable, dendrite-free metal batteries. Shocks in cross flow can also be used for continuous membrane-less ionic separations in the emerging technology of shock electrodialysis, which enables the continuous, low-cost separation of trace multivalent ions (such as heavy metal contaminants) from drinking water or industrial wastewater.

21ATOM04: Unique Plasma-based Sources for Chemical/Isotopic Analysis

Chair: C. Derrick Quarles Jr.
Co-Chair: Benjamin Manard
On-site Chair: C. Derrick Quarles Jr.

(ATOM-04.1) **The LS-APGD/Orbitrap Coupling: Uranium Isotopic Analysis and Beyond**

R. Kenneth Marcus[1], R. Kenneth Marcus[1], Edward Hoegg, David Koppenall, Tyler Williams, Jacob Bills;
[1]*Clemson University*

Isotope ratio (IR) analysis of natural abundance uranium presents a formidable challenge for mass spectrometry (MS): the required spectral dynamic range needs to enable the quantitatively accurate measurement of the 234UO2 species present at ~0.0053% isotopic abundance. Thermal ionization mass spectrometry (TIMS) and inductively coupled plasma - mass spectrometry (ICP-MS), particularly on multi-collector, sector-field platforms, have long been the gold-standards for isotope ratio (IR) analysis due to their abilities to obtain high precision measurements. Unfortunately, whereas these instruments provide high precision measurements, each method is not without its drawbacks. TIMS instruments are large, complex, and expensive, while having low throughput as a result of often-tedious sample preparation processes. ICP-MS instruments require large capital input, both in up-front costs and consumables, requiring argon flow rates of up to 14 L min-1. In both cases, mass resolution limitations to ~10,000 m/□m, create challenges which again lead to the need for extensive sample preparation procedures. We address these challenges by empowering a benchtop Orbitrap Fourier transform mass spectrometer (FTMS) coupled with the liquid sampling – atmospheric pressure glow discharge (LS-APGD) ion source. The LS-APGD microplasma has demonstrated impressive capabilities regarding elemental and IR analysis when coupled with Orbitrap FTMS. First, mass resolution in excess of 70,000 (and up to 1.7 M) alleviate many of the chemical separation needs. Second, by definition, the Orbitrap analysis is "simultaneous" for the ions of interest. Finally, the LS-APGD microplasma provides a high intensity, extremely stable ion beam. Despite successes, there are a number of experimental parameters and data acquisition operations which can have appreciable impact on the isotope ratio performance. In this presentation, we detail methods of optimizing the isotope ratio performance in the LS-APGD/Orbitrap coupling as it applies for uranium isotopic analysis. Comparisons are also made with the benchmark methods. Ultimately, this coupling holds incredible promise in this application, and by extension to other elements.

(ATOM-04.2) **Cold Atmospheric Micro Plasma: A Powerful Tool From Biomaterials to Biomedical Applications**

Prasoon K. Diwakar[1]; [1]*South Dakota School of Mines and Technology*

Cold Atmospheric micro-Plasma (CAP) has shown potential for wide range of applications ranging from biomedical applications (including bacterial disinfection, cancer treatment) to surface functionalization (hydrophobic vs hydrophilic surfaces). CAP is a directed, continuous plasma stream consisting of energetic ions, electrons and reactive species. CAP effluent or species, as the name suggests, cools to room temperature and can be safely used on biological cells and tissues as well as on several surfaces, materials including biomaterials, metals, ceramics, aerosols etc. without having any thermal effects. Cold plasma contains a certain amount of reactive oxygen and/or reactive nitrogen (ROS/RON) that contributes to redox reactions which leads to desirable affects in various applications. These species and their concentration can be controlled to achieve desired results. This presentation highlights novel applications of CAP to cancer and Type 1 Diabetes

therapeutics as well as 2D bio-film surface functionalization. Reactive oxidative species (ROS) produced by cold atmospheric plasma (CAP) will be explored as a potential option for apoptosis of cancer cells as well as for stimulation of cytokines directed for wound healing and anti-inflammatory effects in Type 1 Diabetes. In addition, effect of CAP species on 2D films including surface wettability characteristics will be presented.

(ATOM-04.3) **Vacuum Ultraviolet Radiation from Atmospheric-Pressure Discharges for Optical and Mass Spectrometries**

Jacob Shelley[1], Brian T. Molnar[2], Sunil P. Badal[2], Sanja Dmitrovic[3], James Foley[4], Tom Milster[3]; *[1]Rensselaer Polytechnic Institute, [2]Department of Chemistry and Chemical Biology, Rensselaer Polytechnic Institute, [3]James C. Wyant College of Optical Sciences, University of Arizona, [4]Botanisol Analytics*

Electrical discharges have been critical tools in spectroscopy for over a hundred years. Perhaps the most useful property of electrical plasmas is that the resultant reactive species span a wide range of potential energies (in excess of 20 eV). These high-energy reactive species are well-suited for ionization (mass spectrometry, MS) and optical excitation (spectroscopy). While low-pressure discharges have dominated the field of plasma-based spectrochemical analysis, atmospheric-pressure glow discharges have recently emerged as useful sources for UV-Vis spectroscopy and MS. They are advantageous because they are inexpensive to build/maintain, consume relatively little power (<50 W), and are amenable to a range of sample introduction approaches. Here, we explore the use of direct-current (DC) and alternating-current (AC) atmospheric pressure glow discharges (APGDs) as inexpensive, simple sources of vacuum-ultraviolet (VUV) radiation to enable excitation and ionization for optical and mass spectrometries, respectively. In one case, a helium APGD was used as an APPI source to ionize a range of analytes such as saturated alkanes, amines, alcohols, perfluorinated compounds, polyaromatic hydrocarbons, etc. for detection by mass spectrometry. The effect of discharge current on analyte ion signal was found to increase linearly with discharge current. The VUV emission spectra of these sources were measured and compared to commercially available, low-pressure hydrogen Lyman-alpha (HLA) optical sources. It was found that a DC-APGD sustained in a helium-hydrogen mixture offered superior spectral purity and HLA emission to much more complicated electron-beam sources. The possibility of using this plasma source for VUV Raman spectroscopy will also be discussed.

(ATOM-04.4) **Modulation of the solution-cathode glow-discharge and solution-anode glow-discharge using a rotating magnetic field**

Nicholas Hazel[1], Jaime Orejas Ibanez[2], Steven J. Ray[3]; *[1]University at Buffalo, [2]Department of Physics, University of Oviedo, [3]The State University of New York at Buffalo*

The solution-cathode glow discharge (SCGD) is a simple, low-power, portable plasma sustained directly upon a sample solution that is used for atomic emission spectrometry. By reversing the electrode polarity, a similar plasma can be formed that we call the solution-anode glow discharge. The SCGD and SAGD are miniaturized atmospheric pressure glow discharges sustained in open air between a metal anode and a liquid cathode. Sample solutions are directly sputtered and excited to undergo atomic emission. The discharges require less than 100W and require no external pressurized gas flow. The SCGD has been shown to have limits of detection on par with radially-viewed Inductively-Coupled Plasma Atomic Emission Spectrometry for many elements, and the SAGD has been shown to have further improved LODs for a select few elements including Cd, Ag, and Pb. Here, a rotating, permanent, rare-earth magnetic is used to physically modulate both the SCGD and SAGD. Time resolved voltage, current, and atomic emission measurements were taken while modulating the plasmas at a variety of rotation frequencies, plasma operating conditions (discharge gap, current). Emission characteristics of several elements, emission lines, and prominent background species were examined. Lock –in detection was examined as a means to improve the LODs of several elements while modulating the plasma. Additionally, pictures and video were taken of both the whole plasma and the surface of the solution-plasma interface.

(ATOM-04.5) **Laser scattering investigation into fundamental parameters of µs-pulsed radiofrequency glow discharge under optical emission spectroscopy elemental mapping conditions**

Kevin Finch[1], Gerardo Gamez[1], Harsshit Agrawaal[2], Hanuk Kwon; [1]*Texas Tech University, Department of Chemistry and Biochemistry,* [2]*Texas Tech University*

Glow discharges (GD) are advantageous for the high throughput, direct elemental analysis of solids with inherent depth-profiling capabilities (sputtering) and the ability to analyze light elements where most other techniques fail. Operating the GD using radiofrequency (RF) power further permits the analysis of nonconductive samples in comparison to conventional direct current (DC) powering schemes. However, under traditional GD optical emission spectroscopy (GDOES) plasma conditions, the sputtered atoms are known to mix in the discharge and a corresponding poor lateral resolution is typically observed. Nevertheless, elemental mapping (EM) has been made possible via GDOES by using pulsed-power operation while sustaining the discharge at higher operating pressures. However, under GDOES EM operating conditions, the underlying species behavior and governing mechanisms are not well understood, which leaves a need to perform fundamental systematic studies. The methods of choice to probe these species are laser scattering diagnostic techniques (Thomson, Raman, and Rayleigh) which have inherent spatial and temporal resolution, show minimal-to-no plasma perturbation (if the laser fluence is strictly controlled), and do not require the prior assumption of local thermodynamic equilibrium conditions. Thomson scattering also enables the simultaneous measurement of electron temperature and density, without propagating the error from one calculation to the next, as is unavoidable with other techniques (e.g. Boltzmann plots and Langmuir probes). Here, a recently constructed, transmission-type triple grating spectrograph, will be utilized to obtain spatiotemporally resolved maps of fundamental species parameters, pertaining to µs-pulsed RF GD operated under GDOES EM conditions. The effects of pressure (~7-19 torr), and voltage (~150-300 Vdc) will be studied as a function of spatial position in the plasma (~0-8 mm axially from cathode) and time along the pulse train (~0-80 µs after pulse leading edge). This systematically performed study will allow the gain of much needed insights into the governing mechanisms that permit GDOES EM under RF powering modes.

21BIM05: Evolving Technologies for Clinical Applications

Chair: Bridget O'Donnell
On-site Chair: Bridget O'Donnell

(BIM-05.1) **Plasmonic Gold Nanostar-Mediated Photothermal Heating Treatment**

Ren Abelard A. Odion[1], Yang Liu[1], Stephen Norton[1], Tuan Vo-Dinh[1]; [1]*Duke University*

In fifteen words or less, explain the significance of this contribution (Novel Aspect).: The interaction of light, gold nanostar plasmonics, and heating provide new insight in thermal therapy.

Abstract Text: Recently, gold nanoparticles have become increasingly used for laser-based photothermal treatment of diseases such as cancer. Due to their flexible synthesis tuning for laser absorption, gold nanostars (GNS) make an ideal candidate for photothermal treatment and can easily be coupled with other sensing modalities such as molecular imaging using surface-enhanced Raman spectroscopy (SERS) and Immunotherapy. The latter modality can be exploited with immune check-point inhibitors for a combination treatment called Synergistic Immuno Photo Nanotherapy (SYMPHONY). This technique has recently been shown to have effective and long-lasting immunity against both primary tumors and metastatic growths. However, careful consideration in laser illumination and GNS design must be evaluated in the context of the target tissue to achieve the best photothermal treatment outcome. In particular, the choice of laser wavelength and plasmon GNS absorption must not only match with each other but the tissue absorption of the surrounding tissue needs to be considered. To understand the interplay of optical and thermal effects of GNS mediated heating, theoretical simulations based on Monte Carlo light propagation simulations, analytical solutions to thermal diffusion and experiments using tissue phantoms are evaluated to investigate and understand the

optimal conditions of GNS heating for efficient and specific targeted therapy.

(BIM-05.2) **Rapid 15-minute LIBS-based Assay for Monitoring Onset of Cytokine Storms in COVID-19 Infection**

Xi Wu[1], J. Paul Robinson[2]; *[1]Department of Basic Medical Sciences, Purdue University, West Lafayette, IN 47907, [2]Department of Basic Medical Sciences and Weldon School of Biomedical Engineering, Purdue University, West Lafayette, IN 47907*

In fifteen words or less, explain the significance of this contribution (Novel Aspect).: Rapid detection of cytokines using LIBS shows great potential for clinical evaluation of COVID-19 infection.

Abstract Text: Accumulating evidence suggests that cytokine storm syndrome (CSS) induced by the SARS-CoV-2 may be the ultimate cause of acute respiratory distress syndrome (ARDS), resulting in severe outcomes of COVID-19 and potentially death. Elevated levels of serum interleukin 6 (IL-6) and interferon gamma-induced protein 10 (IP-10) correlate with the occurrence of respiratory failure, ARDS, and adverse clinical outcomes in many COVID-19 patients. The currently available clinical cytokine tests are costly, time-consuming, expensive, and require highly trained staff to execute. There is an unmet need for affordable, robust, rapid, and sensitive tests for cytokine and chemokine levels. Therefore, this study aimed to develop a cost-effective system for the quantitative detection of cytokines that can be used in the point-of-care (POC) format. Our approach combines detection based on laser-induced breakdown spectroscopy with a lateral flow immunoassay (LIBS-LFIA) to deliver a quantitative clinical analysis platform with multiplexing capability. Lanthanide-complexed polymers (LCPs) were selected as the labels to provide the optimal quantitative performance when sensing the signals from the test (T) lines of LFIAs. For a prototype implementation and a proof-of-concept, we targeted IL-6 as it is one of the most critical pro-inflammatory cytokines. Our LIBS-LFIA biosensor can achieve a detection limit of 0.2298 μg/mL of IL-6 within 15 min, demonstrating superiority to several conventional methods. Importantly, we introduce a new direction for LFIA design and optimization based on geometric flow control (GFC) of nitrocellulose (NC) membranes, leading to increased sensitivity. This novel technique enables comprehensive flow control via various membrane geometric features such as the width and the length to improve analytical performance and reduce antibody consumption. The performed experiments also illustrate the importance of the proper choice of NC membranes in the assay design. NC170 and NC120 membranes were selected and optimized for our LIBS-LFIA detection of cytokines. Our research demonstrates a great promise of the LIBS-LFIA approach to bio-detection. It provides evidence that rapid and accurate detection of cytokines for clinical diagnosis and prognosis of COVID-19 and other pathogenic infections using LIBS is highly feasible and compatible with the POC format.

(BIM-05.4) **In Vivo Assessment of Porcine Osteochondral Repair in the Vis-Near Infrared Region**

Shital Kandel[1], William Querido[1], Hannah Zlotnick[2], Ryan Locke[2], Robert Mauck[2], Nancy Pleshko[1]; *[1]Temple University, [2]University of Pennsylvania*

In fifteen words or less, explain the significance of this contribution (Novel Aspect).: Novel nondestructive evaluation of cartilage repair using a Vis-NIR fiber optic probe.

Abstract Text: Cartilage defects and degeneration cause disability and pain in millions of people. Detection of tissue pathology and monitoring progression of repairing cartilaginous tissues can be done by visual arthroscopy whereby a tactile probe is used to assess the tissue. However, such investigations are highly subjective and may result in suboptimal assessment. Visible-near infrared (Vis-NIR) spectroscopy of joint tissues, including articular cartilage and subchondral tissue, by fiber optic probe can provide an approach for quantitative

assessment of tissue. However, understanding the spectra in an in vivo environment is challenging due to overlapping tissue absorbances. Previously, NIR spectroscopy equipped with a fiber optic diffuse reflectance probe was used to investigate cartilage and subchondral bone properties, where information from the NIR first optical window (650 – 950 nm) was useful for characterizing and estimating subchondral bone properties, and thus potentially could be adapted for arthroscopy. Expansion of the range to include the visible region (350-650 nm) incorporates hemoglobin absorbances which could be useful for assessment of repair tissue healing. The objective of the current project is to identify spectral markers in normal and repairing tissue in the Vis-NIR spectral region (350-2500 nm, 28000-4000 cm-1). Full chondral defects were made in Yucatan mini pigs using a 5 mm biopsy punch (under an IACUC protocol) and repair with the microfracture (MFx) technique was performed. Tissues were evaluated at 1 and 3 months post-repair (N = two animals each timepoint). Three Vis-NIR spectra were collected from the defects and nearby normal articular cartilage after euthanasia. In the visible region, hemoglobin absorbance peaks (540 nm and 570 nm) in spectra from 1 month MFx repair tissue were significantly higher compared to 3 month repair tissue, indicating the presence of more blood, and thus less tissue present. In the NIR spectral region, the intensity ratio of water absorbances at 5200/7000 cm-1 was significantly higher in 1 month compared to 3 months repair tissue, indicative of a greater influence of subchondral bone signal in the one month repair tissue. Together, these data provide support for non-destructive monitoring of the progression of cartilage repair using Vis-NIR arthroscopy.

(BIM-05.5) **Label-free Stimulated Raman Scattering Imaging Reveals Silicone Implant Residues In Breast Tissue**

Robert W. Schmidt[1], Ludo van Haasterecht[1], Liron Zada[1], Erik de Bakker[2], Freek Ariese[3]; *[1]Vrije Universiteit Amsterdam, [2]Amsterdam UMC Location VUMC, [3]LaserLaB, Vrije Universiteit Amsterdam*

In fifteen words or less, explain the significance of this contribution (Novel Aspect).: We describe a label-free method to locate and identify transparent silicone (PDMS) in breast tissue.

Abstract Text: Millions of women worldwide have silicone gel breast implants. Long-term structural integrity of these implants is poor, and they can rupture or 'bleed' silicone into the surrounding tissue. Currently, no histopathological technique exists that can specifically detect silicone in tissue, neither before or after staining. Stimulated Raman Scattering microscopy (SRS) is a fast and powerful, label-free imaging technique based on chemical contrast with sub-micron sized resolution. Therefore, silicone distribution images can be easily made with SRS to specifically identify silicone and distinguish it from other chemical components in the tissue. Here we describe a robust method for silicone detection in ex vivo breast tissue [1]. SRS imaging was performed on H&E stained histology slides in the C-H stretching region. These samples had been obtained earlier after implant removal and had been prepared according to standard histopathological protocols. Two optimal wavenumbers were calculated, by minimizing the root mean square error (RMSE) between the silicone and protein (background) spectra. Then, the whole tissue was imaged by a quick scan with a pixel size of 1.60 µm. Finally, selected regions of interest (ROI) with high silicone content or nuclei density were imaged by a high-resolution scan with a pixel size of 0.52 µm. Image processing operations, such as image subtraction, applying threshold and co-registration with a histological color image, were carried out using MATLAB. Data provided by connected component analysis assisted the statistical interpretation of silicone distribution in the tissue. This method was found to be very suitable for identifying silicone debris from leaking implants and quantifying silicone in the surrounding tissue. We are currently investigating the relationship between silicone particulates and the severity of capsular contracture. We are also studying options for applying SRS directly on freshly frozen tissues.

21IR02: Advances in Photothermal Spectroscopy

Chair: Rohith Reddy
Co-Chair: Curtis Marcott
On-site Chair: Rohith Reddy

(IR-02.1) **Photothermal Mid-infrared Spectroscopic Imaging for Disease Diagnosis**

Chalapathi Gajjela[1], Rupali Mankar[1], Sharmin Afrose[2], Ragib Ishrak[2], Xinyu Wu[2], David Mayerich[1], Rohith Reddy[2]; *[1]Department of Electrical and Computer Engineering, University of Houston, [2]University of Houston*

In fifteen words or less, explain the significance of this contribution (Novel Aspect).: We present advances in optical-photothermal imaging for ovarian cancer analysis and identification of bone disorders.

Abstract Text: Vibrational spectroscopy using mid-infrared light enables biochemical identification in tissue. Biomedical samples such as cancerous tissue are chemically heterogeneous, and bulk spectroscopy is inadequate to understand such samples. Mid-infrared spectroscopic imaging (MIRSI) combines the molecular specificity of vibrational spectroscopy with the spatial detail provided by microscopy. Traditionally, MIRSI has been performed using Fourier transform infrared (FT-IR) imaging. The combination of machine learning and MIRSI has facilitated tissue sub-type and cancer grade identification in a label-free and quantitative manner. We will discuss these technologies in the context of emerging MIRSI instrumentation. Innovations in Quantum Cascade Lasers (QCLs) have revolutionized MIRSI, and new techniques such as discrete frequency infrared (DFIR) and photothermal IR imaging have emerged recently. These technologies are more flexible, provide higher resolution, and have important advantages over FT-IR. We will present results comparing FTIR and new MIRSI technologies and discuss the benefits of each technology. Ovarian cancer is one of the deadliest cancers among women in the U.S., with over 22,000 women diagnosed with the disease every year. Early diagnosis of the disease is essential for improving survival. To automate the process of disease diagnosis, we perform MIRSI imaging followed by machine learning. However, this requires data of higher quality and resolution. We use a new technique based on photothermal absorption to improve the resolution by order of magnitude relative to FT-IR imaging and present results of ovarian tissue analysis using the new approach. Bone disorders such as osteosclerosis and collagen deposition have spectroscopic signatures that can be identified using are MIRSI. We present imaging data and results of high-resolution MIRSI of bone samples. We also present the first study to demonstrate the ability to spectroscopically identify thin collagen fibers (≈1μm diameter) and their orientations, critical for accurate grading of human bone marrow fibrosis.

(IR-02.2) **Correlative Photothermal IR and Fluorescence imaging of biological samples**

Kathleen M. Gough[1], Gorkem Bakir[2], Atacenk Baslic[2], Kelsey Gsell[3], Mustafa Kansiz[4], Eoghan Dillon[4], Laurent Kreplak[5], Samuel Veres[3], Sabine Mai[2], Laurent Bozec[6]; *[1]Dept of Chem, Uni. of Manitoba, Winnipeg, Canada, [2]University of Manitoba, [3]Dalhousie University, [4]Photothermal Spectroscopy Corp., [5]Dahousie University, [6]University of Toronto*

O-PTIR spectroscopy and imaging have filled a significant gap in IR analysis of biological materials. Our group has worked mainly with collagenous materials and with mammalian cells; our latest results are presented here. Collagen is one of the most widely studied proteins because of its natural abundance, its unique triple helix molecular structure and its critical role in connective tissues, as well as its role in scar formation and the development of synthetic collagen-based scaffolds. The functional properties of collagen-based mammalian tissues are determined by their complex hierarchical structures, chemical cross linking and post-translational modifications. Positional tendons (e.g. digital extensors and flexors of the hand and foot) enable precise motion; load-bearing tendons (e. g. Achilles) provide the energy storing/release capability for forward motion, including repetitive, highly stressful actions such as running. We are studying collagen from two different tendons of the bovine forelimb: the (energy-storing) superficial digital flexor tendon, and the (positional) common digital extensor (CDE) tendon. For this, we have used Far-field IR with FPA detection at 5000 nm pixel resolution,

nanoFTIR at ~25 nm resolution, and now O-PTIR with 500 nm, oversampled at 100 nm, pixel resolution, on intact tissues and on fibrils. Amide I and Amide II band positions, band shapes and relative intensities are sensitive to molecular conformation, and are readily identifiable through polarized IR spectroscopy (Bakir et al. 2020 Molecules, 25:4295). Our goal is to determine whether there are spectroscopically distinct differences in these functionally distinct collagen fibrils, and to evaluate structural alterations following mechanical damage. The relationship between the structural and mechanical properties is essential for understanding remodeling events under mechanical overload/rupture in tendons and for the development of bioengineered materials. O-PTIR studies are also underway on mammalian cells, where IR spectra and images are correlated with images obtained as bright field (before) and fluorescently stained (after).

(IR-02.4) **Bone quality and mineralization assessment at sub-micron resolution**

Nancy Pleshko[1], William Querido[1], Emily Reiner, Frank Weston[2]; *[1]Temple University, [2]Photothermal Spectroscopy Corp*

Bone is a complex material comprised primarily of collagen and carbonated calcium phosphate. Bone fragility and fracture increases with age, disease, and with use of certain therapeutics. Factors that underlie the increased risk include changes in overall bone mineral density (BMD) and tissue-level bone composition. Variations in bone composition have been widely investigated using vibrational spectroscopy at the microscopic level (6-50 μm spatial resolution). However, since bone is a nanocomposite material, variations of bone composition that contribute to pathology undoubtedly occur on a sub-micron scale. Here, we demonstrate the novel application of optical photothermal infrared (O-PTIR) spectroscopy to assess bone composition at sub-micron resolution. O-PTIR point spectra, hyperspectral images and single-wavenumber images were collected using a mIRage spectrometer (Photothermal Spectroscopy Corp.). To validate the application of this method in assessing bone mineral content, we analyzed bone powders subjected to different degrees of demineralization. To illustrate the application of this method for assessment of relevant bone samples, we analyzed cross-sections of human tibias and femurs, and murine femurs embedded in thick polymethyl methacrylate (PMMA) blocks. Minimal sample preparation was necessary other than ensuring the surface was exposed. Mineral content was quantified based on the phosphate/amide I ratio, using either band areas or second derivative intensities at 1040 and 1660 cm-1, respectively. Our validation showed that quantification of bone mineral content based on O-PTIR spectra was strongly correlated ($r>0.88$, $P<0.05$) to values obtained using standard Fourier transform infrared (FTIR) spectroscopy. Application of this method for analysis of bone sections allowed obtaining single point spectra, line-scans and images from single osteons (cortical bone) and trabeculae (trabecular bone) at 100-500 nm spatial resolution. Differences in tissue mineralization between cortical and trabecular bone, and details of the distribution of mineral and collagen across osteon layers and trabecular regions were apparent. Data from this study establishes the sensitivity of O-PTIR spectroscopy to assess differences in bone tissue composition at sub-micron resolution. The ability to apply this method to a variety of samples with minimal preparation requirements could significantly advance insights into mechanisms and biomarkers of bone fragility and fracture.

21LIBS05: Microanalysis Using LIBS

Chair: François Doucet
On-site Chair: François Doucet

(LIBS-05.1) **LIBS Hyperspectral Imaging Applications**

François Doucet[1], Lütfü Özcan[2], Kheireddine Rifai[2]; *[1]ELEMISSION inc., [2]ELEMISSION INC.*

Laser-Induced Breakdown Spectroscopy (LIBS) is emerging as a valuable asset for multi-elemental micro analysis of surface. Although, the microanalysis advantages have been identified soon after the first LIBS experiments, however, the real recognition of the usefulness of LIBS as a microprobe is rather recent. Scanning Electron Microscope tandem with Energy Dispersive Spectroscopy (SEM-EDS) is still the gold standard when

talking about multi-elemental analysis at microscale or lower. Micro X-ray Fluorescence (μXRF) has helped to enlarge the accessibility of microprobe analysis, nevertheless the instantaneous sensitivity of the technology is limiting the scanning speed due to long dwell-time. LIBS is an Atomic Emission Spectroscopic (AES) based technique, which has the reputation to be far more sensitive than XRF or any other direct photon-based spectroscopic techniques. Absolute sensitivity in the femtogram have been reported by many researchers for single laser-induced plasma. This instantaneous sensitivity enables unmatched scanning speed for microanalysis of surfaces in many application fields. In this communication, we will report the application landscape of LIBS as a microprobe, including tomography, a revolutionary binder design for laser-based analytical application.

(LIBS-05.2) **LIBS for the Identification and Quantification of Water Ice and Platinum Group Elements in Lunar Regolith Simulants**

Frédéric Diotte[1], Myriam Lemelin[1], François Doucet[2]; [1]*Université de Sherbrooke,* [2]*ELEMISSION inc.*

Assessing the abundance, distribution, and form of volatiles within the lunar regolith is frequently mentioned as a main scientific objective for space exploration. A report from Keck Institute for Space Studies (Hayne et al., 2013) states as a requirement the ability to "Determine the concentration of water in the upper few meters of lunar regolith with sensitivity better than 0.5 wt%". Remote sensing missions from the past decades have allowed demonstrating the presence of water at the lunar poles. One main limitation for mapping H20 distribution on the lunar surface is the coarse spatial resolution of past and current orbiting spectrometers. Data of finer resolution would be of great importance to better understand the processes involved in the water cycle on airless bodies. Water could also be used as a key resource for human exploration. Robotic exploration of the lunar poles is therefore seen as a key step to prepare for humans landing on the Moon. In this instance, we explore the capabilities of LIBS for the quantification of water in two icy regolith simulants. A first type of sample was prepared by homogeneously mixing liquid water with the powdered material. The second category includes snow crystals that were mixed with the sample in a solid-solid manner. Univariate regression models were computed to analyze the average Hβ intensity of emission from calibration standards containing varying H20 concentration. No differences between samples containing infiltrated water and snow were observed. Regressions provide a good linear fit (R2=0.91) between 0.5 and 10 wt.% of water ice. This range indicates that a LIBS device on the Moon could be used to quantify concentrations of 5.6 wt.% of water, as were detected by LCROSS (Colaprete et al., 2010) in Cabeus crater. This technique would also be relevant for the quantification of water within other high-priority craters for lunar landing, such as Faustini, Shoemaker, Amundsen, as defined by Lemelin et al. (2021; The Planetary Science Journal, in press) based on ice detections by Li et al. (2018; PNAS). Finally, we will also report the progress made in detection of PGE mixed with regolith under vacuum conditions.

(LIBS-05.3) **Chemical and Mineralogical Characterization of Palladium and Platinum Ore Samples Using Laser-Induced Breakdown Spectroscopy and Micro-XRF**

Nessrine Mohamed[1], Marc Constantin[1], Kheireddine Rifai[2], François Doucet[3], Lütfü Özcan[2], Mohamad Sabsabi[4], Samira Selmani[5], Raphaël St-Cyr[1], François Vidal[5]; [1]*Université Laval,* [2]*ELEMISSION INC.,* [3]*ELEMISSION inc.,* [4]*National Research Council Canada,* [5]*INRS-EMT*

Laser-Induced Breakdown Spectroscopy (LIBS) is increasingly gaining ground in the mining industry due to its rapidity and high sensitivity for all elements. This emerging analytical technique can constitute an efficient alternative to standard techniques used by mining staff to characterize geochemical and mineralogical contents of ore samples. This study demonstrates the use of LIBS for the chemical and mineralogical characterization of Pd and Pt in ore core samples from the Lac des Îles mine (Ontario, Canada). It is the first of its kind allowing the establishment of calibration curves for measuring Pd-Pt contents at low concentrations up to ppm level within ore samples. In this framework, TESCAN Integrated Mineral Analyzer (TIMA), Scanning Electron Microscopy (SEM), Optical Microscopy and Electron Probe Microanalysis (EPMA) were used to calibrate the

LIBS instrument for the mineralogical characterization of Pd-Pt ore samples whereas micro X-ray fluorescence (μ-XRF) mapping was achieved to independently evaluate LIBS mapping results. For Pd and Pt quantitative analyses, the LIBS instrument was calibrated with reference materials. Two rock types were scanned by both LIBS and μ-XRF for their spatial characterization: pyroxenite and gabbronorite. LIBS mineralogical mapping allowed the identification of 9 mineral phases including silicates (chlorite, plagioclase, actinolite and hornblende), sulfides (Pd-bearing pentlandite, chalcopyrite, pyrrhotite and pyrite) and an oxide (ilmenite). LIBS mineralogical maps display not only the spatial distribution of the identified mineral phases but also their abundances in area % over the scanned surfaces of samples. Further, LIBS analysis allowed to establish the distribution chemical map of Pd. It was revealed that Pd distribution was very similar to that of pentlandite from LIBS mineralogical maps. Overall LIBS and μ-XRF maps were in very good agreement for the distribution, composition and abundance of most of the identified mineral phases. The obtained results showed the potential of LIBS to perform extremely rapid (e.g. two samples, acquisition time of 7 minutes for LIBS vs 5 hours and 23 minutes for μ-XRF) high-resolution mapping and automated mineral phase identification of Pd-Pt ore samples. This study contributed to demonstrate how useful LIBS can be for mining industry applications, to both mineral exploration and production.

(LIBS-05.4) **Compression And Data Fusion Strategies For Laser Induced Breakdown Spectroscopy (LIBS) and Plasma Induced Luminescence (PIL) Hyperspectral Images**

Alessandro Nardecchia[1], Ludovic Duponchel[2], Anna de Juan[3], Vincent Motto-Ros[4], Michael Gaft[5]; *[1]Université de Lille, [2]University of Lille, [3]Universitat de Barcelona, [4]Institut Lumiere Matiere University of Lyon, [5]Ariel University*

In fifteen words or less, explain the significance of this contribution (Novel Aspect).: Chemometrics approaches applied to LIBS and PIL hyperspectral images for a deeper interpretation of data

Abstract Text: LIBS imaging is an essential tool for elementary characterization of complex samples in many scientific domains. Unmixing techniques, such as Multivariate Curve Resolution-Alternating Least Squares (MCR-ALS) allow the efficient way to explore the complexity of this kind of data providing multi-elemental spectral signatures and related distribution maps linked to the different constituents. Despite the suitability of the imaging technique, several challenges need to be addressed. Particularly, the huge image size, pixel- and spectral-wise, and also the consequent risk of missing minor components, located in very small areas of the image. Create a data analysis pipeline capable of correcting the instrumental artifacts, removing the redundant information preserving also very specific relevant areas and speeding up the analysis is not only necessary, but crucial. The proposed procedure starts with a detection of abnormal signals followed by a spectral baseline correction and cropping of the image background. For compression, the full image is divided in small blocks, easier to be handled. Then, SIMPLISMA is applied to compress the spectral channels (taking advantage of the fine spectral features of LIBS) followed by a pixel selection. MCR analysis will work only with the double-compressed purest information coming from all sample blocks analyzed in a resized dataset (the 1% of the initial information). The full spectral signatures and complete distribution maps are easily recovered combining the MCR results and the information in the extended pixel and spectral dimensions. On the other hand, by the observation of PIL images, it is possible to notice that some zones of the samples are highlighted by PIL but not by LIBS. This phenomenon happens when the matrix subjected for plasma excitation is capable to emit luminescence. This leads to the interest of fusing together these two correlated effects, with the aim of obtaining a more complete picture of the sample. A similar pretreatment is applied for the PIL dataset. The LIBS and PIL datasets (1100 x 2000 pixels x 2048 spectral channels) selected for this research study is a kyanite, a mineral with composition Al2SiO5 that shows the heterogeneity of several trace elements (mainly iron, calcium, vanadium, titanium and chromium).

(LIBS-05.5) **Analysis of Fluorine in Polymer Samples Using LIBS via Measurement of Molecular Emission Bands**

Andreas Limbeck[1], Maximilian Weiss[1], Zuzana Gajarska[1], Georg Ramer[2], Bernhard Lendl[2], Hans Lohninger[1]; *[1]TU Wien, Institute of Chemical Technologies and Analytics, Getreidemarkt 9/164-I²AC, 1060 Vienna, Austria, [2]TU Wien*

In fifteen words or less, explain the significance of this contribution (Novel Aspect).: Proposed LIBS procedure enables quantitative and spatially resolved analysis of fluorine in polymer samples

Abstract Text: Synthetic polymers are widely used materials with a broad range of applications in all kinds of industries. At the end of their life-cycle, these high performance polymers often end up in the environment, either as packaging waste or in the form of micro-plastics, both of which pose a significant threat to various ecosystems. Since fluorine-containing polymers exhibit an extremely long lifetime in the environment, there is an increasing need for their qualitative and quantitative analysis. However, compared to the elements chlorine, bromine and iodine, the measurement of fluorine is a rather difficult task. Moreover, the enhanced inertness of these polymers requires the use of sophisticated sample pretreatment procedures for sample dissolution, which hampers the application of inductively coupled plasma mass spectrometry (ICP-MS), ion chromatography (IC) or ion-selective electrodes. One of the techniques showing a potential for the direct analysis of fluorine in polymers is laser induced breakdown spectroscopy (LIBS). However, sensitivity of fluorine analysis is limited as a result of the high excitation energy of this element. Recently, it has been shown that molecular LIBS allows to improve the detection sensitivity of such analysis. In this approach a molecular emission of the element of interest is detected instead of the atomic line itself . For analytical purposes, either an element naturally occurring in the sample or an element intentionally added to the sample can be used as a partner for the formation of molecules. In the literature, several approaches of the latter are reported, including spiking of a powdered sample with an additive or introduction of a nebulized liquid standard on the sample surface during the LIBS analysis. As these approaches allow only bulk investigations, we would like to present a novel method for the introduction of the element acting as a molecule building partner for the fluorine detection, opening up the possibility of fluorine imaging in solid samples. Applicability of the proposed methodology is demonstrated for actual research tasks, in particular the quantitative measurement of fluorine in unknown polymer samples, and the mapping of the fluorine distribution in artificial polymer waste.

21PAT02: PAT Pharma/Biotech

Chair: Daniel Hill
On-site Chair: John Wasylyk

(PAT-02.1) **Spectroscopy-based sensors in Biopharmaceutical Manufacturing**

Karin M. Balss[1], Treavor Jones[2], Christopher Mahoney[2], Olav lyngberg[2], Rahel Eberle[2], Marius Müller[2], Raf DeDier[2]; *[1]Janssen Supply Group, LLC, [2]Janssen Pharmaceuticals*

Spectroscopic sensors are playing an increasing role in biopharmaceutical manufacturing. For example, monitoring suspension-based cell culture processes with in-line sensors can provide real time information about nutrient feed, metabolites, biomass, product yield, and quality attributes. Evaluating downstream unit operations can provide information about both the product concentration and its molecular structure, ensuring that quality is maintained during purification, polishing, and formulation. In drug product operations, sensors are providing information about both the product and excipients which are key to establishing the final product quality attributes. Finally raw material evaluations link spectral fingerprints to yield or other performance attributes. This work will provide examples within our group to assess the utility of vibrational spectroscopy sensors amongst different unit operations in drug substance and drug product biopharmaceutical manufacturing. In some examples, comparisons will be made between Raman and infrared spectroscopy for evaluating

concentration and product quality attributes. Finally, we will discuss how these sensors fits into our overall strategy to enable real time release for biopharmaceuticals.

(PAT-02.2) **Application of Online UPLC in ASO Process Development and Manufacturing**

Andrew Argo[1]; [1]*Biogen*

The current approach in monitoring and controlling the deprotection reaction of oligonucleotides requires the use of an offline HPLC with significant sample preparation. Integration of the Waters PATrol in the manufacturing suite allows for real time sampling, monitoring, data processing and reporting during oligonucleotide deprotection to simplify reaction end point determination. Controlling this process with automation software further removes the need for manufacturing associates to tend to the system allowing for a streamlined workflow that promotes an environment that allows for associates to focus on tasks required for successful product purification.

(PAT-02.3) **Improving Biologics Downstream Processes with Real-time Measurements**

Jim Cronin[1]; [1]*Mettler Toledo Autochem*

The complexity of modern biologics paralleled with advances in upstream processes now highlight challenges in many downstream processes. Traditionally, tangential flow filtration (TFF) requires extensive manual interactions and sampling for process characterization. Multiple analytical methods may be required and results are limited by turn-around of analytical services. An alternative method, in-situ infrared spectroscopy, provides fast and quantitative measurements of multiple components, simultaneously and in real time. This presentation will examine technology gaps in current UF/DF measurement practices process characterization; also demonstrating the comparative advantages of in-situ infrared measurements. Examples will highlight the large dynamic range in aqueous systems for ATR-based IR, measuring drug substance and multiple excipient concentrations simultaneously, from below 1 mg/mL to in excess of 300 mg/mL. Notable advantages of in-situ spectroscopy include: • Process fingerprinting as a function of time-resolved parameters • Accelerated UF/DF development characterized by real-time data, without the need for analytical services • Automates the recording of comprehensive and structured data • Process automation and feedback control; enables informed and immediate decisions • Reduces manual interactions and enable scientists to focus on high value tasks

(PAT-02.4) **Raman as a solution for biopharma applications**

John Richmond[1], Brian Marquardt[1], Tom Dearing[1]; [1]*MarqMetrix Inc.*

In fifteen words or less, explain the significance of this contribution (Novel Aspect).: The importance of sampling, stability and reproducibility when applying Raman to monitor a bioprocess

Abstract Text: This presentation will focus on the practical aspects of applying Raman spectroscopy for the measurement and control of biological processes. A successful application of Raman starts with selecting the correct hardware for the required application and measurement. Selection of the system laser wavelength, spectral range and detector response function will be covered. Other factors such as system temperature and intensity stability will also be discussed with regards to calibration and model transfer. Once the hardware system has been determined the next and probably most critical decision is determining the correct sampling interface for the application. The correct sampling interface drives both the capability and reproducibility of the Raman system for accurately measuring the bioprocess attributes. This is especially true in many bioprocessing applications where the sample is heterogeneous. The final consideration is the modeling approach to take with the bioprocess Raman data. The discussion will include effective data pretreatment algorithms, best modeling approaches to determine both consumption of reactants and formation of products and how to use the information for improved process understanding and control.

(PAT-02.5) **Cell Culture Media Monitoring By Time-Gated Raman Spectroscopy**

Amuthachelvi Daniel[1], Miia Mikkonen[1], Mari Tenhunen[1]; [1]*Timegate Instruments Oy*

In fifteen words or less, explain the significance of this contribution (Novel Aspect).: Quantitative analysis of cell culture media spiked with phenylalanine.

Abstract Text: Cell culture productivity is one of the major challenges in the complete production cycle of biotherapeutic proteins. Online monitoring and control of the complex cell culture media alleviates this risk. Although Raman spectroscopy has been extensively explored for the quantitative analysis of cell culture media, native fluorescence of the media solution poses a great challenge. Time-gated Raman spectroscopy resolves the issue by collecting the photons before the advent of fluorescence. In this pilot study, we have spiked cell culture media with different concentrations of Phenylalanine and measured. Multivariate statistical analysis for this dataset yielded a model with limit of detection of Phenylalanine as 0.5603 mMol and limit of quantification as 1.70 mMol.

21RAM02: SERS

Chair: Roy Goodacre
On-site Chair: Zachary Schultz

(RAM-02.1) **Application of SERS sensors for ultrasensitive detection of SARS-CoV-2 biomarkers**

Jaebum Choo[1]; [1]*Chung-Ang University*

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pandemic has caused significant social and economic problems worldwide. Currently, RT-PCR, which detects RNA inside a virus, is used as the standard diagnostic method for SARS-CoV-2 but the total diagnostic time, including sample preparation, gene amplification, and detection, requires approximately 3-4 h. Various rapid kits for immunodiagnosis using antigen-antibody reactions have also been developed and commercialized to shorten the diagnosis time. However, they have not been adopted as the standard diagnostic method owing to their low LoD and poor accuracy. In particular, false-negatives obtained by commercialized immunodiagnostic kits is a severe problem that can aggravate the spread of SARS-CoV-2. To resolve this problem, we developed a new SERS-based immunodiagnostic assay platform capable of quantifying SARS-CoV-2 lysate with a high sensitivity. In this study, a spike protein DNA aptamer was used as a receptor, and a self-grown Au nanopopcorn surface was used as a SERS detection substrate for the sensible detection of SARS-CoV-2. A quantitative analysis of SARS-CoV-2 lysate was performed by monitoring the change in the SERS peak intensity caused by the new binding between the aptamer DNA released from the Au nanopopcorn surface and the spike protein in the SARS-CoV-2 viron. This technique enables detecting SARS-CoV-2 with a LoD of less than 10 PFU/mL within 15 min. The results of this study demonstrate the possibility of a clinical application that can dramatically improve the detection limit and accuracy of the currently commercialized SARS-CoV-2 immunodiagnostic kit.

(RAM-02.2) **Paper-based Diagnostics at the Point-of-Care**

Samuel Mabbott[1], Siddhant Jaitpal[2], Suhash Chavva[2], Monika Schechinger[2], Gerard Coté[2], Mahua Choudhury[3]; [1]*Department of Biomedical Engineering, Texas A&M University; Center for Remote Health and Technologies & Systems, Texas A&M Engineering Experiment Station,* [2]*Texas A&M, Biomedical Engineering,* [3]*Department of Pharmaceutical Sciences, Texas A&M Irma Lerma Rangel College of Pharmacy*

Point-of-care testing enables a diagnosis to be made where healthcare is provided close to or near the patient resulting in immediate and informed decisions about patient care. Paper-based devices, including lateral flow tests capable of detecting disease biomarkers, have significantly impacted point-of-care diagnostics. Many different assay formats targeting a multitude of biomarkers have been successfully translated onto paper,

making diagnostic testing more accessible. In addition, there is a vast array of particles that can be biofunctionalized and integrated into paper-based test formats; however, most currently available tests only utilize colorimetry as a method of measuring biomarker levels. Using dye-modified noble metal nanoparticles, it is possible to measure both colorimetric and spectroscopic (SERS) signals to quantify biomarkers. We have successfully developed two paper-based molecular tests to detect emerging biomarkers associated with HIV and pre-eclampsia at the point-of-care. In my presentation, I will discuss the need for the devices, their development and also outline future advances that need to be implemented to improve these tests.

(RAM-02.4) **Nanostructured Probes for Virus Detection: Reducing Costs while Retaining Selectivity and Sensitivity**

Laura Fabris[1], Hao Wang[2], Kholud Dardir[3], Zhaolin Xue[4], Sasanka Ulapane[3], Kevin Christian[3]; *[1]Rutgers, the State University of New Jersey, [2]Duke University, [3]Rutgers University, [4]University of Massachusetts Amherst*

Emerging viral pathogens such as SARS-CoV-2 can transmit undetected from person to person often without apparent symptoms. This feature largely facilitates its rapid spread and underscores the importance of the development and deployment of infectious disease surveillance systems at the early stages to test, isolate, and trace the viral spread in efforts to contain an outbreak and mitigate damage. Rapid evolution in RNA viruses gives the ability to some viruses to jump from species to species, leading to spillover events. This genetic drift is also responsible for the low effectiveness or the lack of vaccines for certain RNA pathogens. The impact on the healthcare systems worldwide and the repercussions on patients are substantial, as we are witnessing. Understanding viral mutations holds significant importance because of its wide impact on new vaccine design, drug resistance management, and prediction of new pathogenesis. Furthermore, approaches that are designed to study viral evolution can be adapted to implement effective diagnostic platforms. In my talk, I will discuss our results on the implementation of SERS probes for the identification and quantification of viral RNA in intact individual cells, leveraging an ON-OFF SERS signal switching that is triggered by the conformational changes in the sequence-specific oligonucleotides bound to the gold nanostar-based probes. I will show how individual nanostars can provide measurable signal, and that their response is only partially affected by the formation of protein corona in media, cell lysate, or intact cells. Furthermore, I will report on the sensitivity of the probes to base mutations within the target RNA, and on their selectivity toward their intended target, even within individual cells. I will compare the SERS results with those enabled by fluorescence transduction on the same nanostars and on nanoflare systems designed for similar targets. These unique probes are promising because they can be easily adapted to target any viral RNA, individually and in multiplex, to enable the implementation of effective diagnostic platforms with high sensitivity and selectivity and reduced cost.

(RAM-02.5) **Spectrophotometric Characterization of Paper-based SERS Sensors**

Li-Lin Tay[1]; *[1]National Research Council Canada*

In fifteen words or less, explain the significance of this contribution (Novel Aspect).: Total reflectance measurement of plasmonic sensors

Abstract Text: Paper-based SERS sensors fabricated by loading cellulose based paper (or fabric) substrates with colloidal Au or Ag nanoparticles is a versatile platform for chemical detection. Paper-based sensors can be produced economically and provide rudimentary sampling capabilities that is often harder to achieve with the rigid SERS substrates such as plasmonic structures patterned on glass or silicon substrate. The plasmonic characteristics of SERS sensors reveals important information such as the localized surface plasmon resonance is typically measured through specular reflectance in spectrophotometer. Due to the diffuse nature of the paper substrates, specular reflected component from a paper-based SERS sensors can be small and most of the reflected light component are lost in the diffused reflection. In this presentation, we will present the total reflectance measurement of the paper-based SERS substrates and discuss the different contribution from the specular and diffuse-reflectance components. We have fabricated inkjet-printed SERS sensors and tested it

against fentanyl molecules. We will present systematic characterization of the printed sensors and correlate the sensors total reflectance (which is a measure of AuNP loading on the sensor) to the performance of the sensor.

21SPECIAL02: Spectroscopy-based Sensors for COVID-19

Chair: Jean-François Masson
Co-Chair: Rob Chimenti
On-site Chair: Jean-François Masson

(SPEC-02.1) **Spectroscopic and Bioanalytical Tools Provide Insight into Coronavirus Host Entry and Therapeutic Targets**

Susan Daniel[1], Susan Daniel[1]; *[1]Cornell University*

The coronavirus disease 2019 (COVID-19) pandemic has focused attention on the need to develop effective therapies against the causative agent, SARS-CoV-2, and also against other pathogenic coronaviruses (CoV) that have yet to emerge. Virus entry into a host cell is mediated by a single glycoprotein protruding from its membrane envelope, called spike (S). Within S, the region that directly interacts with the membrane is called the fusion peptide, FP, a highly conserved region across the CoV family, and thus a promising drug target for interference. It is the physico-chemical interactions of the FP with the host membrane that anchors it, thus enabling the necessary deformations of the membrane that lead to delivery of the viral genome into the cell when a fusion pore opens. Understanding of thermodynamics, kinetics, and intermolecular interactions are useful to describe FP interactions with the host membrane at the most fundamental molecular level. This knowledge in turn, can be used to facilitate the development of strategies to limit those interactions to stop the spread of infection. In this talk, I will describe our work on understanding the impact of calcium ions on CoV infection. Using cell infectivity, biophysical assays, and spectroscopic methods, we found that calcium ions serve to stabilize the fusion peptide structure during conformational change that then allows its insertion into the host membrane, resulting in increased lipid ordering in the membrane. This lipid ordering precedes membrane fusion and has been shown to correlate with increased fusion activity and higher levels of infection in the presence of calcium. As such, depletion of calcium ions leads to structure and activity changes in the fusion peptide that correlate well with in vitro experiments using calcium-chelating agents to block cell infection.

(SPEC-02.2) **Protein and reaction engineering for rapid COVID-19 antigen and antibody tests**

Hadley Sikes[1]; *[1]MIT*

Lateral flow tests have appealingly low cost of goods and can be very simple to operate. However, many generate plastic waste and their sensitivity is often found lacking. A performance-determining step in developing diagnostic immunoassays is identification of pairs, or sets in the case of multiplexed assays, of affinity reagents that simultaneously capture and label targets and also do not cross-react with one another or complex matrix components. Engineered binding molecules derived from thermophilic organisms will be presented as alternatives to antibodies, human or camelid. Analysis of reaction rates and fluid flow within all-cellulose devices suggested further protein engineering strategies to improve sensitivity. Generalized assay design principles for integrating these engineered proteins into antigen and serology tests will be discussed, with applications to covid-19.

(SPEC-02.4) **Development of Peptide-Based Surface Enhanced Raman Spectroscopy Sensor for the Detection of Severe Acute Respiratory Syndrome- Coronavirus- 2**

Taylor Payne[1], Zachary Schultz[1], Ronit Freeman[2], Stephen Klawa[3]; *[1]The Ohio State University, [2]University of North Carolina, Chapel Hill, [3]University of North Carolina- Chapel Hill*

In fifteen words or less, explain the significance of this contribution (Novel Aspect).: Selective detection of SARS-CoV-2 using SERS sensor with virus-specific peptide capture agent

Abstract Text: COVID-19 remains an ongoing issue across the globe, highlighting the need for a rapid, selective sensor for SARS-CoV-2 and its potential variants. Due to the struggles of current detection methods, including polymerase chain reaction (PCR) assays and antibody tests, there remains a need for a quick, selective, and error-free sensor for SARS-CoV-2 and potential future variants. Surface enhanced Raman spectroscopy (SERS) is a rapid, sensitive vibrational spectroscopy technique that requires minimal sample preparation and gives a highly specific molecular fingerprint. SERS takes advantage of the properties of noble metal nanostructures, which produce a strong localized electric field upon laser excitation, giving enhanced Raman signals from analytes on the surface. SERS could be used to develop a quantitative assay for SARS-CoV-2 that would provide immediate and accurate COVID test results for patients around the world. It is important to consider the challenges associated with SERS detection of viruses, which are large, complex species that can yield different SERS signals based on their orientation on the substrate. Additionally, viruses exist in complex bodily fluids with a number of other biomolecules, which can give undesired signal. Capture molecules can improve reproducibility of SERS signal by forcing the analyte into a consistent orientation on the surface, and they can selectively target the analyte in complex solutions. Antibodies are a common recognition element for sensing virus particles with SERS, but these molecules are large and bulky, producing complex SERS spectra. Instead, small aptamers or peptides, which yield simple SERS signals, can be used to target viruses, allowing the signature of the virus itself to be identified and used for quantification. Here we present the development of an ACE2 mimetic peptide-based SERS sensor for SARS-CoV-2. The unique vibrational signature of the spike protein on the peptide-modified surface was used to construct a multivariate calibration model for quantification. The sensor demonstrated a 300 nM limit of detection and high selectivity in the presence of excess BSA. This work provides the basis for designing a SERS-based assay for the detection of SARS-CoV-2, as well as SERS biosensors for other viruses in the future.

(SPEC-02.3) **IR-Spectroscopic Rapid Virus Detection on Protective Face Masks**

Boris Mizaikoff[1], Vanessa Schorer[2], Julian Haas[3], Robert Stach2[3], Vjekoslav Kokoric[3]; *[1]Institute of Analytical and Bioanalytical Chemistry, Ulm University and Hahn-Schickard, Institute for Microanalysis Systems, Ulm, Germany, [2]Student, [3]Group Head*

In fifteen words or less, explain the significance of this contribution (Novel Aspect).: Infrared spectroscopic signatures of SARS-CoV-2 virus on used protective face masks.

Abstract Text: Reliable direct detection of SARS-CoV-2 is of particular importance in order to contain and control the spread of the virus via targeted measures. While wearing any kind of FFP1/2/3/KN95 protective face mask, with every breath one aspires particles including viruses that are trapped at the membrane layer of the mask. In turn, every expired breath unloads gaseous exhaled breath (EB) matrix, and therein, exhaled aqueous droplet phase (EDP) along with all contained species onto the exhalation valve. Considering that the average respiration rate per minute (BRPM) is 15, within an hour one may collect 900-times particles from the ambient environment onto the mask filter membrane, and 900-times exhaled components onto a filter material located at the exhalation port. Hence, even after only one hour of wearing a protective mask represents a ‘sample’ of enormous information content and significantly enriched species, which is usually disposed of. The question is – how can we capitalize on this information? Hence, we demonstrate the analysis of worn face masks via infrared spectroscopic signatures augmented by multivariate data evaluation/classification schemes. Recently, our research team has demonstrated that infrared spectroscopic analysis directly-on-filter (IR-DoF) is a promising technique for particle identification and quantification. In this novel approach, the same concept was applied for analyzing virus and virus-like particles captured within protective face mask materials [1-3]. 1. R. Stach, T. Barone, E. Cauda, P. Krebs, B. Pejcic, S. Daboss, B. Mizaikoff, Direct infrared spectroscopy for the

size-independent identification and quantification of respirable particles relative mass in mine dusts, Analytical and Bioanalytical Chemistry, Analytical and Bioanalytical Chemistry, 412, 3499–3508, 2020. 2. R. Stach, T. Barone, E. Cauda, B. Mizaikoff, A Novel Calibration Method for the Quantification of Respirable Particles for Mining Scenarios via Fourier Transform Infrared Spectroscopy, Applied Spectroscopy, in press, 2020. 3. T. Barone, T. Lee, E. Cauda, A. Mazzella, R. Stach, B. Mizaikoff, Segregation of Respirable Dust for Chemical and Toxicological Analyses, Archives of Environmental and Occupational Health, accepted for publication, 2020.

(SPEC-02.5) **Evaluation of SARS-CoV-2 Neutralizing Antibodies and Duration of Immunity with a Portable Surface Plasmon Resonance Biosensor**

Maryam Hojjat Jodaylami[1], Abdelhadi Djaileb[1], Pierre Ricard[1], Julien Coutu[1], Danny Brouard[2], Ludovic S. Live[3], Denis Boudreau[4], Joelle N. Pelletier[1], Jean-François Masson[5]; *[1]University of Montreal, [2]Hema Quebec, [3]Affinité Instruments, [4]University of Laval, [5]Université de Montréal*

In fifteen words or less, explain the significance of this contribution (Novel Aspect).: Rapid SPR sensors for neutralization assays provide an indication of the immune response to SARS-CoV-2/vaccination.

Abstract Text: The development of serological tests are critical in order to properly manage and control the spread of the COVID-19 pandemic caused by the severe acute respiratory syndrom coronavirus 2 (SARS-CoV-2). Determining the neutralizing effects of anti-SARS-CoV-2 antibodies and their longevity is essential to identify individuals who are potentially immunized against (re)infection as well as the duration of the protection, whether the immunity is acquired by infection or through vaccination. Neutralizing antibodies prevent viral entry into the host cell by blocking the virion binding to its cellular receptor, angiotensin-converting enzyme 2 (ACE2). The spike (S) protein, a large trimetric glycoprotein with its receptor-binding domain (RBD), is the main and the only structural protein used as the target for neutralizing antibodies. We have developed a pseudo-neutralization assay by using a portable surface plasmon resonance (SPR) biosensor to assess the maturity and the level of neutralizing antibodies produced by the immune system of individuals infected with SARS-CoV-2 within six months post infection. In addition, the emergence of SARS-CoV-2 variants has raised concerns about the extent of neutralizing antibody responses. In these tests, we used SPR sensors, previously developed by our group, to evaluate if neutralizing antibodies at different covalescent stages can inhibit the interaction of the native and some variants of the spike protein (B.1.1.7, B.1.351 and P.1), immobilized on the surface of the SPR sensor, with ACE2.

21AES04: Electrophoretic Bioanalytical and Pharmaceutical Analyses

Chair: Erin Henslee
On-site Chair: Erin Henslee

(AES-04.1) **Proteo-Metabolomic Single-Cell Systems Biology Using Microsampling Enabled In Vivo Subcellular Mass Spectrometry**

Jie Li[1], Camille Lombard-Banek[1], Erika Portero[1], Rosemary Onjiko[2], Chase Singer[1], David Plotnick[2], Reem Al Shabeeb[2], Peter Nemes[1]; *[1]Department of Chemistry and Biochemistry, University of Maryland, College Park, MD, [2]Department of Chemistry, The George Washington University, Washington, DC*

In fifteen words or less, explain the significance of this contribution (Novel Aspect).: We conducted the first proteo-metabolomic single-cell analysis in live embryos without interfering normal embryonic development.

Abstract Text: Understanding cell-to-cell differences is crucial to studying tissue formation and organogenesis.

Therefore, single-cell omics techniques combined with systems biology were developed to provide a panoramic view of cells by integrating different omics. However, in vivo systems biology single-cell analysis is challenging because general sampling techniques are destructive and not compatible with in vivo analysis. Additionally, integrating different omics techniques is difficult and the limited cellular contents available in single cells necessitate techniques to detect low-abundant and unamplified biomolecules. To address these challenges, we developed a bioanalytical technology that combines microsampling with capillary electrophoresis mass spectrometry (CE-MS) to perform dual proteo-metabolomic analysis of single cells in live embryos. Left dorsal-animal (L-D1) and ventral-animal (L-V1) cells in live 8-cell Xenopus laevis frog embryos were identified under a microscope. We scaled microsampling to aspirate ~10 nL of cellular contents twice from both L-D1 and L-V1 cells in the same embryos to perform proteo-metabolomic analysis. To evaluate the effect of microsampling on embryonic development, we conducted survival, morphological, and behavioral assays. We found microsampling minimally interfered with the normal embryonic development, as 95% of microsampled embryos developed into tadpoles, and these tadpoles showed statistical similarity in morphology and visual functions as their wild-type siblings. These results demonstrated our microsampling strategy's capability for in vivo sampling. To detect metabolites and proteins from the microsampled cellular contents which were ~100–1000 times smaller than the amount that is required in typical LC-MS analysis, we used our ultrasensitive custom-built CE-MS platforms which can detect <700 zmol peptides and <60 amol metabolites. We detected ~150 molecular features and 738 proteins from microsampled cellular contents, respectively. By integrating metabolomic and proteomic data, we expanded the coverage of metabolic pathways such as arginine-proline metabolism. Moreover, the integrated multi-omic data revealed molecular cell heterogeneity between L-D1 and L-V1 cells, revealing the notable chemical difference among early-stage pluripotent cells with different tissue fates. In summary, we developed a technology to enable, for the first time, dual proteo-metabolomic analysis of single cells in live embryos. The technology is also scalable to other biological systems for drug discovery, molecular biology, and neuroscience research.

(AES-04.2) **ESSENCE - Novel modular electrochemical analytical point-of-care/point-of-use platform for medical diagnostics to screening emerging contaminants across different matrices like blood to source water**

Sagnik Basuray[1], Yu Hsuan Cheng[2], Li Zhenglong[2]; *[1]New Jersey institute of Technology, [2]New Jersey Institute of Technology*

In fifteen words or less, explain the significance of this contribution (Novel Aspect).: Modular POC/POU novel electrochemical platform architecture to detect biomolecules to chemical moieties in any matrix

Abstract Text: ESSENCE has a three-dimensional electrode architecture. In this 3D electrode architecture, a nonplanar interdigitated microelectrode ("NP-IDμE") sandwiches a microfluidic channel of dimensions 50 mm length *500 μm width* 100 μm. Our method to fabricate this 3D electrode is simple. NP-IDμE electrode architecture consists of three layers; the top and bottom glass layers are decorated with microelectrodes (μEs) with a middle layer of double-sided polypropylene tape with the desired channel pattern sandwiched between μE layers. The room temperature, operator-independent instrumentation, and fabrication protocol for NP-IDμE allow us to pack NP-IDμE with different dielectric materials like metal-organic-framework (MOF) or conducting materials like carbon nanotubes (CNT). ESSENCE can overcome current electrochemical sensors' selectivity and sensitivity limitations due to the improved electric field penetration compared with the traditional planar interdigitated electrode. Thus, ESSENCE has six significant benefits over the current generation of electrochemical sensors. The electrode nanoporosity facilitates the development of shear forces, significantly increases selectivity, high signal-to-noise ratio (SNR), increases convective transport of the analyte of interest to the sensing element, thus overcoming diffusion limitations and reducing assay times. The sensitivity and device selectivity can be decoupled using the flow rate as a tuning design parameter. The EIS signal is shifted to a higher frequency range (1kHz to 100MHz) and results in a fast response with a higher

signal-to-noise ratio in Electrochemical Impedance Spectroscopy(EIS) measurements. The modular fabrication of the ESSENCE chip allows ESSENCE to target different biomolecules from DNAs to protein cancer biomarkers by simply changing the packed transducer material. ESSENCE has a switchable electrode system for usage in multiple configurations. ESSENCE has high selectivity and sensitivity for DNA (fM sensitivity, selective against non-target DNA), breast cancer biomarker proteins (p53, pg/L sensitivity, selective against non-target HER2). Using different nanoporous metal-organic framework (MOF) materials (e.g., MIL-101 (Cr, Fe), MIL-100 (Fe, Cr, Al), UiO-66)) allows us to detect perfluorooctane sulfonate (PFOS) from 100 ng/L to 5 ng/L in different matrices like industrial leachate. ESSENCE has also been automated, uses additive manufacturing for easy operability, replication, and usage in other academic and non-academic labs. ESSENCE can detect toxins and emerging contaminants in the air.

(AES-04.3) **Electronic Single-Molecule Sensing for Glycomics and Genomics Using Chemically Tailored Nanopores**

James T. Hagan[1], Brian S. Sheetz[1], Jason R. Dwyer[1]; [1]*University of Rhode Island*

In fifteen words or less, explain the significance of this contribution (Novel Aspect).: Biopolymer properties and sequence are accessible using molecularly tailored single-molecule-sensitive nanopore sensors

Abstract Text: Nanopores offer all-electronic single-molecule sensing of unlabelled (or labelled) molecules in solution with minimal preparatory workflow. Nanopore tools allow for the detection of ions, small molecules, and (bio)polymers; for the characterization of analyte properties such as physical size (e.g. polymer length) and charge; and for the potential for biopolymer sequencing. These nanoscale tools must be carefully designed for their specific application since nanopore resistive-pulse sensing involves forcing analytes into a confined volume not much larger than the analytes, themselves. I will discuss how we can customize nanopore dimensions by electrically-controlled thin-film-removal and molecularly-precise coating addition and how we can optimize nanopore performance by chemical decoration—and then how machine learning can augment these efforts. I will then present work we have done to improve nanopore DNA sequencing and to develop nanopores to deliver glycan fingerprinting, characterization, and even sequence sensitivity. I will conclude by presenting examples of nanopore analysis of importance to quality assurance assays of glycan-based pharmaceuticals.

(AES-04.4) **DNA-analyte bioconjugates enable rapid electrochemical sensing of steroid hormones, peptide-based drugs, and protein biomarkers in clinically-relevant ranges**

Christopher J. Easley[1], Asanka Gurukandure Gedara[1], Niamat Khuda[1], Madelyn james[1], Mainul Mazumder[1], Nan Shi[1], Rashad Karimov, Kacey Ortiz; [1]*Auburn University*

In fifteen words or less, explain the significance of this contribution (Novel Aspect).: Our DNA nanostructure platform is a generalizable electrochemical sensor for clinically-relevant analytes and minimal workflow

Abstract Text: Sensors based on electrochemistry (EC) provide low cost, miniaturization, and adaptability to the point-of-care (POC). There has been renewed interest in EC biosensors for the multitude of biomarkers that are not EC-active, do not undergo enzymatic conversion, or are not suited for potentiometry. Aptamer-based EC sensors are even capable of sensing in living animals with temporal resolution as low as a few seconds. While these sensors can already impact human health, most method development has been target-focused, lacking generalizability. Presently, the clinical EC toolbox is a conglomerate of targeted methods, and there is a pressing need to develop an EC platform amenable to rapid, generalizable, quantitative readout of multiple classes of clinically relevant targets.

Our group began to address this need by developing a DNA-nanostructure sensor platform for general-purpose sensing (J. Am. Chem. Soc. 2019, 141, 11721-11726). Initially, the sensors were validated with biotechnology controls and with a small molecule immunomodulatory drug in human serum. In this presentation, we discuss the expansion of the generalizability of our sensor platform using varied DNA-analyte bioconjugates within the DNA-nanostructure.

For peptide sensing, DNA-peptide conjugates were synthesized, purified, the ligated to the DNA-nanostructure. Sensors were validated for quantifying exendin-4 (4.2 kDa)—a human glucagon-like peptide-1 receptor agonist important in diabetes therapy—for the first time using direct EC methods, with an LOD of 6 nM. Sensors for larger proteins were made using DNA-epitope conjugates. The antibody-binding epitope of creatine kinase MM (CK-MM) was conjugated into the nanostructure, allowing CK-MM sensing in the 10 to 100 nM range. Finally, DNA-steroid bioconjugates have been incorporated into the sensors, allowing sensing of testosterone in the range of 3 to 50 nM, and preliminary evidence shows that 30 nM cortisol can be easily detected with these sensors.

All of these sensors were functional in 98% human serum, and the detection ranges overlap with the clinical/therapeutic ranges, boding well for future applications in biosensing or therapeutic drug monitoring. Overall, this new DNA nanostructure platform provides a generalizable sensor with minimal workflow, direct-readout, and the capability to expand EC sensing to a wide variety of clinically important analytes.

(AES-04.5) **Deterministic iDEP Ratchet Devices for High-throughput Organelle Separation**

Domin Koh[1], Ricardo Ortiz[1], Mukul Sonker[1], Alexandra Ros[2]; *[1]Center for Applied Structural Discovery, [2]Center for Applied Structural Discovery, The Biodesign Institute. School of Molecular Sciences. Arizona State University*

In fifteen words or less, explain the significance of this contribution (Novel Aspect).: This offers a size-based organelle fractionation with a size range tunable by changing electric parameters

Abstract Text: Studying organelle size is very useful in investigating the diseases like Alzheimer's disease, obesity, diabetes, cancer because the cellular dysfunction associated with such diseases causes the organelle size-variation. As a result, the size-selective isolation of organelle is important to explore diseases at the biomolecular level and development of innovative therapeutic interventions. The conventional method of isolating organelle is cumbersome and extraction steps leading to significant sample loss. Previously, we demonstrated a novel DEP-based deterministic ratchet migration phenomenon using an insulator-based dielectrophoretic (iDEP) microfluidic device containing an array of insulating posts to for size-based separation of polystyrene beads and organelles with excellent resolution. The size range of this novel approach is tunable by simply modifying the electrical driving conditions. Here, we further developed devices for high throughput (HT) organelle fractionation that can process large sample volumes >5 μL and up to 106 organelles per run so that a sufficient amount of purified organelles can be recovered for further characterization and biomolecular assessment. We also optimized the electrical driving parameters in-silico using numerical modeling and tested them experimentally, as required for HT devices with continuous flow. We explored size-based particle fractionation at different flow rates using a mixture of polystyrene 270 nm and 880 nm microbeads and obtained good fractionation at a flow rate of 20 nL/min overlaid with the electrical driving forces inducing the ratchet migration. Then, we used a mixture of mitochondria isolated from HepG2 cells (588 ± 98.2nm) and cells with knockdown of Mfn-1 genes causing smaller mitochondria size (207 ± 103nm) to demonstrate size-based organelle fractionation at a constant flow rate (20 nL/min). The result shows good agreement between the numerical model and the experimental data (microbeads and mitochondria) that the larger particles experienced ratchet migration and smaller particles were not affected by ratchet mechanism. Consequently, the migration velocity of small particles was faster than the large particles which migrated toward the opposite outlet. In the

future, the size distribution of the sample collected at each outlet will be analyzed to study the separation efficiency by counting the number of each particle.

21BIM01: Biophotonics Technologies Fighting Infections at the Point of Care

Chair: Ute Neugebauer
Co-Chair: Juergen Popp
On-site Chair: Karen Esmonde-White

(BIM-01.1) **AI-powered high-throughput imaging to fight COVID-19**

Julia Gala de Pablo[1]; [1]*University of Tokyo*

A characteristic clinical feature of COVID-19 is the frequent occurrence of thrombotic events. Furthermore, many cases of multiorgan failure have been found thrombotic in nature. To study COVID-19-associated thrombosis, I introduce a new type of technology that provides the landscape of circulating platelet aggregates in COVID-19. This is made possible by large-scale single-cell image-based profiling and temporal monitoring of the blood of COVID-19 patients. Surprisingly, our analysis shows the anomalous presence of excessive platelet aggregates in nearly 90% of all COVID-19 patients, including those who were not clinically diagnosed with thrombosis. Additionally, results indicate a strong link between the concentration of platelet aggregates and the severity and mortality of COVID-19. Finally, high-dimensional analysis and comparison with other diseases reveal that COVID-19 behaves as a product of thrombosis (localized) and infectious diseases (systemic), as a cause of systemic thrombosis.

(BIM-01.2) **Planar Waveguide Biosensor for Point-of-Care Fluorescent Immunoassays**

Christopher Myatt[1]; [1]*LightDeck Diagnostics; USA*

Optical waveguides are an optimal biosensor platform. Evanescent illumination, where the light field extends a fraction of the wavelength above the sensor surface, provides a clean signal for surface reactions with minimal background. By only illuminating a volume extending above the surface by a few hundred nanometers, evanescent waveguide sensors often don't require wash. Signal develops quickly and results can be quantitated in a few minutes. We will present the architecture and design features of the LightDeck® Diagnostics evanescent planar waveguide sensor, and one of the applications: a 5-minute test for antibodies to the SARS-CoV-2 virus. The LightDeck(R) COVID-19 Total Antibody test for detecting SARS-CoV-2 antibodies in blood uses the point-of-care LightDeck® platform. Validation included a retrospective study to establish performance characteristics of the LightDeck COVID-19 Total Antibody Test using the LightDeck® Analyzer. The Clinical Agreement Study was designed to determine the sensitivity, or Positive Percent Agreement (PPA), to detect antibodies against the SARS-CoV-2 virus in 95 de-identified serum or plasma samples from RT-PCR positive COVID-19 subjects. Specificity was determined in a Cross Reactivity Study using 547 samples that were collected prior to the COVID-19 pandemic. Matrix equivalency studies compared serum, plasma, venous whole blood and fingerstick whole blood. RESULTS: Sensitivity (PPA) was 97.9% (93/95 PCR+ samples, 95% CI 92.6% - 99.4%). Specificity (NPA) was 99.6% (545/547 PCR- samples, 95% CI 98.7% - 99.9%). Results are reported as signal to cutoff value, defined as the cutoff index (COI). Correlation coefficient R2 of COI values from venous whole blood samples to plasma samples was 0.93, and correlation coefficient R2 of COI values from venous whole blood samples to fingerstick samples was 0.98. CONCLUSION: The 5-minute point-of-care LightDeck COVID-19 Total Antibody Test demonstrated performance characteristics competitive with those of assays designed for a central lab. Additional studies with non-laboratory users are underway. This project has been funded in whole or in part with Federal Funds from the Department of Health and Human Services Office of the Assistant Secretary for Preparedness and Response; Biomedical Advanced Research and Development Authority, under Contract No. 75A50120C00130.

(BIM-01.3) **Biophotonics Light The Way For SARS-CoV-2 Diagnostics**

Stefanie Deinhardt-Emmer[1], Volker Deckert[2], Bettina Loeffler, Juergen Popp[3]; *[1]Jena University Hospital, [2]Leibniz IPHT, [3]Leibniz Institute of Photonic Technology*

COVID-19 indicates infections with the novel SARS-CoV-2 as a systemic disease. Therefore, diagnostics has to be adapted due to the broad organ tropism. Our study presents a broad viral tropism and highlights innovative biophotonic technologies for the detection of SARS-CoV-2. The viral distribution within the human body was carried out in deceased COVID-19 patients. For this, standard laboratory diagnostic was used. However, innovative approaches are needed to detect SARS-CoV-2 in various tissues. To improve the diagnostic, we have developed 2 strategies. For the Raman-spectroscopic techniques, functionalized magnetic beads with ACE2 receptors were used. Due to the strong interaction of the spike protein with ACE2, only this virus remains on the surface of the magnetic beads during sample preparation. A subsequent Raman spectroscopic investigation was performed to distinguish a control virus (Influenza A). Since the spectra are quite similar, two-dimensional correlation spectroscopy (2D-COS) is applied to achieve reliable discrimination. For the scanning probe microscopy-based methods, we used size-matched nanoscale investigation of size and shape parameters of single SARS-CoV-2 virions. Those experiments were correlated with near- and far-field spectroscopic tools for the specific identification of virions. Our results demonstrate high viral loads in the lungs and lower viral loads in other tissues of deceased COVID-19 patients. Using a sample-sample correlation approach, an overall specificity of 89 % and sensitivity of 99 % shows that Raman spectroscopy, in combination with a suitable sample preparation strategy, is a powerful tool for identifying viruses. The size of SARS-CoV-2 virions could be confirmed by the correlation of the topographic information of virions. This provides a basis for label-free identification of single virions by simple topographic investigation; subsequent TERS experiments on such particles reveal the surface composition of the particles. Our study demonstrates the dissemination of viral RNA throughout the body, resulting in viremia and multiorgan dysfunction. By using Raman-spectroscopic techniques, the determination of viruses from different specimens is possible. In addition, the AFM-based experiments provide a fast pre-characterization method for nanometer-sized pathogens.

(BIM-01.4) **Biophotonic analysis of the immune response enables rapid detection of infection**

Ute Neugebauer[1], Natalie Arend[2], Anuradha Ramoji[3], Daniel Thomas-Rüddel, Oleg Ryabchykov[4], Michael Kiehntopf, Frank Bloos, Thomas W. Bocklitz[5], Iwan Schie, Michael Bauer[6], Juergen Popp[3]; *[1]Friedrich Schiller University Jena, [2]Leibniz-IPHT, [3]Leibniz Institute of Photonic Technology, [4]Leibniz Institute of Photonic Technology (Leibniz-IPHT), Albert-Einstein-Straße 9, 07745 Jena, Germany; Research Campus Infectognostics e.v., Philosophenweg 7, 07743 Jena, Germany; Institute of Physical Chemistry and Abbe Center of Photonics (IPC), Friedrich-Schiller-University Jena, Helmholtzweg 4, 07743 Jena, Germany;, [5]Leibniz Institute of Photonic Technology (Leibniz-IPHT), Albert-Einstein-Straße 9, 07745 Jena, Germany; Research Campus Infectognostics e.v., Philosophenweg 7, 07743 Jena, Germany Institute of Physical Chemistry and Abbe Center of Photonics (IPC), Friedrich-Schiller-University Jena, Helmholtzweg 4, 07743 Jena, Germany;, [6]Jena University Hospital*

Biophotonic technologies show a high potential to provide portable and cost-competitive solutions, which deliver in-depth information about biological specimen in only short analysis time. In this contribution, it will be demonstrated that Raman spectroscopy is ideally suited to characterize immune cells in a direct, label-free and non-destructive manner to extract valuable information about the immune cell's response to pathogens and pathogen-associated molecular patterns. This can provide insights into the cause of infection, but also be used to identify dysregulated immune responses as in sepsis. Peripheral blood contains several subtypes of leukocytes which all have their specific roles in the immune response of the body. The major subtypes could be successfully differentiated by means of Raman spectroscopy [1, 2]. The activation of THP-1 monocytes after in-vitro stimulation with the pathogen-associated molecular pattern lipopolysaccharide (LPS) could be followed by means of Raman spectroscopy over a time-course of 16 hours, revealing metabolic changes that could be

confirmed by gene transcription analysis and quantification of the expression of the two cytokines TNF-α and IL-1β [3]. The Raman-based approach was successfully translated to reveal LPS-induced changes in T lymphocytes isolated from spleen of an endotoxemia mouse model [4]. In order to fight invading pathogens, leukocytes have developed specialized response mechanisms. Different pathogens, such as bacteria, fungi or viruses, trigger different signaling cascades resulting in activated immune cells. Raman spectroscopic analysis of in-vitro stimulated primary human neutrophils enabled a reliable differentiation of bacterial and fungal infection of using an in-house built high-throughput screening Raman microscope. Translation of the Raman-spectroscopic analysis to human diagnostics was assessed in a clinical trial characterizing the peripheral leukocytes from patients with either sterile inflammation, infection without organ dysfunction or sepsis [6].
References: [1] Anal. Chem. 2012, 84(12), 5335-5342. [2] Anal. Chem. 2018, 90, 2023−2030 [3] Integrative Biology 2019, 11(3), 87–98. [4] ImmunoHorizons 2019, 3 (2) 45-60. [5] Anal. Chem. 2020, 92, 10560−10568. [6] Critical Care Explorations 2021, accepted.
Acknowledgements Financial support by the EU ("HemoSpec", CN611682; "Raman4Clinics", Grant No. 861122: "ImageIN"), BMBF (CSCC, FKZ 01EO1502), Leibniz Society (InfectoOptics) and DFG (JSMC, JBIL) are highly acknowledged.

(BIM-01.5) **Raman spectroscopy determines conformational changes in sub-30 nm drug-delivery systems with an impact on biodistribution.**

Irina Muljajew[1], Sophie Huschke[2], Anuradha Ramoji[3], Stephanie Hoeppener[1], Christine Weber[1], Juergen Popp[3], Michael Bauer[2], Ulrich S. Schubert[1], Adrian T. Press[2]; *[1]Friedrich-Schiller University Jena, [2]Jena University Hospital, [3]Leibniz Institute of Photonic Technology*

In fifteen words or less, explain the significance of this contribution (Novel Aspect).: Raman spectroscopy determines conformational changes in sub-30 nm drug-delivery systems with an impact on biodistribution.

Abstract Text: Nanocarriers such as micelles below 50 nm in diameter have favorable tissue penetration properties, which allow them to reach even poorly perfused tissue, e.g., hypoxic tumor aerials, increasing the selective accumulation of pharmaceutics as well as the effectiveness of therapies. The distribution of micelles is highly dependent on their surface characteristics. Already minor temporary changes of ion-interaction during the preparation of nanocarriers can determine cell-type specificity in the liver. Particularly in small carrier systems, conventional methods such as cryo-transmission electron microscopy cannot reveal surface changes anymore. In such cases, we demonstrated that Raman spectroscopy is a powerful tool to investigate the molecular arrangement of nanocarriers for biomedical applications as small as 30 nm in diameter. [1] In this study, we investigated dye-loaded micelles of 10 nm diameter formed from amphiphilic graft copolymers composed of a hydrophobic poly(methyl methacrylate) backbone and hydrophilic oligo (2-ethyl-2-oxazoline) (OEtOx) side chains with a degree of polymerization of 15. Despite the high molar mass of the individual macromolecules (Mn ≈ 20 kg mol-1), backbone end group modification by attachment of a hydrophilic anionic fluorescent probe strongly affected the in vivo performance. The end group was modified by the attachment of four methacrylic acid repeating units. Although those micelles appeared similar in dynamic light scattering and cryo-transmission electron microscopy, the cellular recognition of those micelles by immunocompetent cells investigated by intravital confocal laser scanning microscopy in mice was significantly altered. Raman spectroscopy visualized a significant difference in the arrangement of the OEtOx-based surface and condensation of the core segment reducing interactions with immunocompetent cells that would otherwise clear large numbers nanocarriers. The observations correlated with in vivo microscopic findings that micelles formed from polymers with anionically charged, thiol, or hydrophobic end groups altered the micelles' structure. These changes had been sufficient to influence cell-type specificity and stealth properties in the liver. Therefore, end group modifications might be critical for the passive targeting of drugs to different liver cells.

21IR06: Advances in Photothermal Spectroscopy II

Chair: Rohith Reddy
On-site Chair: Rohith Reddy

(IR-06.1) **Fluorescence-Guided Optical Photothermal Infrared (O-PTIR) Spectroscopy**

Craig Prater[1], Mustafa Kansiz[2], Oxana Klementieva[3], Ferenc Borondics[4], Kevin Kjoller[1], Roshan Shetty[1]; *[1]Photothermal Spectroscopy Corp, [2]Photothermal Spectroscopy Corp., [3]Department of Experimental Medical Science, Faculty of Medicine, Lund University, [4]Soleil Synchrotron*

In fifteen words or less, explain the significance of this contribution (Novel Aspect).: Enables chemical analysis with sub-micron spatial resolution of fluorescently labeled regions of cells and tissue

Abstract Text: We have developed a new multimodal microscope enabling "Fluorescence-Guided" Optical Photothermal Infrared (O-PTIR) spectroscopy. O-PTIR has proven itself a breakthrough in IR microspectroscopy, providing submicron IR spectroscopic spatial resolution (~20x better than traditional IR), in a non-contact, far-field optical configuration. We have now coupled O-PTIR with fluorescence microscopy, a long established, cornerstone technique in life science research. The new fluorescence guided O-PTIR instrument allows: (1) a sample to be analyzed via fluorescence microscopy to map sample regions that are labeled one or more fluorophores; and (2) use fluorescence images to direct infrared spectroscopic analysis in the vicinity of the fluorescently labeled regions. Because the O-PTIR technique uses visible light to measure IR absorption, it achieves a spatial resolution at the same scale as fluorescence microscopy, thus allowing direct correlation between the two techniques without the need for any sample registration. Furthermore, we have shown that O-PTIR measurements can be carried out directly on the fluorescently labelled sample without interference, owing to the extremely low concentrations of fluorophores typically employed. The Fluorescence-Guided O-PTIR instrument has been used to perform infrared spectroscopic analysis on fluorescently labelled cells and tissues. This new technique holds the promise of enabling correlative, in situ spectroscopic analysis of fluorescently labelled proteins, for example those related to neurodegenerative diseases.

(IR-06.2) **Fluorescence-Detected Mid-Infrared Photothermal Microscopy**

Minghe Li[1], Aleksandr Razumtcev[1], Ruochen Yang[1], Youlin Liu[1], Jiayue Rong[1], Andreas C. Geiger[1], Romain Blanchard[2], Christian Pfluegl[2], Lynne Taylor[1], Garth Simpson[3]; *[1]Purdue University, [2]Pendar Technologies, [3]Purdue*

In fifteen words or less, explain the significance of this contribution (Novel Aspect).: Fluorescence-detected photothermal infrared (F-PTIR) microscopy reveals infrared spectral information with fluorescence microscopy resolution.

Abstract Text: Fluorescence-detected photothermal mid-infrared (F-PTIR) spectroscopy is demonstrated herein and used to characterize chemical composition within phase-separated domains of pharmaceutical materials. Infrared and Raman spectroscopic imaging are powerful techniques for generating detailed chemical images based on a sample's spectrum. A previous study on optically detected photothermal infrared (O-PTIR) improved the spatial resolution by probing the temperature-induced refractive index change but are potentially prone to high background in scattering media. Fluorescence-detected photothermal mid-infrared (F-PTIR) spectroscopy is proposed, providing dual-level chemical discrimination based on both fluorescence and infrared absorption. F-PTIR relies on the intrinsic sensitivity of the fluorescence quantum efficiency to temperature. Therefore, fluorescence can serve as a sensitive probe (SNR over 100) for reporting on highly localized and selective infrared absorption. The theoretical spatial resolution of F-PTIR is ultimately limited by fluorescence microscopy and the thermal diffusivity of the sample instead of the infrared wavelength. Following proof-of-concept measurements with model systems of silica gel and polyethylene glycol particles, F-PTIR measurements were used to probe chemical composition within phase-separated domains of ritonavir within

copovidone polymer matrices of relevance in the production of pharmaceutical final dosage forms.

(IR-06.3) **Polarization sensitive photothermal mid-infrared spectroscopic imaging of human bone marrow tissue**

Rupali Mankar[1], Chalapathi Gajjela[1], Carlos Bueso-Ramos[2], Cameron Yin[2], David Mayerich[1], Rohith Reddy[3]; *[1]Department of Electrical and Computer Engineering, University of Houston, [2]Department of Hematopathology, MD Anderson Cancer Center, [3]University of Houston*

In fifteen words or less, explain the significance of this contribution (Novel Aspect).: Clinically-viable, label-free approach to study fibrosis in bone marrow using polarization-sensitive photothermal mid-infrared spectroscopic imaging.

Abstract Text: Collagen quantity and integrity play a major role in understanding diseases such as myelofibrosis (MF). Label-free mid-infrared spectroscopic imaging (MIRSI) has the potential to quantify collagen while minimizing the subjective variance observed with conventional histopathology. Polarization-sensitive Infrared (IR) spectroscopy provides chemical information while also estimating tissue dichroism. Quantitative chemical and structural information can potentially aid MF grading and improve pathological agreement on the diagnosis by quantifying chemical and structural information of collagen fibers. We are presenting the first study of polarization-dependent spectroscopic variations in collagen from human bone marrow samples. We translate polarization-sensitive IR studies performed on animal models into a clinically viable method for analyzing human clinical biopsies. We developed a new polarization-sensitive optical photothermal mid-infrared (O-PTIR) spectroscopic imaging scheme that enables sample and source independent polarization control. O-PTIR provides 0.5μm spatial resolution, enabling the identification of thin (≈1μm) collagen fibers that were not separable using fingerprint wavenumbers from Fourier Transform Infrared (FTIR) imaging at diffraction-limited resolution (≈5μm). We also propose quantitative metrics to identify fiber orientation from discrete band images (amide I and amide II) measured under three polarizations. Previous studies have used a pair of orthogonal polarization measurements, parallel and perpendicular to the fiber axis, to demonstrate polarization dependence of fiber orientation with IR imaging. However, the use of two orthogonal polarizations is inadequate for the identification of collagen orientation in clinical samples since human bone biopsies containing collagen fibers with multiple orientations. Here, we address this challenge and demonstrate that three polarization measurements are necessary and sufficient to resolve orientation ambiguity in clinical bone marrow samples. We have quantified fiber orientation into a single metric using Jones calculus on polarization-sensitive IR images. Quantitative metrics for collagen fiber spread will aid more robust diagnosis and improve pathological agreements on the results. Our study is the first study to demonstrate the ability to spectroscopically identify thin collagen fibers (≈1μm diameter) and their orientations, critical for accurate grading of human bone marrow fibrosis.

(IR-06.4) **Review of Life Science Applications using submicron O-PTIR and Simultaneous Raman microscopy – A new paradigm in Vibrational Spectroscopy**

Mustafa Kansiz[1], Alice Spadea[2], Jayakrupakar Nallala[3], Cassio Lima[4], Howbeer Muhamad-Ali[4], Joanna Denbigh[5], Jayne Lawrence[6], Gorkem Bakir[7], Peter Gardner[8], Nick Stone[3], Roy Goodacre[4], Kathleen M. Gough[9], Oxana Klementieva[10]; *[1]Photothermal Spectroscopy Corp., [2]NorthWest Centre for Adv. Drug Delivery (NoWCADD) School of Health Sciences & Division of Pharmacy and Optometry Faculty of Biology, Medicine and Health Uni of Manchester, UK, [3]Biomedical Physics, School of Physics and Astronomy, Uni of Exeter, UK, [4]Department of Biochem. and Systems Biology, Inst. of Systems, Molecular and Integrative Biology, Uni of*

Liverpool, UK, [5]Seda Pharmaceutical Development Services, [6]NorthWest Centre for Adv. Drug Delivery (NoWCADD) School of Health Sciences Uni of Manchester & Division of Pharmacy and Optometry Faculty of Biology, Medicine and Health Uni of Manchester, UK, [7]University of Manitoba, [8]Manchester Institute of Biotechnology & Dept of Chem. Eng. and Anal. Sci, Sch of Eng., Uni of Manchester, UK, [9]Dept of Chem, Uni. of Manitoba, Winnipeg, Canada, [10]Department of Experimental Medical Science, Faculty of Medicine, Lund University

In fifteen words or less, explain the significance of this contribution (Novel Aspect).: A brief review of biomedical applications using the emerging technique of O-PTIR will be presented

Abstract Text: The recent advent of Optical Photothermal IR (O-PTIR) spectroscopy, has enabled for the first time, true submicron infrared microscopy in far-field reflection mode, generating "FTIR transmission-like" spectral quality, without spectral artefacts and distortions such as Mie Scattering associated with traditional FTIR or other emerging QCL based IR microscopy systems. Furthermore, it is now possible to combine O-PTIR with Raman for correlative IR & Raman microscopy. Photothermal spectroscopy is not new and has been exploited for decades with techniques such as PhotoAcoustic Spectroscopy (PAS) and AFM-IR (nano-IR). Where O-PTIR differs to is that it uses an optical (green laser) probe for detection, being analogous to the microphone in PAS and the AFM tip in AFM-IR. The use of this optical probe is the key enabling breakthrough in O-PTIR allowing for non-contact measurements, providing for advantages in capabilities relative to traditional FTIR/QCL microscopy but also in instrument architecture, thus enabling the first combined (correlative) IR and Raman (IR+Raman) platform that provides for simultaneous IR and Raman spectral information at the same time, from the same spot with the same submicron spatial resolution. These unique and exciting synergistic capabilities are now spawning interest in life science applications [1-2]. A broad range of life science applications, which are otherwise impossible with traditional FTIR/QCL microscopy, will be presented, ranging from live cell imaging in water, to ultra-high resolution images of breast tissue calcifications, amyloid aggregates in neurons (neurites and dendritic spines), individual collagen fibrils with polarized IR and individual isotopically labelled bacterial cells and more.

(IR-06.5) **nano-FTIR correlation nanoscopy for organic and inorganic material analysis**

Stefan Mastel[1], Tobias Gokus[2], Artem Danilov[3], Sergiu Amarie[1]; *[1]attocube systems AG, [2]neaspec GmbH, [3]attocube Systems AG*

In fifteen words or less, explain the significance of this contribution (Novel Aspect).: Correlation scanning probe techniques to complement nanoscale IR measurements for next generation sample characterization

Abstract Text: Scattering-type Scanning Near-field Optical Microscopy (s-SNOM) is a scanning probe approach to optical microscopy and spectroscopy, bypassing the ubiquitous diffraction limit of light to achieve a spatial resolution below 20 nanometers. s-SNOM employs the strong confinement of light at the apex of a sharp metallic atomic force microscopy (AFM) tip to create a nanoscale optical hot-spot. Analyzing the scattered light from the tip enables the extraction of the optical properties of the sample directly below the tip and yields nanoscale resolved images simultaneous to topography [1]. In addition, the technology has been advanced to enable Fourier-Transform Infrared Spectroscopy on the nanoscale (nano-FTIR) [2] using broadband radiation from the visible spectral range to THz frequencies. Recently, the combined analysis of complex nanoscale material systems by correlating near-field optical data with information obtained by other scanning probe microscopy (SPM)-based measurement methodologies has gained significant interest. For example, the material-characteristic nano-FTIR spectra of a phase-separated polystyrene/low-density polyethylene (PS/LDPE) polymer blend verifies sharp material interfaces by measuring a line profile across a ca. 1 µm sized LDPE island. Near-field reflection/absorption imaging at 1500cm-1 of the ca. 50nm thin film allows to

selectively highlight the distribution of PS in the blend and simultaneously map the mechanical properties like adhesion of the different materials [3,4]. Further, we present results that correlate the near-field optical response of semiconducting samples like graphene (2D) or functional SRAM devices (3D) in different frequency ranges (mid-IR & THz) to Kelvin Probe Force Microscopy (KPFM) measurements. Thus, s-SNOM systems represent an ideal platform to gain novel insights into complex material systems by different near-field and AFM-based method. [1] F. Keilmann, R. Hillenbrand, Phil. Trans. R. Soc. Lond. A 362, 787 (2004). [2] F. Huth, et al., Nano Lett. 12, 3973 (2012). [3] B. Pollard, et al., Beilstein J. of Nanotechn. 7, 605 (2016). [4] I. Amenabar, et al., Nature Commun. 8, 14402 (2017).

21LIBS04: LIBS Analytical Applications II

Chair: Vassilia Zorba
On-site Chair: Matthieu Baudelet

(LIBS-04.2) **193nm-Excimer Tandem LIBS and LA-ICP-MS – the case of geological samples**

Jhanis J. Gonzalez[1], Alan Koenig[2], Charles Sisson[2], Chunyi Liu[2], Jong Yoo[2], Diep Trieu[2], Robb Hunt[2], Rick Russo[3]; *[1]Applied Spectra, Inc. / Lawrence Berkeley National Laboratory, [2]Applied Spectra, Inc., [3]Applied Spectra, Inc*

This instrumentation addresses the needs of the scientific community for new technology to analyze heterogeneous materials faster and simpler with improved sensitivity and precision. Inductively coupled plasma – mass spectrometry (ICP-MS) is one of the leading technologies for elemental and isotopic analysis at trace (ug/g) and ultra-trace (ng/g) levels. However, ICP-MS analysis does not provide analysis for every element that can be present in geological samples, sometimes requiring several sample preparation methods to characterize the sample fully. In addition to the dry ablated mass aerosol that is transported to the ICP, laser ablation creates an optical plasma, in which chemical information of the sample is available as emission spectra. The measurement technique is known as laser-induced breakdown spectroscopy (LIBS). The innovation that such an instrument provided to the scientific community allowed for the simultaneous measurements of LIBS and LA-ICP-MS data. This instrument approach offers the analyst the ability to measure every element in a heterogeneous sample rapidly with spatial resolution, using one instrument. Our approach of an integrated instrument for measuring every element on the periodic chart has significant potential for many dual-use commercial applications, including biological imaging, geochemical age dating, mining, energy, and advanced industrial manufacturing. Some of these capabilities have already been explored and demonstrated for Nd:YAG nanosecond laser and femtosecond based laser in several peer-review articles over the last few years. These articles covered a wide range of applications from coal and petroleum products [1-2,9], archeological samples [3], polymer samples [4], biological tissues [5-6], food and related products [7-8], samples of forensic interest [10] and minerals [11]. In this presentation we introduce this multi sensory technique to Excimer (193nm) based laser system and the impact on the analysis of Geological samples.

(LIBS-04.3) **Tracing the provenance of minerals using advanced machine learning methods on LIBS spectra**

Prasoon K. Diwakar[1]; *[1]South Dakota School of Mines and Technology*

Laser-induced breakdown spectroscopy (LIBS) is a powerful multi-elemental analytical technique used for the detection of a variety of samples including solids, liquids, gases, and aerosols. The method utilizes a pulsed laser which generates a plasma resulting in ablation of minuscule amounts of the sample followed by its atomization, excitation, and ionization resulting in emission spectra. In this study, LIBS methodology is applied to a variety of complex geo-samples in conjunction with Raman spectroscopy. These complex samples result in generation of intricate spectral results hindering the identification of samples with certitudes. Rich spectral data collected using LIBS, in conjunction with other spectroscopy methods, can be utilized to decipher the

qualitative and quantitative composition of a sample which can facilitate provenance tracking. Application of machine learning algorithms like PCA, clustering, DFA, SVMs, and ANNs, etc., to this spectral data, can enhance its capability of discrimination/identification of sample-composition and their source-tracing considerably. Advanced machine learning approach has been developed and applied on LIBS spectra for classification and identification of complex samples and future direction of data analysis will be presented.

(LIBS-04.4) **Ionic Be II and Molecular BeO Emission for Beryllium Quantitative Analysis**

Michael Gaft[1], Lev Nagli[1], Andrey Gorychev[1], Yosef Raichlin[1]; [1]*Ariel University*

In fifteen words or less, explain the significance of this contribution (Novel Aspect).: Molecular emission of BeO is free from self-absorption unlike Resonance Be II emission doublet.

Abstract Text: Strong ionic Be II emission doublet at 313.01 and 313.04 nm in Laser-Induced Plasma (LIP) is well known for trace beryllium quantities analysis. Still, being a resonance line, it subjects to intense self-absorption (SA). The line at 313.04 nm is two times more sensitive to SA than the line at 313.1 nm because of their degeneracies difference. Thus, a reduction in doublet intensities ratio (DIR), i.e., the stronger line relative to the weaker line, from the theoretical ratio expected in optically thin conditions indicates that SA is present. Indeed, the DIR depends on Be concentration. At trace Be amounts, where the SA is absent, the line at 313.04 nm is twice higher than the line at 313.11 nm. Such ratio remains to approximately 0.005 % BeO, where it starts to decrease to 1.1 evidently because of SA. It makes this doublet unsuitable for Be evaluation at higher concentrations, typical for minor (0.1 – 1.0 %) or major (≥1.0 %) levels. Quantitative analysis of Be at elevated concentrations may be accomplished using BeO molecular emission previously not used for analytical purposes in LIP. These molecules are characterized by very intensive blue-green emission series located mainly in the 470-480 nm spectral range. It is related to B1Σ -X1Σ transition, where the strongest vibrational lines peak at 470.9 (0,0), 473.3 (1,1), 475.5 (2,2), 477.6 (3,3) and 479.5 (4,4) nm, 505.4 (0,1), 507.5 (1,2), 509.5 (2.3) and 511.2 (3,4) nm. Very weak emission bands also present at 442.81 (1,0), 445.2 (2,1), and 447.5 (3,2) nm. In our experimental setup, molecular BeO emission demonstrates a Limit of Detection (LOD) near 0.05 % of BeO at Single Pulse (SP) and 0.01 % at Double Pulse (DP) modes. The absence of SA for BeO molecular emission is presently proved in the 0.05 – 5.0 % range, which is quite suitable for minerals and alloys analysis.

21PAT03: PAT for Industrial R&D

Chair: Xiaoyun (Shawn) Chen
Co-Chair: Mark Rickard
On-site Chair: Mark Rickard

(PAT-03.2) **Elucidation of Lithium Acetyl Phosphate Synthesis Using Process Analytical Technology**

Joseph P. Smith[1], Jennifer Obligacion[1], Zachary Dance[1], Justin Lomont[1], Nicole Ralbovsky[1], Xiaodong Bu[1], Benjamin Mann[1]; [1]*Merck*

In fifteen words or less, explain the significance of this contribution (Novel Aspect).: Deep scientific understanding of lithium acetyl phosphate synthesis was accomplished using in situ analytical methodologies

Abstract Text: Acetyl phosphate salts are common phosphate donors for enzymatic phosphorylation with kinases and are used in a variety of industrial biocatalytic processes. Due to their ever-increasing demand, synthesis of high-quality acetyl phosphate salts is critical for pharmaceutical process development, especially given that common impurities such as acetic acid and phosphate can inhibit the intended reaction. Current offline analytical methodologies for investigating acetyl phosphate reagent quality are limited due to the hydrolytically unstable nature of the chemical species, resulting in its potential degradation before complete analysis may occur. It is therefore advantageous to synthesize high purity acetyl phosphate using process

analytical technology (PAT) tools to monitor reaction progress in real time. In this work, several PAT tools were employed specifically for the investigation of lithium acetyl phosphate synthesis, including in situ Fourier Transform Infrared (FTIR) spectroscopy, pH and temperature sensing, online optical microscopy, and focused beam reflectance measurements (FBRM). These PAT tools offer a significant benefit by providing insight toward reaction optimization, including through observing the timing of temperature and pH changes, realizing a particle size target, and directly monitoring the consumption of reagents and formation of products. Results indicate these online in situ methods are successful for detailed reaction monitoring and process characterization for the production of quality lithium acetyl phosphate, allowing for real time reaction understanding.

(PAT-03.3) **Globally monitoring 9,000+ molecular groups in whole crude using spectroscopy**

Bryan Bowie[1], Bryan Bowie[1]; [1]*ExxonMobil*

Fast determination of the composition of whole crude and refinery streams is a valuable tool as it can help avoid disruptions and assess its value. However, the composition is often comprised of tens of thousands of different molecules making it far too complex to thoroughly evaluate in a period of minutes. Structure oriented lumping models (SOL) has been used in the past and allows for the simplification of a complex composition. Each whole crude or refinery stream can be described using ten thousand or so of these lumps reducing the complexity. The FT(N)IR spectrum of an uncharacterized refinery stream can be described as a linear combination of library of FT(N)IR spectra of known refinery streams whose compositions have been characterized. This creates a blend of known spectra which represents the unknown. Likewise, their respective compositions can also be blended together. Furthermore, a least-squares approach permits easy inclusion of new samples with minimal need for optimization and rehashing of more complex models such as PLS. Least-squares models can also be further augmented with input from other online systems such as density and sulfur to improve accuracy. An FT(N)IR spectrum can be collected in a matter of minutes allowing for a rapid determination of composition. Replicating this at several refineries across the globe, allows for larger scale optimization.

(PAT-03.4) **Combi-Fiber Probes for Multispectral System and Multiwavelength Sensor Applications**

Viacheslav Artyushenko[1], Andrey Bogomolov[2], Iskander Usenov[1], Tatiana Sakharova[1], Alexey Bocharnikov[1]; [1]*art photonics GmbH,* [2]*Global Modelling kG*

In fifteen words or less, explain the significance of this contribution (Novel Aspect).: Combi-Fiber probes for fusion spectroscopy: FTIR, Raman, NIR & Fluorescence and for Multiwavelength Sensors

Abstract Text: Fiber optics spectroscopy provides compact, flexible, and cost-effective solutions to match the fast growing demands of industrial process control, remote environment monitoring and biomedical diagnostics. Fiber probes enable industrial reaction monitoring in-line in a harsh environment that is not possible to perform by common lab techniques. Fiber probes assisted reaction monitoring does not need any sampling and can be made at high or low temperatures, in vacuum or under high pressure and vibrations, in aggressive or toxic media. Customized probe design and length can be adapted to various chemical reactors and process-interfaces with optical coupling to different types of spectrometers: FTIR, QCL, NIR, Raman, Fluorescent, etc. Here we´ll present the latest results on the development and application of fiber optic probes designed for the key spectroscopy methods used in a broad spectral range 0.3-17µm for the analysis of ATR-absorption, transmission, Raman scattering, diffuse reflection, fluorescence in liquid, solid and gaseous media. Multi-Spectral Fiber (MSF) system enables to analyze a reaction medium by 4 key spectroscopy methods either alone for their comparison or in any combinations with a single or a combi-fiber probe. This helps to achieve the most sensitive and accurate control of process parameters on-line or in-line. Several examples of multimodal approach will be presented for industrial and biomedical applications. Multichannel NIRaman fiber optic probes (patent pending), have been developed by art photonics in cooperation with Company M.A.C. (Measure Analyze Control BV) to measure in-situ NIR diffuse reflectance and Raman scattering of solids, powders or

liquids. The data fusion from the simultaneous measurement by both methods enables the hybrid modelling opportunities to enhance the accuracy of the media analysis to the level which would be impossible for them being used separately. Another innovative combi-probe has been designed for the simultaneous analysis of various liquid and solid samples using ATR-absorption in Mid InfraRed in combination with fluorescence spectra collected from the same spot. Data of fluorescence spectra induced by electron transitions can be fused as complimentary data to Mid IR-absorption details specific for distinct molecular vibrations, - and it result in much better media composition analysis.

(PAT-03.5) **MEMS-based NIR spectrometer technologies for efficient on-line process analysis: field application and performance comparison**

Markus Brandstetter[1], Robert Zimmerleiter[1], Paul Gattinger[1], Thomas Reischer[2], Michael Rossbory[3];
[1]Research Center for Non-Destructive Testing (RECENDT) GmbH, [2]Metadynea Austria GmbH, [3]Software Competence Center Hagenberg

In fifteen words or less, explain the significance of this contribution (Novel Aspect).: Successful application reports of MEMS spectrometers under real-world conditions; performance comparison of latest technology variants

Abstract Text: Fully-integrated spectrometers based on Micro-Electro-Mechanical-System (MEMS) technology are on the fast track in NIR spectroscopy, as they combine ruggedness, miniature size and very low hardware costs. These highly attractive features make them particularily interesting for on-line process analysis. Their metrological characteristics, such as signal-to-noise ratio and accuracy are getting close to those achieved with classical NIR process spectrometers. Many variants of MEMS spectrometers are available, each of them in conjunction with different measurement geometries. In this contribution we present a thorough performance comparison of various Fabry-Perot (FP), Fourier-Transform (FT) and Digital Light Processing (DLP) spectrometers modules under realistic conditions with a focus on fiber-based systems. This comparison reveals both positive and negative features of each approach but also proves sufficient performance of the MEMS spectrometer technology. Further, we present on-site industrial application examples of MEMS-based NIR spectrometers for online process analysis, including the determination of the formaldehyde concentration in formalin in a melamine- and phenol-formaldehyde resin production plant as well as full-process control of an industrial scale batch reactor. Another practical application example that will be presented is the on-line monitoring of a cascaded injection process for the production of free-form fiber reinforced plastic composites.

21RAM05: Raman Microscopy

Chair: Katsumasa Fujita

(RAM-05.1) **CANCELLED On self-assembling intracellular Raman reporters and plasmonic nanocavities**

Ishan Barman[1]; *[1]Johns Hopkins University*

This presentation does not yet have a recording. Please wait 20 minutes to play the next presentation following this one. The potential for organizing molecules into supramolecular structures to tailor Raman signals has been surprisingly underappreciated. In this talk, we present a smart strategy for intracellular self-assembly of Raman reporters. Specifically, by leveraging the in situ click condensation reaction, we propose a new class of Raman reporters that exhibit distinct intracellular signatures in furin-overexpressing cells, and demonstrate its use for targeted detection of tumor cells in vitro and in a subcutaneous xenograft tumor model. As the spectral fingerprint is unique to this Raman reporter, it permits unambiguous tumor detection in comparison to other optical methods, such as fluorescence-based imaging, where tissue autofluorescence may lead to false-positive

signals. Additionally, we will also discuss highly precise and robust nanocavities, termed DNA-silicified template for Raman optical beacon (DNA-STROBE), that are engineered by using silicified DNA scaffolds for spatial organization of discrete plasmonic nanoparticles. The ultra-small mode volume of the DNA-STROBE constructs promotes single-molecule occupancy enabling surface-enhanced Raman spectroscopy (SERS) observations of single molecule activity even at elevated background concentration, significantly relaxing the restrictive pico- to nanomolar molecular concentration condition typically required for such investigations.

(RAM-05.2) **High-Resolution Chemical Imaging of Cells and Tissues**

Lu Wei[1], Lu Wei[1], Chenxi Qian, Kun Miao, Li-En Lin; [1]*California Institute of Technology*

Innovations in high-resolution optical imaging have allowed visualization of nanoscale biological structures and connections. However, super-resolution fluorescence techniques, including both optics-oriented and sample-expansion based, are relatively limited in quantification and throughput especially in tissues from photobleaching or quenching of the fluorophores, and lowefficiency or non-uniform delivery of the probes. Here, I would like to present our recent efforts in developing a general sampleexpansion vibrational imaging strategy for label-free high-resolution (to below 100 nm) chemical imaging in cells and tissues. With further adoption of machine learning training, we successfully obtained label-free multi-component and volumetric prediction of nucleus, blood vessels, neuronal cells and dendrites in complex mouse brain tissues

(RAM-05.4) **Quantitative Imaging Of Minor Groove Binders In Mammalian Cells Using Raman Microscopy**

Christian Tentellino[1], William J. Tipping[1], Leah McGee[1], Laura M. Bain[1], Fraser J. Scott[1], Colin J. Suckling[1], Corinna Wetherill[1], Karen Faulds[1], Duncan Graham[1]; [1]*University of Strathclyde*

In fifteen words or less, explain the significance of this contribution (Novel Aspect).: Quantitatively imaging of minor groove binders in live cells by Raman microscopy at sub-cellular resolution.

Abstract Text: Quantitative drug imaging in living cells is one of the major challenges within the drug development pipeline. The development of an analytical technique capable of quantitatively investigating the uptake and distribution of a drug would improve the identification of drug candidates early in the development stage thanks to a better assessment of these in preclinical evaluation studies. Herein, we introduce Raman microscopy as a powerful technique for the quantitative imaging of Minor Groove Binders (MGBs) in mammalian cells and investigate the influence of minor structural changes on the pharmacodynamic and pharmacokinetic properties of MGBs. This study demonstrates the unique capacity of Raman spectroscopy to analyse drug-cell responses in a label free quantitative manner. We have synthesised bioisosteric MGBs modified to include a small Raman active tag (alkyne) within the structure. Raman analysis was carried out on several mammalian cells resulting in clear identification of the alkyne tags within the MGBs. A comparison between live and fixed cells showed a redistribution of the MGBs across the cells. Fixation remarkably alters the intracellular localisation of pH sensitive MGBs. The intracellular uptake and distribution of MGBs was related to their different physicochemical properties caused by the minor structural changes between different molecules. The intracellular concentration of MGBs was estimated in several cell lines. With the attempts to further define the mechanism responsible for the intracellular localisation of MGBs, several studies were carried out to demonstrate which one between active transport, passive diffusion and facilitated diffusion is the mechanism responsible for the intracellular uptake. The stronger Raman scattering measured at cellular level led the project to further investigate its sub-cellular localisation. A study of colocalization using lysotracker Red DND-99 showed a partial lysosomal distribution for one of the MGB. This study highlights the potential of Raman spectroscopy to quantitatively investigate MGBs in mammalian cells, follow their intracellular biological dynamics as well as facilitating further drug development and optimisation.

(RAM-05.5) **Low Temperature Raman Imaging of Micron-Size Droplets**

Qishen Huang[1], Peter J. Vikesland[1]; [1]*Virginia Tech*

In fifteen words or less, explain the significance of this contribution (Novel Aspect).: The study applies Raman microscopy to investigate ion distribution upon phase transition in droplets in-situ.

Abstract Text: Atmospheric droplets exhibit heterogeneity due to their distinct mixing states and specific phases. Ice nucleation is a common and stochastic atmospheric process that may enhance the differences between atmospheric droplets. Raman microscopy has recently been shown to be a convincing tool to investigate ice nucleation and the phase transitions of cloud droplets. In this study, we investigated differences in the distributions of chemical moieties within micron-size droplets by recording spatial maps of sulfate, ice crystals, and gold nanoparticles (AuNPs) via Raman imaging at 293K and 223K. We observed an even distribution of sulfate in ammonium sulfate (AS) solutions and droplets at 293K, as well as in supercooled AS droplets at 223K. Spatially enriched sulfate, expelled from ice crystals, appeared in frozen droplets and bulk solutions at 223K. Interestingly, a fraction of the frozen droplets exhibited spatially enriched sulfate distributions while others froze evenly. Our experimental setup favors homogeneous nucleation, nonetheless a higher percentage of evenly distributed droplets were found for lower initial AS concentrations. Using optical and Raman images of the AS droplets, we determined that >93% supercooled droplets were evenly distributed, while >90% of frozen droplets were spatially enriched due to ice nucleation. The remainder of the supercooled droplets could contain partially nucleated ice, and the balance of frozen droplets might experience glass formation. We also investigated the distribution of pH sensing functionalized AuNPs in frozen AS droplets and observed an independent pattern of AuNPs that differed from either the AS distribution or the locations where ice crystals formed. The relative peak intensity of the Raman spectrum of AuNPs changed at 223K compared to its room temperature spectrum, which suggested that the crystallization of AuNPs at 223K altered the spectral behavior of the functionalized molecule. We demonstrate the capability of Raman imaging to determine chemical distributions in micron droplets at low temperature.

21SPR01: Sensing and Actuating Chemistry

Chair: Amanda Haes
On-site Chair: Nan Jiang

(SPR-01.1) **Plasmonic Platforms for Polaritonic Chemistry**

Matthew Sheldon[1]; [1]*Texas A&M University*

We are developing experimental platforms and spectroscopic techniques to probe vibrational strong coupling (VSC) between molecules and resonant infrared (IR) nanophotonic architectures, in order to understand how this coupling can fundamentally control chemical reactivity, as well as enable new classes of light-matter interaction. This method of altering the potential energy surface of a chemical process via coherent, electromagnetic perturbation of vibrating bonds has also been termed “polaritonic chemistry”. We employ a combined experimental strategy leveraging expertise in (1) the design of IR “metasurfaces” composed of plasmonic metal substrates that provide tailorable VSC to molecules within their optical near-field; and (2) continuous wave (CW) electronic Raman spectroscopic techniques that enable analysis of several non-equilibrium, dynamic electronic effects in the metal substrate in parallel with conventional molecular Raman spectroscopy. Taken together, these tools allow studies into new regimes of spectral bandwidth (e.g. simultaneous multi-mode coupling), coupling strength, and time domains (e.g. studies of long lived and steady-state phenomena) that have been inaccessible using conventional optical cavities and time-resolved spectroscopies performed to date. Vibrational strong coupling is fundamentally interesting because it is a coherent interaction between radiation and molecular motion. The direct manipulation of a molecular process using externally controlled forcefields to obtain a desired outcome, i.e. “coherent control” or “quantum control”, has been a long-standing goal connected to the central aims of chemical science. VSC using plasmonic

substrates offers a rationalizable experimental platform for attaining this goal. Thus, the primary objective of our research is to explore the limits of chemical analysis and chemical control at interfaces within this framework, by understanding new classes of chemical and physical phenomena that leverage the coherent interactions between controllable features of the engineered surface geometry and the molecular systems under study.

(SPR-01.2) **A Surface Enhanced Raman Scattering (SERS) Sensor for Rapid Detection of a Plant Disease Biomarker**

Jing Zhao[1], Chen Song, Yu Lei; [1]*University of Connecticut*

Microbial diseases in plants could lead to decreased production, and thus it is important to detect them at early stages. When plants get infected by pathogens, they could emit unique volatile organic compounds (VOCs), which could be used as disease biomarkers. In this work, methyl salicylate (MeSA) is chosen as the target, as it is released abundantly when plants have pathogen infection. In order detect MeSA vapor with high sensitivity, surface enhanced Raman scattering (SERS) spectroscopy was employed using a portable Raman spectrometer. Au nanoparticles functionalized were SERS probe molecules used as the SERS substrates. Upon exposure to the MeSA vapor, the SERS signal of the probes changes in a few minutes due to the interaction between MeSA and the SERS probes. The SERS sensor can detect MeSA vapor pressure as low as a few ppbs. This study demonstrates the capability of the SERS sensing platform for non-invasive, rapid detection of volatile biomarkers for crop health.

(SPR-01.3) **Hybrid Plasmonic Reactors for Photocatalysis and Microbe Inactivation**

Bjoern Reinhard[1]; [1]*Boston University*

Hybrid plasmic nanomaterials comprising a noble metal nanoparticle and photocatalysts located in the vicinity of the nanoparticle provide opportunities for enhanced photocatalysis and, thus, actuating chemistry. We have integrated the transition metal complex tris(bipyridine)ruthenium(II) [Ru(bpy)3]2+ into a self-assembled lipid layer around a silver nanoparticle, which localizes the photocatalyst in an electromagnetic sweet spot. The localized surface plasmon resonance (LSPR) of the silver nanoparticle overlaps with the metal-to-ligand charge transfer (MLCT) band of the [Ru(bpy)3]2+, facilitating an efficient excitation of the photocatalyst. The lipid membrane around the silver nanoparticle serves as a spacer that ensures sufficient separation between photocatalyst and noble metal nanoparticle to avoid strong quenching of the photoexcited state but, at the same time, positions the photocatalyst close enough to the nanoparticle to ensure strong electromagnetic enhancement of the photoexcitation. The fundamental working principles of the hybrid materials will be analyzed, and selected applications in photocatalysis for water treatment, solar energy conversion, and microbe inactivation will be discussed.

(SPR-01.4) **Colloidal Magnesium Plasmonics for Sensing and Catalysis**

Emilie Ringe[1], Jeremie Asselin[1], Christina Boukouvala[1], Elizabeth Hopper[1], Vladimir Lomonosov[1], Thomas Wayman[1], John S. Biggins[1]; [1]*University of Cambridge*

Recently, alternatives to the rare and expensive noble metals Ag and Au have been sought for more sustainable and large-scale plasmonic utilization. Mg supports plasmon resonances in the UV, visible and NIR, is one of the most abundant elements in earth's crust, and is fully biocompatible, making it an attractive framework for plasmonics. This talk will first very briefly review the unusual shapes obtained in colloidal synthesis, including single crystal and twinned nanoparticles. The discussion will then move to the surface chemistry and reactivity of magnesium, proving that the thin oxide layer formed is self-limiting in air and only minimally affects the nanoparticle's plasmonic behavior. The deliberate addition of protective shells, and their effectiveness in protecting Mg from oxidation and reaction with water will be presented. Then, the optical response of Mg nanoparticles is overviewed, highlighting Mg's ability to sustain localized surface plasmon resonances across a

broad range of energies. We will discuss how we use these plasmon resonances in surface-enhanced spectroscopies as well as light-enhanced catalysis, the latter using a bimetallic structure enabled by galvanic replacement.

(SPR-01.5) **Nanoscale Imaging of Catalytic Processes on Gold-Platinum and Gold-Palladium Nanoplates**
Dmitry Kurouski[1]; [1]*Texas A&M University*

In fifteen words or less, explain the significance of this contribution (Novel Aspect).: Tip-enhanced Raman imaging revealed the underlying physical cause of unique catalytic activity of bimetallic catalysts.

Abstract Text: Bimetallic nanostructures possess unique chemical properties. This makes them broadly used in a large variety of research fields ranging from optical sensing to light-driven catalysis. Chemical properties of bimetallic nanostructures are optimized by empirical synthetic approaches that are time and labor-consuming. Primarily, because their optical properties and nanoscale structural organization cannot be directly accessed by commonly used analytical techniques, such as absorption spectroscopy. Much more productive and focus optimization of catalytic reactivity of bimetallic nanostructures can be made if the relationship between structure and catalytic reactivity at the nanoscale will be understood. My group showed that tip-enhanced Raman spectroscopy (TERS) could be used for nanoscale spatiotemporal characterization of catalytic processes of gold-platinum and gold-palladium nanoplates (Au@PtNPs and Au@PdNPs). We found that these bimetallic catalysts exhibited unique reactivity and selectivity that was not evident for their monometallic analogs, gold nanoplates (AuNPs). We also found that catalytic reactivity and selectivity of bimetallic nanostructures can be controlled by the intensity of rectified electric field that in turn can be controlled by light intensity. This discovery opens up an avenue for direct optical control of catalytic reactions that can be used in a large variety of synthetic procedures.

21ATOM07: New trends in atomic spectroscopy analysis

Chair: Mauro Martinez
On-site Chair: Mauro Martinez

(ATOM-07-1) **Lithium Isotope Analysis via High-Resolution Continuum Source Atomic Absorption Spectrometry**
Carlos Abad[1], Alexander Winckelmann[1], Dalia Morcillo[1], Sascha Nowak[2], Silke Richter[1], Jochen Vogl[1], Jens Riedel[1], Sebastian Recknagel[1], Ulrich Panne[1]; [1]*Federal Institute for Materials Research and Testing (BAM),* [2]*MEET Battery Research Center, University of Münster*

In fifteen words or less, explain the significance of this contribution (Novel Aspect).: Faster alternatives for lithium isotope analysis by monitoring the isotope shift in atomic spectra

Abstract Text: Alternative methods for lithium isotope analysis by using high-resolution atomic absorption spectrometry (HR-CS-AAS) are proposed herein. They are based on the well-known isotope shift of approximately 15 pm for the electronic transition 22P←22S at around the wavelength of 670.8 nm, which can be measured by state-of-the-art HR-CS-AAS. Isotope analysis can be used for (i) the traceable determination of Li concentration [1] and (ii) isotope amount ratio analysis based on a combination of HR-CS-AAS and spectral data analysis by machine learning (ML)[2]. In the first case, the Li spectra are described as the linear superposition of the contributions of the respective isotopes, each consisting of a spin-orbit doublet, which can be expressed as Gaussian components with constant spectral position and width and different relative intensity, reflecting the isotope ratio in the sample. The procedure has been validated using human serum certified reference materials. The results are metrologically comparable and compatible to the certified values. In the second case, for isotope amount ratio analysis, a scalable tree boosting ML algorithm (XGBoost) was employed

and calibrated using a set of samples with 6Li isotope amount fractions ranging from 0.06 to 0.99 mol mol−1. The training ML model was validated with certified reference materials. The procedure was applied to the isotope amount ratio determination of a set of stock chemicals and a BAM candidate reference material NMC111 (LiNi1/3Mn1/3Co1/3O2), a Li-battery cathode material. These determinations were compared with those obtained by MC-ICP-MS and found to be metrologically comparable and compatible. The residual bias was −1.8‰, and the precision obtained ranged from 1.9‰ to 6.2‰. This precision was sufficient to resolve naturally occurring variations. To assess its suitability to technical applications, the NMC111 cathode candidate reference material was analyzed using high-resolution continuum source atomic absorption spectrometry with and without matrix purification. The results obtained were metrologically compatible with each other. References [1] A. Winckelmann et al., Determination of Lithium in Human Serum by Isotope Dilution Atomic Absorption Spectrometry, Preprint ChemRxiv, (2021) [2] A. Winckelmann et al., High-Resolution Atomic Absorption Spectrometry Combined with Machine Learning Data Processing for Isotope Amount Ratio Analysis of Lithium, Preprint ChemRxiv, (2021)

(ATOM-07-2) **Multi-element matrix-match hydroxyapatite reference material for laser ablation ICP-MS and LIBS, a solution for quantitative tooth analysis for environmental medicine.**

Mauro Martinez[1], Christine Austin[1], Manish Arora[1]; [1]*Icahn School of Medicine at Mount Sinai*

In fifteen words or less, explain the significance of this contribution (Novel Aspect).: Quantitative metal analysis on teeth has a high impact in epidemilogy studies

Abstract Text: Laser ablation ICP-MS and LIBS have become in an important tool of analysis in medicine, measuring element distributions in teeth, bones, hair and soft tissue. Increasingly, these tools are being used to reconstruct environmental exposure histories in large epidemiological studies . However, to achieve quantitative analysis, that will enable standardization of methods and inter laboratory comparisons across studies, a reference material that imitates the chemical and optical sample properties of the sample is required. Doped powder pellets can be chosen to match the chemical composition, crystalline structure, mineral hardness, laser-surface response and other properties. In this work, we propose the synthesis of hydroxyapatite sintered pellet reference materials doped with various elements of interest. The preparation process involves doping during synthesis followed by sintering to create pelletized ceramics. We show that these materials have good chemical homogeneity and optical properties that emulate those of calcified biological tissues, as teeth. These new standard materials open a door for the standardization of quantification methods for environmental medicine and clinical research.

(ATOM-07-3) **Evaluation of the Influence of the Particle Size and Coating on the Stability of AuNPs in Suspension Using Single Particle ICP-MS**

Antonio R. Montoro Bustos[1], Karen E. Murphy[1], Michael R. Winchester[1]; [1]*NIST*

In fifteen words or less, explain the significance of this contribution (Novel Aspect).: The stability of AuNPs at environmentally relevant concentrations is rigorously assessed for the first time

Abstract Text: The outstanding physical and chemical properties exhibited by engineered nanoparticles (NPs) are related to many factors, including their chemical composition and surface structural characteristics. The fate, transport, stability and potential risks of NPs are directly related to their physicochemical properties, including composition, particle size and shape. Among them, surface chemistry and agglomeration/aggregation state are critically determined by particle size and the nature of the coating surrounding the NPs, which plays a pivotal role in the interactions of NPs with their environment. The expected low levels of engineered NPs in environmental systems precludes reliable detection by conventional analytical strategies. Thus, novel

approaches for reliable in situ NPs characterization are needed. In this context, single particle inductively coupled plasma-mass spectrometry (spICP-MS) is considered an emerging and promising analytical technique for the ultrasensitive detection and characterization of metal-containing NPs. Particularly, spICP-MS offers exceptional potential for providing information about particle size, agglomeration/aggregation state and particle number concentration (PNC) at mass concentration levels down to ng L-1. Additionally, simultaneous detection of dissolved, pristine NPs, and their agglomerated/aggregated species can be carried out in a single analysis by using spICP-MS. This communication presents, for the first time, a rigorous assessment of the influence of particle size and surface coating on the stability of different commercially available gold NP (AuNP) suspensions at very low concentrations (ng L-1). Thus, the long-term stability of AuNP suspensions of different sizes and surface coatings over 120 days in high purity water at pH 7.4 is evaluated through spICP-MS sizing, and PNC and dissolved fraction quantification results. Preliminary spICP-MS analysis did not reveal a significant influence of the coating on the long-term stability of the particle size and PNC of AuNPs under study. In general, while a variability of 10 % in mean particle diameter was found, a larger variability was observed for PNC.

(ATOM-07-4) **Discrimination of Engineered, Natural, and Incidental Cerium Nanoparticles in Particle Mixtures using sp-ICP-TOFMS**

Sarah E. Szakas[1], Alexander Gundlach-Graham[1], Richard Lancaster[1]; [1]*Iowa State University*

In fifteen words or less, explain the significance of this contribution (Novel Aspect).: Novel approach to quantitative analysis of natural, engineered, and incidental cerium nanoparticles through statistical discrimination.

Abstract Text: Discrimination of natural nanoparticles (NNPs) and engineered nanoparticles (ENPs) is necessary to monitor anthropogenic particles against natural backgrounds. However, quantitative detection of NNPs and ENPs with similar major-element composition becomes difficult in mixtures, and NNP-rich samples (such as soil and water) hinder detecting low concentrations of ENPs. Recently, single particle Inductively Coupled Plasma Time-of-Flight Mass Spectrometry (sp-ICP-TOFMS) has been used to discriminate CeO_2 ENPs from Ce-NNPs in soil extracts through elemental fingerprinting.[1] It was found that the presence of lanthanum (La) in Ce-NNPs provided enough discriminating power for separation. In this study, we expand on Ce-rich particle classification by sp-ICP-TOFMS through the analysis of mixtures of CeO_2 ENPs, Ce-NNPs, and Ce-containing incidental nanoparticles (INPs) created via high-temperature sparks from striking ferrocerium “flint” from a common disposable lighter. We found that the INPs produced from the lighter have high mass fractions of both Ce and La, which obscures the use of simple binary Ce-only and Ce-La particle classification to separate Ce-NPs. Instead, we must use other rare earth elements (REEs) in particles to separate INPs from NNPs, namely Nd, Pr, and Th. We show that mass ratios of Ce to REEs in NNPs are homogeneous even as particle size is heterogeneous and that element-signal ratios from individual Ce-NNPs follow Poisson statistics. Based on these observations, we can predict the likelihood that a given Ce-only or Ce-La particle signals are from ENPs, INPs, or NNPs. We describe the statistical basis of our Ce-NP discrimination approach and demonstrate its application on mixtures containing constant amounts of bastnaesite (Ce-NNPs) and differing amounts of CeO_2 ENPs and lighter- spark INPs. Ce-NNPs were found with a constant number concentration of ~$4x10^4$ particles per mL across all samples, while ENPs and INPs were detected proportional to their dilution, with number concentrations ranging from ~$4x10^3$ to ~$1x10^5$. We discuss the application of this approach for quantitative analysis of anthropogenic Ce-NPs from environmental samples. (1) A. Praetorius, A. et al., Environ. Sci.: Nano, 2017,4, 307-314

(ATOM-07-5) **Ultra-high Throughput Nanoparticle Characterization and Elemental Mapping through Glow Discharge Optical Emission Spectroscopy**

Gerardo Gamez[1], Aldo Hernandez[1], Layan Shabaneh[1], Kevin Finch[1]; [1]*Texas Tech University, Department of Chemistry and Biochemistry*

In fifteen words or less, explain the significance of this contribution (Novel Aspect).: Ultrahigh-throughput nanoparticle characterization is enabled by GDOES elemental mapping for the first time

Abstract Text: The use of nanoparticles (NP) has become widespread in lots of areas, including energy conversion, biomedical diagnostics and therapeutics, disinfection, catalysis, etc. In addition, the increased NP use has resulted in their growing proliferation in the environment. Thus, alternative techniques for NP analysis and characterization, including elemental mapping (EM), are needed. EM allows monitoring the NP distribution in relevant samples, and can be used as an element specific detector for techniques that yield spatial distributions of NP according to size, shape, or even surface functionality. Typical EM techniques, however, require excessively long times for analysis. In contrast, glow discharge optical emission spectroscopy (GDOES) allows EM with ultra-high throughput, within seconds, while yielding depth resolved information over time. Herein, the development of a GDOES EM method to permit analysis of NPs from dried solution residues will be described. The effects of various experimental parameters, including GDOES pulsing, pressure, and sample substrate materials, on the analytical figures of merit (FOM) will be presented. In addition, the FOM for different NP sizes and compositions will be reported. Preliminary data indicates that detection limits for silver NPs are within the requirements for studying their interaction and uptake by single cells. Finally, the use of hollow cathode strategies for potential FOM enhancements will be investigated and discussed.

21AWD05: SAS Lester W. Strock Award Symposium Honoring Uwe Karst

Chair: Uwe Karst
On-site Chair: C. Derrick Quarles Jr.

(AWD-05.1) **Droplets and Plasmas as Sources of Novel Chemistry to Probe the Chemical Origins of Life**

Jacob Shelley[1], Montwaun D. Young[2], Brian T. Molnar[2], Sunil P. Badal[2], Morgan F. Schaller[3], Karyn Rogers[4]; [1]*Rensselaer Polytechnic Institute,* [2]*Department of Chemistry and Chemical Biology, Rensselaer Polytechnic Institute,* [3]*Department of Earth and Environmental Sciences, Rensselaer Polytechnic Institute,* [4]*Rensselaer Astrobiology and Research Education Center, Rensselaer Polytechnic Institute*

Determining or theorizing the origins of life on Earth has been a long-standing philosophical and scientific endeavor for humans. Many efforts of the past several decades have focused on the chemistry that could have existed on the early Earth to yield complex molecules seen in modern life and often viewed as necessary precursors for life formation (e.g., amino acids, nucleotides, and carbohydrates). The classic Miller-Urey experiments of the 1950s generated excited in use of ions and plasma (to mimic lightning) to synthesize complex organic molecules from atmospheric precursors. Unfortunately, those experiments utilized a reduced atmosphere, while the modern geological consensus is that Hadean Earth atmosphere was oxidized. More recently, it has been shown that gaseous ions and highly charged droplets can enhance reaction rates of carbon-nitrogen and carbon-oxygen bond formation by several orders of magnitude over solution-phase systems. The Hadean Earth likely had numerous sources of gaseous ions and charged droplets, including photoionization, frequent impact events, lightning, and crashing waves, that could result in such unique chemistry. This presentation will focus on the use of charged microdroplets and electrical plasmas in realistic early Earth conditions to enable rapid, efficient synthesis of biologically relevant species. Charged microdroplets were produced by means of electrospray ionization (ESI) where reactants, such as activated nucleotides or amino acids, dissolved in water with cations (e.g., H+, Na+, Mg2+). Products (oligonucleotides and peptides) formed in the droplets were recorded in real-time with mass spectrometry (MS). The area between the ESI needle and

MS was purged with CO2 and N2 to mimic the oxidized Hadean atmosphere. Reaction yields and rates could be estimated based on the MS results. Unique gas-phase ion-molecule chemistry was probed using an atmospheric-pressure glow discharge and gaseous reactants (e.g., PAHs, amines, and hydrocarbons) in an oxidized atmosphere. Formation of carboxylic acids, ketones, and aldehydes were rapidly formed and recorded in real time with MS. These reactions products could be collected and characterized offline. Lastly, these results will be discussed in the context of the astrobiology literature to postulate scenarios for complex (bio)molecule formation on the early Earth.

(AWD-05.2) **New Insights into the Protein Corona of Gold Nanoparticles in Living Cells.**

Jörg Bettmer[1], Gergo P. Szekeres, Nerea. Fernández-Iglesias, María Montes-Bayón[1], Janina Kneipp[2];
[1]University of Oviedo, [2]Humboldt-Universität zu Berlin

The understanding of the formation of the intracellular protein corona surrounding nanoparticles is nowadays essential for many applications related to nanomedicine. The so-called hard protein corona considered as the proteins with highest affinity to the surface of the nanoparticle has here a deterministic effect on its intracellular processing. Therefore, the aim of this work is to point out an analytical strategy to characterize the hard protein corona in living cells on the example of different cell lines after incubation with gold nanoparticles. In contrast to most of the research work found in the literature, we developed an analytical procedure that enabled us to study this processing after extracting the intact bioconjugates from living cells [1]. Cells from cell lines HCT-116 and A549 were incubated with 30 nm citrate-stabilized gold nanoparticles and the combined experiments of mass spectrometry and surface-enhanced Raman scattering revealed different incorporation mechanisms [2]. Moreover, the analysis of the proteinaceous composition of the corona gave insight into the entire "past" of the nanoparticles. [1] G.P. Szekeres, N. Fernández-Iglesias, J. Kneipp, M. Montes-Bayón, J. Bettmer. J. Proteom. 212 (2020) 103582. [2] G.P. Szekeres, S. Werner, P. Guttmann, C. Spedalieri, D. Drescher, V. Živanović, M. Montes-Bayón, J. Bettmer, J. Kneipp. Nanoscale 12 (2020) 17450-17461.

(AWD-05.3) **Development of a diagnostic tool for the determination of Wilson disease**

C. Derrick Quarles Jr.[1], Marcel Macke[2], Uwe Karst[3], Bernhard Michalke[4], Hans Zischka[5], Patrick Sullivan[1];
[1]Elemental Scientific, Inc., [2]University of Münster, Institute of Inorganic and Analytical Chemistry, [3]Institute of Inorganic and Analytical Chemistry, University of Münster, [4]Helmholtz Center Munich, [5]Helmholtz Center Munich & Technical University Munich

Copper is an essential element for biological functions, however, there are several copper related diseases such as Menkes or Wilson disease that can result from copper deficiency or overload, respectively. In the case of Wilson disease, it is typically determined from physical tests that were triggered because of family history or the disease was onset causing medically relevant side-effects. Most medical issues arise from elevated "free copper" levels typically in the liver and/or brain, which can be toxic to cells. If Wilson disease goes untreated for too long it can lead to liver damage, resulting in surgery to remove portions of the liver or worst case require a liver replacement. The monitoring of Wilson disease is most commonly done by measuring the total Cu values in serum and subtracting the ceruloplasmin (copper transport protein) values to determine what portion is considered "free copper". We present here a potential diagnostic tool for direct determination of total, bound, and free copper in serum using an automated sample introduction and liquid chromatography (LC) system (prepFAST IC) in combination with an inductively coupled plasma-mass spectrometer (ICP-MS). This LC-ICP-MS method was validated using serum from either control (Atp7b+/-) or Wilson disease (Atp7b-/-) rats. The free copper/bound copper ratio was found to be 6.4% in healthy control rats, 38% for healthy Wilson rats, and 34% for diseased Wilson rats. These results presented here suggest that this method could be used as a diagnostic tool for Wilson disease or other copper related diseases.

(AWD-05.4) **Analytical Strategies for the Elemental Characterization of nano- and microstructures**

David Clases[1], Thomas Lockwood[2], Emma Camp[2], Samantha Goyen[2], Sarah Meyer[2], Philip Andrew Doble[1], Raquel Gonzalez de Vega[2]; *[1]University of Technology Sydney , [2]University of Technology Sydney*

Analytical Chemistry is an essential bridge between life- and environmental sciences, medicine, and nanotechnology that provides novel and innovative perspectives on contemporary and important problems associated with medical research, climate change and environmental pollution. Specifically, inductively coupled plasma – mass spectrometry (ICP-MS) is a mature technology with utility beyond the sole investigation of elemental concentrations in aqueous solutions. ICP-MS offers unique detection modes and signal acquisition strategies with elegant solutions for rapid sampling and generation of accurate statistical models to improve descriptions of properties and behaviors of nanoparticles and colloidal systems. Single particle (SP) ICP-MS is becoming the gold standard for developing models of particle number concentrations, size distributions, particle-particle interactions, longitudinal stability, and the environmental fate. This work demonstrates how recent technological innovations may be combined to investigate relevant nano- and microstructures in terrestrial and aqueous environments. These structures include engineered and natural nanoparticles, microplastics and cells. Dedicated software solutions for novel SP ICP-MS applications will be presented and brought into context with new directions in hyphenated SP ICP-MS. Further, the utility of different technological advances including tandem mass spectrometry will be demonstrated by investigating the number and size of nano- and micropollutants in complex matrices. Applications and examples will consist of studies on natural and engineered nanomaterials in the catchment of urban metropoles, the analysis of microplastics in environmental matrices, and the characterization of algae in threatened coral reefs.

(AWD-05.5) **Elemental and molecular bioimaging of nanoparticles and their impact on biological tissues**

Ilona D. Nordhorn[1], Antje Vennemann[2], Michael Sperling[3], Martin Wiemann[2], Uwe Karst[3]; *[1]University of Münster, Institute of Inorganic and Analytical Chemistry, [2]IBE gGmbH Institute for Lung Health, [3]Institute of Inorganic and Analytical Chemistry, University of Münster*

Nanoparticles with their unique physical and chemical properties offer great benefits for various applications such as drug delivery, electronics and biosensing. On the downside, nanoparticles can also pose a potential threat to the human health. Particularly noteworthy here is the exposure to nanoparticles by inhalation, which can occur, for example, at industrial workplaces through the inhalation of dusts and exhaust gases. Inhaled nanoparticles can be deposited in the lungs and induce inflammation processes in the tissue. Furthermore, inhaled nanoparticles can translocate to extrapulmonary organs with their effects there being widely unknown. Analytical methods that provide reliable and detailed information on the behaviour of nanoparticles in organisms can contribute to a comprehensive risk assessment of pulmonary nanoparticle exposition. Here, we present elemental and molecular bioimaging techniques that allow to investigate the distribution of nanoparticles and to reveal physiological changes in the exposed organ tissues. As an element-specific technique, the hyphenation of laser ablation and inductively coupled plasma-mass spectrometry (LA-ICP-MS) was used to analyse organ thin sections after intratracheal instillation of metallic nanoparticles differing in size and elemental composition. Distribution images obtained for elements introduced by nanoparticles revealed specific distribution patterns in the tissues of spleen and kidney samples. Furthermore, single particle ICP-MS analysis with sample introduction via laser ablation was employed to obtain size distributions of nanoparticles directly in tissue samples without prior sample digestion. With this technique, particulate and ionic species can be distinguished, allowing to address possible dissolution or aggregation of nanoparticles in biological tissues and, in case of nanoparticle translocation, to identify the size of translocated nanoparticles. As a molecular bioimaging technique, matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS) was used to investigate the impact of nanoparticles on the exposed organs. A special focus was directed the detection of phospholipids in lung samples, as changes in their distribution and ratios may indicate nanoparticle-induced pathological processes.

21BIM02: Translation of Multimodal Imaging Technologies into Clinical Routine

Chair: Thomas Bocklitz
Co-Chair: Anuradha Ramoji
On-site Chair: Andrew Whitley

(BIM-02.1) **FLIM in the Operating Theater**

Laura Marcu[1]; [1]*University of California Davis*

Precise targeting of malignant tissues during interventions is critical to both the efficacy and safety of the surgical therapy and patient outcome. Such precision would be greatly facilitated by information rich diagnostic imaging of targeted tissue at the time of intervention. Unfortunately, the ability to obtain information about the targeted tissues in real-time is currently limited. A new generation of optical spectroscopy and imaging techniques combined with computational advances (i.e. machine-learning and artificial intelligence) now holds great promise for vast improvement in the area of intraoperative diagnostics and smart guided personalized therapeutic interventions. Here, I will present clinically-compatible multispectral fluorescence lifetime imaging (FLIm) techniques and studies demonstrating that tissue autofluorescence lifetime properties can be associated with distinct tissue pathologies. I will show the ability of FLIm to operate in conjunction with surgical robots and microscopes used in standard-of-care and present studies conducted in patients in the operating theatre. Current results demonstrate FLIm's potential for intraoperative delineation of brain tumors and brain radiation necrosis as well as head and neck cancer including image-guided augmented reality during trans-oral robotic surgery (TORS). Challenges and solutions for practical and broader implementation of FLIm in surgical oncology are discussed.

(BIM-02.2) **Detection of COVID-19 in Saliva Using Label-Free Raman Spectroscopy**

Frederic Leblond[1], Katherine Ember[2], Katherine Ember[2], Myriam Mahfoud[2], Frederick Dallaire[2], Esmat Zamani[2], Trang Tran[2], Arthur Plante[2], Francois Daoust[2], Nassim Ksantini[2], Tien Nguyen[2], Fabien Picot[2], Mame-Kany Diop[3], Israel Veilleux[2], Gabriel Beaudoin[1], Guillaume Sheehy[2], Audrey Laurence[2], Sandryne David[2], Jeremie Kerouac[1], Thomas Regouffre[1], Jean-Francois Martin[1], Julie Lanthier[1], Antoine Filiatrault[1], Frederique Leblond[1], Gabriel Potvin[1], Dominique Trudel[3], Frederic Leblond[1]; [1]*Polytechnique Montréal,* [2]*Polytechnique Montréal, Centre du recherche du CHUM,* [3]*Centre du recherche du CHUM*

To date, there are two main ways of detecting SARS-CoV-2: PCR tests from nasopharyngeal swabs or saliva, and blood tests for antibodies against the virus. Both methods require tailored biochemical reagents which can be costly, are often back-ordered and limited in supply. Such analyses often require hours for a diagnosis, which could lead to asymptomatic viral transmission in airports, schools, hospitals and workplaces. Primers and antibodies may need to be re-adapted as new variants of concern emerge. We are addressing the limitations of current SARS-CoV-2 screening strategies by using laser-based Raman spectroscopy (RS) to detect the intrinsic biomolecular signature of saliva and use its fingerprint as an indication of COVID-19 infection. We collected 550 saliva samples from a COVID-19 testing clinic (37 positive by PCR) with information about symptoms (asymptomatic, respiratory, non-respiratory) and confounding factors. Our preliminary microscopy results suggest that we can discriminate between COVID positive and negative saliva. We imaged a subset of 40 matched samples (1:1 ratio between COVID-positive and negative) using a Renishaw InVia Raman microscope. A machine learning model was trained and validated (support vector machine, SVM, 5-fold cross-validation) using 12 features to minimize over-fitting and ensure confidence in model generalizability to new data. The model performed with 75% sensitivity and 70% accuracy and could be tweaked (by modifying the receiving-operating-characteristic curve, ROC curve, parameter) to lead high sensitivity (90-95%) but lower specificity (60-65%). It was shown to perform at both high sensitivity and specificity (>95%) for those with no symptoms or non-respiratory symptoms (e.g. nausea, fever). As people with respiratory symptoms (e.g. cough, running nose) are considered highly contagious and should not be in public spaces, this is very promising for a screening

tool. We are replicating this using a low-cost, custom-built system. We are also currently testing saliva from hospitalised patients over time (ongoing). The simplicity and rapidity of the new test would allow improved pandemic control through real-time on-site testing. Increased accessibility would allow medics to rapidly identify outbreaks in remote regions. The new system will be adaptable to other biofluids and the detection of other diseases.

(BIM-02.3) **Translation of Multimodal Imaging Technologies into Clinical Routine**

Thomas W. Bocklitz[1], Oleg Ryabchykov[2], Pranita Pradhan[3], Shuxia Guo[4], Tobias Meyer[4], Juergen Popp[5], Thomas W. Bocklitz[1]; *[1]Leibniz Institute of Photonic Technology (Leibniz-IPHT), Albert-Einstein-Straße 9, 07745 Jena, Germany; Research Campus Infectognostics e.v., Philosophenweg 7, 07743 Jena, Germany Institute of Physical Chemistry and Abbe Center of Photonics (IPC), Friedrich-Schiller-University Jena, Helmholtzweg 4, 07743 Jena, Germany;, [2]Leibniz Institute of Photonic Technology (Leibniz-IPHT), Albert-Einstein-Straße 9, 07745 Jena, Germany; Research Campus Infectognostics e.v., Philosophenweg 7, 07743 Jena, Germany; Institute of Physical Chemistry and Abbe Center of Photonics (IPC), Friedrich-Schiller-University Jena, Helmholtzweg 4, 07743 Jena, Germany;, [3]University of Jena, [4]Leibniz Institute of Photonics Technology, [5]Leibniz Institute of Photonic Technology*

Multimodal imaging is the combination of multiple different imaging techniques and due to the selection of a specific combination of imaging techniques, multimodal imaging can be tailored to solve a given problem. The drawback of such a combination of imaging techniques is that the multimodal image is hard to analyze manually. That is partly because humans can only process the images sequentially, but also that it is hard to use correlations between images manually. To circumvent this problem machine learning methods can be used to translate the images into information, which is interpretable in the application context. This contribution presents two examples how multimodal imaging in combination with machine learning (ML) / deep learning (DL) can be beneficial in clinical routine diagnostics. The first application of multimodal imaging is the combination of different immuno-histochemical and histochemical stainings for breast cancer diagnostics [1]. We could show that these images can be used together in a data fusion approach to improve cancer diagnostics. Furthermore, we could compare two transfer-learning strategies for this task and found that a feature extraction based transfer learning was better suited probably due to the low sample size of the study. In a second study we could show how nonlinear multi-contrast microscopic imaging, e.g., a multimodal imaging approach combining coherent anti-Stokes Raman scattering (CARS), two-photon excited fluorescence (TPEF) and second harmonic generation (SHG), can be translated into hematoxylin-eosin stain (HE) images in a non-supervised manner using cycle generative adversarial networks (GANs) [2]. Both studies show the benefit of tailoring ML and DL methods to as specific multimodal imaging combination and how this can help surgeons and pathologists in clinical routine tasks. References: [1] Pradhan, P. et al. Proceedings of the 10th International Conference on Pattern Recognition Applications and Methods - Volume 1: ICPRAM, SciTePress, 2021, 495-506. [2] Pradhan, P. et al. Biomedical Optics Express, Optical Society of America, 2021, 12, 2280-2298 Acknowledgements: Financial support by the EU; the BMBF, the Leibniz Society, the Free State Thueringia and the DFG are highly acknowledged.

(BIM-02.4) **Raman Spectroscopy Imaging to follow Septic Liver Restoration**

Anuradha Ramoji[1], Adrian T. Press[2], Johann von Below[3], Michael Bauer[2], Juergen Popp[1]; *[1]Leibniz Institute of Photonic Technology, [2]Jena University Hospital, [3]Institute for Physical Chemistry and Abbe Center of Photonics, Friedrich-Schiller University, Helmholtzweg 4, 07743 Jena, Germany,*

Systemic infections frequently cause liver and other organ failure rendering a life-threatening syndrome commonly known sepsis. The chemical metabolism of the liver gets affected by the infection resulting in excessive lipid accumulation along with an elevated protein metabolism. Biophotonic methods have been extensively applied for tissue imaging to extract biochemical image in a label-free manner and discriminating

healthy from pathologically affected tissue. Here we apply vibrational spectroscopy methods to follow disease relevant biochemical changes in the liver tissue and assess efficiency of the treatment. The liver tissue was harvested from mice with sepsis induced through injection of a characterized human stool suspension (peritoneal contamination model). Post-infection mice were treated with an inhibitor of Phosphinositol 3-kinase known to protect liver function [1]. Sham mice, injected with a sterile saline solution were used as control [2]. On freshly prepared liver cryosections (10 µm) Raman and FTIR spectroscopy imaging were performed. False colour Raman and FTIR images were generated using vector component analysis (VCA). The vibrational spectroscopy images depict changes in the biochemical distribution between sham and mice with sepsis. The morphochemical image analysis with VCA allowed a clear distinction of untreated and inhibitor treated mice. Only mice treated with the PI3K inhibitor showed a similar Raman and FTIR lipid and protein profile as sham control mice. Hence showing the capability of label-free vibrational spectroscopy imaging for visualizing the biochemical composition of liver in health and disease condition and to assess the outcome of the treatment. Acknowledgement: The BMBF via the Integrated Research and Treatment Center "Center for Sepsis Control and Care" (FKZ 01EO1502), the Jena Biophotonic, Imaging Laboratory (PO 563/29-1, BA 1601/10-1) and Research Campus InfectoGnostics (FKZ 13GW0096F) are gratefully acknowledged for providing the infrastructure. Reference: [1] Recknagel et al. 2012, PLoS Med. [2] Adrian T. Press et al., bioRxiv, 2021, https://doi.org/10.1101/2021.01.20.427305.

(BIM-02.5) **From whole body to sub-cellular imaging by applying single bimodal fluorinated probes compatible with both MRI and Raman imaging**

Renzo Vanna[1], Cristina Chirizzi[2], Carlo F. Morasso[3], Alessandro Caldarone[3], Matteo Tommasini[2], Fabio Corsi[3], Linda Chaabane[4], Francesca Baldelli Bombelli[2], Pierangelo Metrangolo[2]; *[1]Institute for Photonics and Nanotechnologies – CNR, Politecnico di Milano, [2]Politecnico di Milano, [3]Istituti Clinici Scientifici Maugeri IRCCS, [4]Neuroscience Division, IRCCS Ospedale San Raffaele*

In fifteen words or less, explain the significance of this contribution (Novel Aspect).: Here we demonstrate the potentialities of new bimodal fluorinated probes for MRI / Raman imaging

Abstract Text: In the current clinical practice and biomedical research fields, several successful imaging tools allow to localize specific probes (contrast agents) at different imaging scales (from sub-cellular to whole body size), using distinct imaging techniques (e.g., fluorescence, MRI or PET imaging) and corresponding specific molecular probes. Besides, what it is not obvious today is the visualization of a single probe with different imaging approaches, focused on different imaging scales. As an example, common strategies used to detect specific MRI probes from whole body to cellular or tissue level include the combination of both MRI contrast agents and fluorescence molecules, with limitations related to stability, durability, accuracy, and technical complexity. Here we reveal the capability to detect fluorinated (19F) probes in vivo by 19F-MRI, across the whole body, and microscopically, at tissue and cellular level, by confocal Raman imaging. This is possible thanks to unique features of perfluorinated molecules clearly showing both MRI and Raman signals. We firstly demonstrated the possibility to detect these bimodal probes at sub-cellular level and in multiplexing modalities using Raman imaging. Thereafter, a mice model with multifocal neural inflammation in the spinal cord (resembling human multiple sclerosis) was employed to follow the inflammation sites on whole body by in vivo MRI, after treatment with fluorinated probes. Next, fresh frozen sections of spinal cord were collected and directly analysed by Raman microscopy without further labelling or staining. The Raman analysis not only allowed the direct localization of the fluorinated probe in the tissue at high resolution but also revealed the biomolecular composition of the surrounding tissue portions, thanks to intrinsic label-free features. These data may open the possibility to use such type of fluorinated probes for research and clinical purposes. The chemical versatility of these fluorinated molecules may permit further chemical functionalization and subsequent targeting. For instance, the detection of tumours by MRI, followed by their intraoperative or ex-vivo

localization by non-invasive Raman imaging is a possible application.

21IR05: Probing Orientation/Anisotropy by Infrared Spectroscopy

Chair: Takeshi Hasegawa
On-site Chair: Richard Crocombe

(IR-05.1) **Template-Free Orientation Control in Thin Films of Small-Molecule Organic Semiconductors**
Nobutaka Shioya[1], Takafumi Shimoaka[1], Takeshi Hasegawa[1]; [1]*ICR, Kyoto University*

Rod-shaped molecules represented by pentacene and perylene tetracarboxylic diimide typically form a polycrystalline thin film with the long axis of the molecule oriented perpendicular to the substrate surface, i.e., end-on orientation. The face-on oriented thin film, on the other hand, where the molecular plane is parallel to the substrate, has never been found on an inert substrate such as silicon. As a result, the face-on orientation has long been believed to be generated only on specific surfaces such as graphene. A low-temperature deposition technique is a candidate for obtaining the metastable face-on crystalline film on an inert surface. In the present study, the face-on orientation of various organic semiconductors has been realized for the first time, and the molecular orientation is identified by means of p-polarized multiple-angle incidence resolution spectrometry (pMAIRS) and two-dimensional grazing incidence X-ray diffraction (2D-GIXD). The pMAIRS spectra clearly discriminate the face-on thin film structure from the conventionally known end-on one. The present study demonstrates that the molecular orientation of small-molecule organic semiconductors can be controlled without a template layer.

(IR-05.2) **Determination of the orientation of the axis of α-helical α-synuclein(61-95) with residue level resolution in monolayer by pMAIRS**
Chengshan Wang[1], Chengshan Wang[1]; [1]*Middle Tennessee State University, USA*

20~30 % of genomes has been reported to encode membrane proteins, which only contribute 2.4 % to the solved protein databank. Challenges stem from the measurements of NMR and X-ray crystallography which are the two major techniques to determine a protein's structure. For example, lots of membrane proteins cannot form single crystal structure required by X-ray crystallography. As for NMR, the measurements were hindered by the low tumbling rates of vesicles. Furthermore, membrane proteins usually form monolayer structure around cell membrane/vesicles whereas neither X-ray crystallography nor NMR can address protein's structure in monolayer with satisfactory resolution. Therefore, surface FT-IR techniques such as p-polarized Multiple Angle Incidence Resolution Spectroscopy (pMAIRS) were developed. Here, pMAIRS was shown to be able to provide residue level resolution results of α-synuclein (α-syn) segment peptide even in monolayer. α-Syn is a protein containing 140 amino acid residues and constitutes three domains: N-terminal with residues 1–60; the nonamyloid component (NAC) which spans residues 61–95; and C-terminus containing residues 96–140. Both α-syn and its NAC part (i.e., α-syn(61–95)) are detected in Lewy bodies which is the hallmark deposition of Parkinson's disease. In addition, α-syn(61–95) was also found to coaggregate with β-amyloid in the senile plaques in the brain of Alzheimer's disease patients. In this study, α-syn(61-95) was found to form a stable Langmuir monolayer at the air-water interface, which was amphiphilic and widely used to mimic cell membranes/vesicles. From circular dichoism results, α-syn(61-95) transform from unstructured conformation in aqueous solution to α-helix at the interface. In addition, the overall tilted angle of the axis of the α-helical α-syn(61-95) at the interface was 30.1 º measured by pMAIRS. Furthermore, 13C isotopic label was introduced to the backbone carbonyl at position of 93G and a new 13C amide I band was generated in the pMAIRS result. The tilted angle of the axis at 93G was found to be almost 0 º. Therefore, pMAIRS can address the tilted angle for a protein/peptide with 13C isotopic label with residue level resolution even in monolayer, and can supplement X-ray crystallography nor NMR for membrane proteins.

(IR-05.3) **Infrared Spectroscopy Study of Self-Assembled Monolayers**

Christian Pellerin[1], Fadwa Ben Amara, Antonella Badia; [1]*Université de Montréal*

Infrared spectroscopy is an especially powerful tool to probe anisotropy in partially organized materials. In this presentation, we apply IR reflection absorption spectroscopy (IRRAS) to elucidate the origin (bulk or interface) of the macroscopic properties of self-assembled monolayers (SAMs) on gold surfaces. Contact angle measurements and electrochemical impedance spectroscopy both show marked odd-even effects for SAMs of CH3(CH2)nSAu (with n = 6−19), where the hydrophobicity and dielectric constant vary gradually with the length (n) and but sharply with the parity (nodd vs neven) of the polymethylene chain.1 IRRAS results indicate that the chain length dependencies originate from changes in the polymethylene chain conformation (bulk), as probed using CH2 stretching peaks. In contrast the odd−even variations arise primarily from a difference in the chemical composition of the interface due to changes in CH3 group orientation for nodd vs neven. We also study the impact of the roughness of the gold substrate on the presence and amplitude of the odd-even effects. Contrary to common expectations, we observe clear parity effects even for SAMs prepared on sputtered gold substrate of high roughness. IRRAS shows that the bulk conformational order again improves with n and that it is in fact higher for SAMs on the rougher substrate. On the other hand, the parity effect on the orientation of the CH3 groups is attenuated compared to the smoother substrate, explaining the weaker odd-even effects on surface wettability. Finally, our undergoing work seeks to establish if the same bulk and interface ordering mechanisms are at play in the odd-even effects observed for functional SAMs terminated by a much larger ferrocene tail group. 1. Journal of the American Chemical Society 2020 142, 13051

(IR-05.4) **Micro ATR-FTIR imaging to analyze spatial distribution of molecular orientation in films**

Ryohei Ishige[1], Ryohei Ishige[1]; [1]*Tokyo Inistitute of Technology, Japan*

Microscopic attenuated-total-reflection (ATR) Fourier-transform infrared (FTIR) spectroscopic imaging based on focal plane array (FPA) detector is the most suitable methods to directly observe micrometer(μm)-scale spatial distribution of chemical composition in soft matters (polymer films, biomaterials, etc.). However, the intensity of ATR-FTIR spectra strongly depends on the contact condition of the sample with the internal reflection element (IRE), which makes quantitative analysis difficult. Thus, we focused on dichroic ratio of the absorbance spectra acquired by using a linear polarizer combined with the ATR-FTIR imaging (pATR-FTIR imaging) apparatus. The dichroic ratio does not depend on the contact condition and provides not only molecular orientation distribution but also the distribution of the invariant of the absorbance value independent from the orientation. The invariant is directly proportional to the concentration of chemical groups and enable to analyze chemical-composition distribution quantitatively. In this study, the pATR-FTIR imaging method was established through the quantitative analysis of molecular orientation of aromatic polyimides (PIs) and their precursor of poly(amic ester)s (PAE) in the biaxially oriented films prepared from its lyotropic liquid crystal (LC) precursor. Uniaxial orientation order parameter, S, and a mean square cosine value of roll angle ψ, f(ψ), were used to quantify the degree of orientation of the main chain of the polymers and that of aromatic imide plane, respectively. It was revealed that the films are apparently homogeneous in the spatial distribution map of the S, while the films exhibited obvious heterogeneity in that of f(ψ), in which “face-on” and “edge-on” orientation domains coexist. The results indicate that orientation direction of the rigid PI chains is fixed in the averaged orientation direction of the oriented PAE matrix during thermal imidization, while the aromatic imide ring can be rotated around the main chain. Furthermore, cracks in the PI film were successfully visualized sensitively by pATR-FTIR imaging, and the orientation distribution inside the cracks was investigated. Through this structural study, we validated the quantitative capability of the micro pATR-FTIR imaging method thorough the analyses of highly oriented PI films and demonstrated its ability to investigate μm-scale heterogeneity on the basis of molecular orientation analyses.

(IR-05.5) **Infrared spectroscopic polarimetry of anisotropic thin films and structured surfaces**

Karsten Hinrichs[1], Timur Shaykhutdinov[2], Christoph Kratz[2], Jörg Rappich[3], Andreas Furchner[3]; *[1]Leibniz-Institut für Analytische Wissenschaften - ISAS e.V., Germany, [2]Leibniz-Institut für Analytische Wissenschaften - ISAS e.V., [3]Helmholtz-Zentrum Berlin*

This contribution focuses on infrared spectroscopic polarimetry for detailed investigations of structural or material anisotropic properties of thin films, aggregates and surfaces. Different polarimetric approaches from the nano-scale (AFM-IR) to the macro-scale (Mueller-Matrix Ellipsometry, Laser Infrared Ellipsometry) are discussed. Such polarimetric methods are of high interest in a wide field of applications, as anisotropy and structure are essential for physical, chemical and functional properties of materials in optoelectronic, polymer, plasmonic, and bio-related research. Combining the infrared polarimetric measurements with analytical and numerical optical simulations enables detailed characterizations of spectra–structure correlations to determine various sample parameters (thickness, geometry, molecular orientation, anisotropy, ordering). Results from investigations of structured surfaces, molecular aggregates, thin polymer and oxide films are discussed. Particularly the availability of mid-IR quantum cascade lasers (QCL) significantly broadens the number of applications, allowing for infrared polarimetric studies from macroscopic to nanoscopic length scales. Currently we are working on the extensions of the IR laser-ellipsometric analysis for the investigation of non-cyclic processes at solid–liquid interfaces in microfluidic flow cells. Support by EFRE 1.8/13 is acknowledged.

21LIBS06: LIBS in environmental and heritage science

Chair: Rosalba Gaudiuso
On-site Chair: Daniel Diaz

(LIBS-06.2) **LIBS, an open field of research for heritage science applications**

Vincent Detalle[1], Vincent Detalle[1], Xueshi Bai, Jessica Auber- -Le Saux, Michel Menu; *[1]C2RMF*

Laser-induced breakdown spectroscopy (LIBS) is a versatile elemental analytical technique that elements of principle were described as early as 1962, just after the invention of the laser. Over the last 50 years, LIBS has become a technique in its own right and has been applied for the analysis of many elements of the periodic table in many fields such as the study of cultural heritage since late in 90’s years. This talk will aim to present the evolution of libs since 1990 in cultural heritage and the new trends and orientations of LIBS in the framework of the new heritage science approaches. The cultural heritage materials are unique then more complex, often mixture and altered comparing with industrial ones. They need a laser with a good shot-to-shot energy reproducibility, a suitable wavelength to minimized the destructivity and an optimized focalization to ensure local analysis or controlled mapping or in-depth measurement. At the same time, other techniques were developed helping to improve the ability of LIBS analysis, such as the laser becomes more stable, the spectrometers have a higher resolution, ICCD camera is faster and easier to control. They have made LIBS become more maturated, compact and portable insuring quantitative analysis and improving material identification. One of the force of LIBS is its capability to detected and measured light elements and to be coupled with others analytical laser-based techniques. We will present recent development in the context of archeological prospect of the carbon measurement that is the way and the ferment of the orientation of positioning LIBS for the future in the context of heritage science challenges. We aim to inspire more people working on cultural heritage, for whom LIBS may be brand-new elemental analysis technique, and aim to motivate more LIBS researcher to continue developing LIBS technique in order to address more issues on conservation/restoration and archaeology, etc. Keywords: laser-induced breakdown spectroscopy (LIBS), cultural heritage science, Art conservation/restorations, archaeology

(LIBS-06.3) **Identification of Mahogany and Lookalike Wood Samples Using Laser-induced Breakdown Spectroscopy**

Richard R. Hark[1], Randy Wilkinson[2], Chandra S. Throckmorton[3], Aniko Bezur[1]; *[1]Yale University, Institute for the Preservation of Cultural Heritage, [2]Fallon & Wilkinson, LLC, [3]Signal Analysis Solutions*

Laser-induced breakdown spectroscopy (LIBS) has been used to analyze a wide variety of cultural heritage objects made from materials such as ceramics, metals and metal alloys, stone and minerals, and glass. LIBS can provide qualitative or quantitative elemental data on everything from paintings and statues to fossils and archeological artifacts. The method offers several attractive advantages such as the ability to simultaneously detect all elements, high throughput in situ analysis, relatively low limits of detection (low ppm range), stratigraphic interrogation of objects with coatings or crusts, and standoff capabilities to reach inaccessible areas. LIBS is a micro-destructive technique, producing a small ablation spot (~100 microns) on the surface of the sample. The analysis area is often not detectable without the aid of a microscope, especially on certain surfaces, and conservation scientists and conservators are routinely required to calculate if the damage from a minimally invasive procedure is worth the potential information to be gained. Commercial handheld LIBS instruments are now available that allow investigations to be carried out in the field or in a museum gallery making the approach even more attractive and affordable. The goal of an ongoing collaborative project with the Yale University Art Gallery (YUAG) is to use a combination of LIBS, mass spectrometry, and machine learning techniques to see if it is possible to discriminate between the three species of mahogany (Swietenia) and between mahogany and wood species with a very similar appearance with a goal of being able to identify woods found in the furniture collection. Mahogany was a valuable commodity sourced from the Caribbean in the 18th- and early 19th-centuries that was used in high-end furniture made in Great Britain and North America. However, there are many tropical hardwoods that are known by the appellation “mahogany” and distinguishing between the various species is challenging, especially once the wood has been incorporated into a piece of furniture. This presentation will highlight details of the project and the very promising results that have been obtained after analysis of 400 examples of mahogany and lookalike woods as well as 27 furniture samples.

(LIBS-06.5) **LIBS-based chemical analysis of vent gases from battery cells at high temperature**

Daniel Diaz[1], David Hahn[2]; *[1]University of Arizona, [2]University of Arizona (USA)*

In fifteen words or less, explain the significance of this contribution (Novel Aspect).: First time report of analysis of highly toxic gases released from batteries at high temperatures

Abstract Text: Batteries are indispensable power supplies for sectors such as industrial, commercial, transportation, and utilities. Depending upon their technology and power, batteries pose different levels of chemical, fire, explosion, and electrical hazards during their use, storage, charging, and disposal. For instance, battery overheating and fires that release dangerous gases with unknown health effects can be started by external heating or fire. Characterization of battery vents and fires by-products is an active and challenging research area from the public health, fire department, utility-scale storage owner, and manufacture standpoints. In this research, a laser-induced breakdown spectroscopy probe was used to analyze vent products during controlled heating and decomposition of commercial 3.8-V, 350-mAh, lithium-ion (Li-ion) battery cells. Li-ion battery cells were heated in a N2-atmosphere tubular chamber up to about 170°C to induce thermal decomposition. The LIBS setup included a 1064-nm Nd:YAG laser, a Czerny-Turner spectrometer, and an ICCD camera. Gases and particles released from the battery cells were introduced in a 6-way chamber with optical accesses for the LIBS probe and visual inspection. Through time-resolved temperature and LIBS intensity measurements estimations of the temperature at which venting occurred and the duration of the venting episodes were obtained. Atomic emission lines with relatively high intensities from Carbon, Hydrogen, Fluorine, Lithium, Phosphorous, and Sodium were detected at different times during the heating experiments and the evolution of the thermal decomposition of the battery cells. A discussion of the potential thermal transformations and chemical reactions that occurred during the tests is also presented.

21PMA07: End-to-end analytical development in Cell and Gene Therapy

Chair: Deniz Temel
On-site Chair: John Wasylyk

(PMA-07.3) **Addressing the issues around low throughout testing for AAV characterization using SECMALS.**

Vikas Bhat[1], Nicole McIntosh, Geoffrey Berguig, Omair Karim, Rolando DeAngelis, Christa Cortesio, Ayesha Khan, Daniel Gold, John Maga; [1]*BioMarin Pharmaceutical Inc.*

Adeno associated virus (AAV) capsids are a leading modality for in vivo gene delivery. Complete and precise characterization of capsid particles, including capsid and vector genome concentration, is necessary to safely and efficaciously dose patients. Size exclusion chromatography (SEC) coupled to multiangle light scattering (MALS) offers a straightforward approach to comprehensively characterize AAV capsids. We demonstrate that this method provides detailed AAV characterization information, including but not limited to aggregation profile, size-distribution, capsid content, capsid molar mass, encapsidated DNA molar mass, and total capsid and vector genome titer. Currently, multiple techniques are required to generate this information, with varying accuracy and precision. A series of equations for SEC-MALS are used in tandem with intrinsic properties of the capsids and encapsidated DNA to quantify multiple physical AAV attributes in one 20-minute run with minimal sample manipulation, high accuracy, and high precision. These novel applications designate this well-established method as a powerful tool for product development and process analytics in future gene therapy programs.

21RAM04: Clirspec Biomedical Raman Session

On-site Chair: Laura Fabris

(RAM-04.1) **Selective sampling Raman spectroscopy techniques for ex-vivo intra-operative assessment of surgical margins and lymph nodes in breast cancer surgery**

Ioan Notingher[1]; [1]*University of Nottingham*

Selective Raman spectroscopy combines fast tissue imaging and Raman spectroscopy to obtain high spatial resolution molecular maps of tissue ex-vivo and detect cancer cells. We will present results supporting the development of selective Raman spectroscopy for intra-operative assessment of surgical margins and lymph nodes in breast cancer surgery. We will describe a range of imaging modalities, including auto-fluorescence and high-wavenumber Raman imaging, that can be used for efficiently guiding the more specific “fingerprint” Raman measurements to the areas of tissue that is more likely to contain tumour cells. This strategy allow significant reduction of the number of Raman spectroscopy measurements while providing tissue images at high spatial resolution. We will describe the methods for discrimination of residual cancer from normal breast and lymphoid tissue, optimisation and estimation of diagnosis accuracy (sensitivity and specificity). Preliminary tests on tissue specimens and full wide-local excision tissue specimens indicated diagnosis accuracy above 90% can be obtained within 20-30 minutes for tissue areas as large as 4 x 6 cm2. While these results demonstrate the feasibility of selective sampling Raman spectroscopy for supporting clinical intra-operative decision making, we will also discuss the potential for clinical integration and remaining challenges related to reducing tissue analysis time.

(RAM-04.2) **High-throughput Raman flow cytometry for directed evolution**

Julia Gala de Pablo[1], Matthew Lindley[1], Akihiro Isozaki[1], Kotaro Hiramatsu[1], Walker Peterson[1], Tomoko Abe[2], Kotaro Ishii[3], Keisuke Goda[1]; [1]*University of Tokyo,* [2]*RIKEN Nishina Center,* [3]*RIKEN Nishina Center for Accelerator-Based Science*

Flow cytometry is an essential tool for single-cell analysis. Analyzing thousands of cells is especially important for biotechnological applications, where the target cell is rare but outperforms the majority of the cells on a

certain measurable (e.g., productivity of high-value chemicals). Fluorescence-flow cytometry allows analyzing thousands of single cells based on their fluorescence signal using fluorescent staining. However, the need for fluorescent labels is problematic due to its low specificity for small biomolecules, cytotoxicity of staining protocols, and autofluorescence interference. Raman spectroscopy obtains a biochemical fingerprint of single cells in a label-free, non-destructive manner. However, its small cross-section results in slow signal acquisition and low throughput, hindering the interrogation of large cell populations. Coherent Raman scattering methods such as coherent anti-Stokes Raman Scattering (CARS) enhance the light-matter interaction, enabling faster acquisitions and allowing high-throughput implementation. Here, we use Fourier-transform CARS (FT-CARS) to obtain a Raman spectrum every 42 μs in the fingerprint region (1-3) and analyze cells based on their vibrational characteristics. Integration of a rapid-scan FT-CARS spectrometer and a microfluidic device with acoustic focusing enables Raman flow cytometry at 200 cells/s. With this method, we demonstrated high-throughput flow cytometry of various microalgae such as Chromochloris zofingiensis, Euglena gracilis, and Haematococcus lacustris based on their intracellular contents of carbohydrates, proteins, chlorophyll, and carotenoids. We also show that our FT-CARS flow cytometer can characterize differences of metabolic activity among Euglena gracilis clones generated by ion beam mutagenesis. We believe that the combination of ion-beam mutagenesis and Raman flow cytometry opens a new path to metabolic engineering, that is, creating and characterizing cells with specific phenotypes in a label-free manner. [1. K. Hashimoto, M. Takahashi, T. Ideguchi, K. Goda, Sci. Rep. 6, 21036 (2016). 2. K. Hiramatsu et al., Sci. Adv. 5, eaau0241 (2019). 3. K. Hiramatsu, K. Yamada, M. Lindley, K. Suzuki, K. Goda, Biomed. Opt. Express. 11, 1752 (2020).]

(RAM-04.3) **Raman Spectroscopy and Semi-supervised Learning as a Method Towards Enhanced Radiation Treatment in Patients Receiving High Dose Rate Brachytherapy**

Kirsty Milligan[1], Xinchen Deng[1], Ramie Ali-Adeeb[1], Phillip Shreeves[1], Alexandre Brolo[2], Jeffrey Andrews[1], Julian Lum[3], Andrew Jirasek[1]; *[1]University of British Columbia, [2]University of Victoria, [3]BC Cancer*

In fifteen words or less, explain the significance of this contribution (Novel Aspect).: Raman spectroscopy and semi-supervised learning can reveal radiation response patterns in real patient samples.

Abstract Text: In radiotherapy no standard method currently exists to monitor and predict patient specific radio sensitivity of normal tissue and tumours, which vary considerably among individuals. As a result, patients who may be able to tolerate higher doses of radiation therapy are treated with a standard dose, thus possibly undermining the maximal efficacy of the treatment. Our group has shown that Raman spectroscopy (RS), combined with principal component analysis (PCA), can be used to identify biochemical changes associated with radiation exposure. However, PCA often has limitations when used to interpret Raman spectra. Difficulties can arise in deciphering the overall contribution to sample variation from individual bio-components within a spectrum. We demonstrate an alternative approach in which a library of reference spectra containing individual cellular bio-components are used as inputs to group and basis restricted non-negative matrix factorisation (GBR-NMF). Using GBR-NMF we have successfully reproduced previously known metabolite response profiles in post irradiated MCF7 breast cancer cells such as those demonstrated for glycogen by Matthews et al. using PCA. We here show that with GBR-NMF we now gain the ability to map profiles of other biologically relevant chemicals. Our current research has focussed on the application of RS, combined with GBR-NMF and supervised learning approaches, as a tool in the development of treatment response monitoring in patients receiving interstitial high dose rate brachytherapy (HDR-BT) as a monotherapy for intermediate risk prostate cancer. Brachytherapy is an attractive alternative to external beam radiation therapy due to shorter treatment regimens and minimised side effects with comparable results. We have used the RS-GBR-NMF approach to elucidate biochemical expression patterns across a preliminary group of patients, identify clusters of individuals with similar profiles and shown some correlation of these expression profiles to the following; pre-treatment clinical prognostic indicators, treatment response and PSA levels in the months and years following treatment. The ability to identify HDR-BT induced responses within individuals opens up a number of new treatment pathways that could be exploited to both increase the radio sensitivity of the tumour as well as the possibility to

explore new, combination therapies.

(RAM-04.4) **Intraoperative Assessment of Breast Tissue Calcifications Load Using Transmission Raman Spectroscopy (TRS): a Prelude to Whole Breast Scanning InVivo**

Ben Gardner[1], Pavel Matousek[2], Adrian Ghita[1], Jennifer Haskell, Nick Stone[3]; *[1]University of Exeter, [2]STFC Rutherford Appleton Laboratory, [3]Biomedical Physics, School of Physics and Astronomy, Uni of Exeter, UK*

Breast cancer is the most common cancer found in the UK (2021), with annual incidence of ~55,000 patients. While there is a successful NHS Breast Screening Programme (NHSBSP), there remains a clinical need for improvements to address specific limitations. The programme is restricted to older patients >47-50 years, due to its inability to probe denser breast tissue found in younger women. Moreover, it doesn't provide a clear malignancy status resulting in 80% of the invasive biopsies taken being benign. Emerging evidence also shows that for every life saved, three patients undergo treatment for what would be a non-fatal cancer. What is clearly needed to reduce the highlighted issues is a non-invasive adjunct to mammographic screening. Emerging studies have demonstrated that in breast tissue sections, vibrational spectroscopy (IR and Raman) can identify between two common types of calcifications (I & II). But, also correlate the level of carbonate substitution in type II calcifications with the overall pathology status, thereby providing a cancer grade. Transmission Raman spectroscopy (TRS), has been shown to probe deeply within scattering samples such as soft tissue non-invasively and non-destructively. The work of Ghita et al demonstrated it is possible to measure clinically relevant amounts of calcifications in a sample volume expected in breast surgeries. He we report on a trial at the Royal Devon & Exeter Foundation Trust using TRS to analyse breast tissue removed during routine breast surgery. To date n=~150, samples have been measured, primarily wide local excisions. Immediately following surgical excision, the sample is X-rayed and rapidly mapped using TRS. The work to date shows that high quality spectra can be acquired rapidly from breast tissue ~1-5cm thickness, and importantly that the non-ideal sample quality is not prohibitive i.e. specimens contains surgical dyes, tissue burning (diathermy). Finally, in samples which exhibit high calcification load it is possible to see the corresponding Raman spectrum associated with calcifications. This ongoing work shows the great potential of Raman spectroscopy to be used in future in vivo devices for rapid non-invasive measurements of bulk breast tissue, with the aim of whole breast scanning in the near future.

(RAM-04.5) **Raman and Fluorescence Characterization of Plaques in Alzheimer's Brain Tissue**

Freek Ariese[1], Ben Lochocki[1], Jeroen J.M. Hoozemans[2], Johannes F. De Boer[1]; *[1]LaserLaB, Vrije Universiteit Amsterdam, [2]Dept. of Pathology, Amsterdam University Medical Center*

In fifteen words or less, explain the significance of this contribution (Novel Aspect).: Plaque areas showed green autofluorescence and resonance Raman spectroscopy revealed the presence of carotenoids.

Abstract Text: Amyloid-beta (Aβ) deposits, or plaques, are a biological hallmark in the post mortem diagnosis of Alzheimer's disease (AD). They are characterized by the occurrence of protein misfoldings (β-sheets). Post mortem AD and healthy control brain tissue was obtained from the Netherlands Brain Bank. Freshly frozen tissue of the CA1 region was cut in 20 µm sections and mounted on CaF2 microscope slides and dried overnight. Afterward, we followed a sequence of imaging modalities to superimpose results from the same areas: autofluorescence, spontaneous Raman mapping, stimulated Raman scattering (SRS) and finally fluorescent staining with Thioflavin-S for confirmation of suspected plaque areas. Auto-fluorescence images were acquired using a fluorescence microscope with an excitation wavelength of 470 nm. RGB color images were obtained for wavelengths above 500 nm and showed a large number of bright yellow lipofuscin-like deposits (not AD-related) but also some areas with green autofluorescence; only the latter were confirmed as (cored) plaques. Using spontaneous Raman as well as Stimulated Raman Scattering (SRS) mapping, those same

plaque areas showed a red-shift of the Amide-I band, as expected for amyloid beta structures. Surprisingly, we also obtained strong (near) resonance Raman signatures of carotenoids in the cored plaque areas when applying 532 nm excitation; no carotenoid signals were observed under non-resonance conditions at 785 nm. We hypothesize that these carotenoids are part of an inflammatory response. We are currently studying these phenomena in more detail, for a broader range of plaque types.

21SPR02: Plasmonic Sensors

Chair: Emilie Ringe
On-site Chair: Nan Jiang

(SPR-02.1) **Plasmonic Mapping for Sensor Design**

Amanda J. Haes[1], Hoa Phan, Claire Vinson; [1]*University of Iowa*

Spatially resolved localized surface plasmon resonance (LSPR) and surface-enhanced Raman scattering (SERS) microscopies are used simultaneously to assess and minimize plasmonic variations of carboxylated gold nanostars on electrospun polymer films. Novel to this study, the spatial distribution and electromagnetic properties of gold nanostars deposited on the polymer are locally quantified using LSPR microscopy mapping and second derivative LSPR spectral analysis. As a result, spectral complexity arising from background variations are eliminated thus enabling quantification of local nanostar density. Next, uranyl intensities collected using SERS are shown to be insensitive to local nanostar densities that range from 140 to 200 pM·cm. This important finding provides a non-biased guideline for selecting sensing regions on electrospun polymer films. By considering interactions between gold nanostars, the polymer substrate, and the analyte and correlating spatially resolved spectral measurements at an interface, realization of a user-friendly sensor that minimizes sampling bias is possible

(SPR-02.2) **SPR Sensors for COVID Antibodies to Measure Seroprevalence, Immunity and Other Ventures**

Jean-François Masson[1], Abdelhadi Djaileb[2], Maryam Hojjat Jodaylami[2], Julien Coutu[2], Pierre Ricard[2], Danny Brouard[3], Ludovic S. Live[4], Denis Boudreau[5], Joelle N. Pelletier[2]; [1]*Université de Montréal,* [2]*University of Montreal,* [3]*Hema Quebec,* [4]*Affinité Instruments,* [5]*University of Laval*

The need to develop clinical tests and rapid sensors for SARS-CoV-2 became evident early in the pandemic to monitor active infections and evaluate seroprevalence. While nucleic acid and antigen tests serve to detect active infections, antibody tests are an essential tool later in the pandemic to monitor past infections and to provide an indication of the immune response of an individual to COVID-19 and to vaccination. To address the need for antibody tests, we have developed surface plasmon resonance (SPR) sensors to detect antibodies expressed towards the nucleocapsid (N) protein and to the spike (S) protein and its receptor binding domain (RBD). The SPR sensors were optimized with animal antibodies spiked in human serum as a surrogate model and then tested with a series of clinical samples (serum, plasma or dried blood spots) collected from PCR-positive individuals and compared to a control group to validate the sensitivity and specificity of the sensors in comparison to ELISA assays. We then applied the SPR sensors to determine the maturation of the affinity of the antibodies in the 24-week period post infection, and if time permits before the presentation, following vaccination. Finally, we developed an in vitro surrogate neutralization assay where the spike protein (the native and a few variants) was immobilized to the SPR sensors to evaluate if convalescent sera inhibited the interaction of spike with ACE-2.

(SPR-02.3) **Probing angstrom-scale interfacial characteristics in organic/2D heterostructures via tip-enhanced Raman spectroscopy**

Nan Jiang[1], Nan Jiang[1], Linfei Li; [1]*University of Illinois at Chicago*

Two-dimensional (2D) monolayers hold promise for a variety of nanoelectronic and quantum device technologies. To realize its full potential, 2D materials need to be seamlessly interfaced with other materials, thus motivating atomic-scale characterization of 2D material-based heterostructures. Here, we measured the angstrom-scale interfacial properties using ultra-high vacuum tip-enhanced Raman spectroscopy (TERS). In addition to identifying the vibrational signatures of adsorbed organic molecules, TERS reveals subtle ripples and compressive strains of the 2D material lattice underneath the molecular layer. The induced interfacial strain is demonstrated by ~1 nm beyond the molecular region by virtue of ~5 Å chemical spatial resolution. In addition to high-resolution chemical analysis, atomically precise control of interfaces coupling with TERS was achieved to realize the full technological potential of 2D heterostructures. Molecular manipulation experiments allow local tuning of 2D materials strains with magnitudes as small as ~0.6%, which could be widely applied to other atomically engineered heterostructural materials with potential utilities for investigations of fundamental science and device applications.

September 29, 2021

21AES02: Electrokinetic Fundamentals

Chair: Christopher Harrison
On-site Chair: Christopher Harrison

(AES-02.1) **Development of Nanopore Characterization System and Method to Explore Unique and Novel SiNx Nanopore Surface Coatings**

Brian S. Sheetz[1], James T. Hagan[1], Jason R. Dwyer[1]; [1]*University of Rhode Island*

In fifteen words or less, explain the significance of this contribution (Novel Aspect).: Surface chemistry inside of a SiNx nanopore and a method to characterize these unique surfaces

Abstract Text: Solid-State Silicon based nanopores have come a long way but still suffer from instability issues, signal masking levels of noise and the constraints of nanofabrication. We explore covalent surface modifications to mitigate these obstacles, as well as explore the design and development of a method to characterize nanopores with unique surface coatings in a timely manner. I will outline some of the surface chemistries that are used along with the key design elements that were vital to realizing the method as an application.

(AES-02.2) **Fluid Elasticity Enhanced Insulator-based Dielectrophoretic Focusing of Particles and Cells in A Constriction Microchannel**

Mahmud Kamal Raihan[1], Micah Baghdady[2], Heston Dort[2], Joseph A. Bentor[1], Amir Malek[2], Xiangchun Xuan[2]; [1]*Clemson University,* [2]*CLemson University*

In fifteen words or less, explain the significance of this contribution (Novel Aspect).: Particle focusing in DC i-DEP with buffer solution improves with a small addition of PEO

Abstract Text: Focusing particles and cells into a tight stream is often an important step prior to detecting, analyzing and sorting them. We demonstrate the addition of a small amount of polyethylene oxide (PEO) polymer into a buffer solution can significantly enhance the insulator-based dielectrophoretic (iDEP) focusing of particles in a constriction microchannel. We attribute this enhancement to the viscoelasticity of the PEO solution that may affect both the electrokinetic and dielectrophoretic particle motions. We study the parametric effects of polymer concentration, polymer molecular weight, particle size, and particle type on the iDEP

focusing under pure DC electric fields. We also demonstrate the application of such fluid elasticity-enhanced iDEP focusing to yeast cells.

(AES-02.3) **Fluid Rheological Effects On Electroosmotic Flow And Dielectrophoretic Particle Focusing And Trapping In A Post-array Microchannel**

Joseph A. Bentor[1], Colin McNeely[1], Mahmud Kamal Raihan[1], Amirreza Malekanfard[1], Xiangchun Xuan[2]; *[1]Clemson University, [2]CLemson University*

In fifteen words or less, explain the significance of this contribution (Novel Aspect).: Understanding how rheological factors affect particle manipulation and flow patterns in electric driven flow

Abstract Text: Our recent studies show that fluid rheological properties (e.g., viscoelasticity and shear thinning) may affect the electroosmotic flow and dielectrophoretic particle focusing and trapping in a single-constriction microchannel. We report in this work an experimental study of such fluid rheological effects in a post-array microchannel, which contains an ordered 2D array of constrictions. We employ three types of polymer solutions with distinct rheological properties including viscoelastic polyethylene oxide, shear thinning xanthan gum, and viscoelastic/shear thinning polyacrylamide solutions. We compare both the electroosmotic flow pattern and dielectrophoretic particle focusing and trapping in each of these non-Newtonian fluids with those in the Newtonian buffer solution. The goal is to understand if the polymer dynamics (e.g., elongation and relaxation) around interconnected constrictions may have additional impacts upon the fluid flow and particle motion.

(AES-02.4) **Glycan sensing and sequencing with native and chemically customized nanopore single-molecular sensors.**

James T. Hagan[1], Brian S. Sheetz[1], Jason R. Dwyer[1]; *[1]University of Rhode Island*

In fifteen words or less, explain the significance of this contribution (Novel Aspect).: Characterizing and distinguishing between diverse glycans using machine learning and chemically tuned nanopore single-molecule sensing.

Abstract Text: Even though glycans are among the most abundant biopolymers present in nature, their complex chemical structure is challenging to both detect and sequence with conventional instrumentation. Broadening the analytical techniques available is an important step to reducing this burden and the resources required. Our work has focused on proving and improving nanopore sensors as a useful tool for the analysis of unmodified glycans. Voltage-driven passage of analytes into these electrolyte-filled nanofluidic channels — nanopores — generates current disruptions that can be used for single-molecule detection, are directly related to the molecule's physical properties (e.g. molecular structure, charge), and can be used as a fingerprint for identification. Thus, nanopores have potential to facilitate high level glycan characterization similar to the expensive and complicated instrumentation used today. We use silicon nitride (SiNx) nanopores formed by controlled dielectric breakdown (CDB). This approach applies simple low-voltage electronics to create nanopores on demand. By tuning the electrolyte bath — using an additive such as bleach — we can modify the nanopore characteristics and downstream sensing performance. We can similarly tune these nanopores after fabrication with conventional synthetic techniques. Light activated hydrosilylation was used to provide a one-step method for covalent attachment of an organic surface coating providing highly customizable sensing conditions and a broad palette of functional groups at the researcher's disposal. Their demonstrated applications include further synthetic steps, such as immobilization of proteins, to light responsive coatings which can change the effective size and stop or allow for translocation. The current analyte scope of untreated and chemically tuned nanopores include neutral (e.g. maltodextrin) and anionic (e.g. heparin, pectin) glycans

ranging from hexasaccharides to polysaccharides tens to hundreds of saccharide units in length. When the resulting measurements were integrated with machine learning and principal component analysis, chemically similar samples have been differentiated and correctly identified, even leading to sequence specific characterization. The use of chemically-tuned nanopores and machine learning thus promises to advance both nanopore science and glycomics.

(AES-02.5) **Self-Pumping Membranes Driven by Asymmetric Electrocatalytic Reactions**

Jeffrey L. Moran[1], Yuhang Fang[2], David M. Warsinger[2]; [1]*George Mason University,* [2]*Purdue University*

In fifteen words or less, explain the significance of this contribution (Novel Aspect).: Electrokinetic phenomena underlie self-pumping nanoporous membranes, potentially enabling nanoscale manipulation of fluids without external power.

Abstract Text: When two dissimilar metals are connected together in the presence of hydrogen peroxide, they can generate spontaneous fluid flows that result in self-propulsion of freely suspended Pt/Au particles, or pumping in the case of Pt and Au surfaces fixed to a surface. In the latter case, this can lead to novel phenomena such as catalytic micropumps. Here, we model conduct detailed numerical simulations of a membrane containing nanoscale cylindrical pores with Pt coated onto one side and Au on the other. These membranes pump fluid through the pores via catalytic reactions. The catalytic reactions result in a concentration gradient of H+, which in turn generates an electric field that drives electroosmotic flows that actuate the self-pumping. Electric double layer overlap is shown to be detrimental to self-pumping. For large pore radii, such as 6 microns, electric double layer overlap is no longer a concern and the velocity of self-pumping can exceed 20 microns per second. This work highlights the potential of utilizing catalytic reactions to pump liquid via membranes without external power, enhances the understanding of the physics underlying self-pumping flow, and provides guidance on the designing the next generation of self-pumping devices.

21ATOM03: Laser Ablation

Chair: Todor Todorov
On-site Chair: C. Derrick Quarles Jr.

(ATOM-03.1) **Characterization of new float glass standards for use in forensic comparisons using laser ablation inductively coupled plasma mass spectrometry**

Jose Almirall[1], Katelyn Lambert, Anuradha Akmeemana, Ping Jiang; [1]*Florida International University*

Consensus concentration values for seventeen (17) major and trace elements typically present in soda-lime glass manufactured using the “float” process and used in the quantitative analysis and forensic comparison of glass samples were determined using LA-ICP-MS for a new set of reference glass materials. We report the results from an international collaboration including 7 laboratories to evaluate the homogeneity of three new glasses and report the consensus values of 17 elements at three concentration levels. These Corning Float Glass Standards (CFGS) were manufactured at low, medium, and high concentrations of 32 elements typically encountered in float glass samples as found in forensic casework. Eight (8) sets of independent results from LA-ICP-MS analyses using the standard test method of analysis (ASTM E2927-16e1) and one set of micro-X-ray Fluorescence Spectrometry (μXRF) data (using method ASTM E2926-17) resulted in typically < 3 % relative standard deviation (RSD) within each lab and < 5 % RSDs among all labs participating in the study for the concentration ranges using sampling spots between 50 μm - 100 μm in diameter. The new set of float glass is intended for use as matrix-matched calibration standards in the forensic analysis and comparison of float glass by LA-ICP-MS using a standard test method. The results suggest that the new calibration standards are homogeneous for most elements at the small sampling volumes (~ 90 μm deep by ~ 80 μm in diameter)

reported and show excellent agreement among the different participating labs. Consensus concentration values are determined using a previously reported calibration standard (FGS 2) and checked with a NIST 1831 SRM®. A collaboration with National Institute of Standards and Technology (NIST) scientists to certify these glasses as SRMs, including the certification of the quantitative analysis of the minor and trace element content for future distribution by NIST is ongoing.

(ATOM-03.2) **Elemental Bioimaging for the Simultaneous Determination of an Antitumor Agent and a Target-Specific Gadolinium-Based Contrast Agent in Liver Tissue**

Katharina Kronenberg[1], Julia Werner[2], Peter Bohrer[2], Fabian K. Lohhöfer[2], Rickmer F. Braren[2], Philipp M. Paprottka[2], Uwe Karst[1]; *[1]Institute of Inorganic and Analytical Chemistry, University of Münster, [2]Klinikum rechts der Isar, Technical University of Munich*

In fifteen words or less, explain the significance of this contribution (Novel Aspect).: First simultaneous quantification of antitumor and contrast agent comparing different liver tumor stages by LA-ICP-MS.

Abstract Text: Liver cancer is the sixth most occurring cancer type worldwide. Symptoms and signs of liver cancer usually appear late in the course of the disease, which is often medicated by the antitumor agent cisplatin. Due to the late diagnosis survival rates are poor and therefore, often palliative therapies are chosen. Hence, an early and reliable diagnosis of liver carcinomas and tumor stages plays an important role in suggesting an appropriate therapy and increasing survival rates. In this context, magnetic resonance imaging in combination with liver-specific contrast agents significantly improves the detection and characterization of liver lesions. This study focuses on the simultaneous determination of cisplatin and the liver-specific gadolinium-based contrast agent gadoxetic acid in liver lesions. Rats with liver tumors were medicated with gadoxetic acid as well as cisplatin and sacrificed 15 minutes after injection. The resection of tumor tissue with surrounding liver tissue allows the direct comparison of gadolinium, platinum, and other endogenous elements in both tissue types. A 213 nm laser ablation system (LA) hyphenated to inductively coupled plasma-mass spectrometry (ICP-MS) revealed quantitative and spatially resolved element information in the tissue thin sections. Element distributions were correlated with different pathological tissue types, which were examined by hematoxylin and eosin staining. Furthermore, gadolinium and platinum were selectively quantified in regions of interest, providing information on how the uptake of gadoxetic acid and cisplatin differs in different tumor regions and stages.

(ATOM-03.3) **U/Pb age fast-mapping of titanite by LA-ICP-MS**

Alicia Cruz-Uribe[1], Jesse Walters; *[1]University of Maine*

With recent advances in fast washout laser ablation cells, strides have been made to develop fast-mapping of trace elements and isotopes for geochronology. Most successes have involved zircon and monazite; titanite has proved more difficult. Here we present some of the successes and challenges faced in producing U/Pb maps of titanite grains by fast-mapping laser ablation inductively coupled plasma mass spectrometry. Recently, fast-mapping at the University of Maine MAGIC Lab (MicroAnalytical Geochemistry and Isotope Characterization Laboratory) has been developed for trace elements in many materials (garnet, calcite, and others) using a 5x5 μm spot size. Application of this method to U/Pb age mapping of titanite highlights a number of known issues. First, at small spot sizes, the focus of the laser on the surface of the grain is critical. Grain flatness becomes even more critical when measuring Pb isotope ratios; 207Pb/206Pb ratios tend to be anomalous along cracks and grain edges, which leads to erroneous U/Pb ages. Second, the physical process of fast-mapping involves rastering sequential parallel horizontal lines across the grain in order to produce an image of the entire grain surface. Each line produces a very small ejecta rim outside the crater, which is then incorporated into the next line scan. This effect is pronounced in the Pb isotopes at small spot sizes (5 μm), which then affects the age calculations and produces age maps with horizontal artifacts. This artifact is mitigated by increasing the spot

size; at larger spot sizes (7–10 μm), each pixel physically consists of more material, and so the incorporation of ejecta on 207Pb/206Pb is lessened. Coeval mapping of key trace elements (Y, Zr) with U and Pb isotopes enables ratio calculations to show visually where isotopic ratios deviate from trace element zones. Calculated Y/206Pb maps of large polymetamorphic titanite (>1 mm) in calc-silicate gneiss from western Maine, USA, reveal areas where recrystallized titanite has incorporated the Pb isotope composition of older titanite not in equilibrium with matrix Pb, resulting in mixed U/Pb ages.

(ATOM-03.4) **A calibration approach to size and localize CeO2 nanoparticles in remote organs using microsecond LA-spICP-MS**

Svenja B. Seiffert[1], Antje Vennemann[2], Erik Niehaves[1], Sabrina Kroeger[3], Martin Wiemann[2], Uwe Karst[4]; *[1]BASF SE, University Muenster, [2]IBE gGmbH Institute for Lung Health, [3]BASF SE, [4]Institute of Inorganic and Analytical Chemistry, University of Münster*

In fifteen words or less, explain the significance of this contribution (Novel Aspect).: LA-spICP-MS offers outstanding possibilities to determine aggregation or dissolution of nanoparticles directly in tissue sections.

Abstract Text: Nowadays, nanomaterials are used in a wide range of applications such as consumer products, medical applications and in the field of catalysis. In the latter, automotive industries utilize CeO2 nanoparticles due their special redox properties. Markable amounts of CeO2 nanoparticles may thus be released into ambient air via the exhaust gases, posing a potential risk to human health. Several animal studies demonstrated that CeO2 nanoparticles induce acute and chronic lung inflammation and, once in the blood circulatory system, are distributed in remote organs. However, the distribution and size of CeO2 nanoparticles in remote organs are not well understood as classical analytical methods provide limited information. They often only allow to obtain either the size of nanoparticles via digestion of the underlying tissue or spatially resolved information. Here, we overcome this bottleneck by using laser ablation and single particle inductively coupled plasma-mass spectrometry (LA-spICP-MS) to investigate dissolution or aggregation by sizing and localizing of CeO2 nanoparticles directly in tissue sections. Additionally, microsecond dwell times allow to distinguish between signals for dissolved material and nanoparticles, thus leading to improved particle resolution. As no CeO2 nanoparticles with narrow size distribution are commercially available, a quantification strategy using aqueous dissolved standards and matrix-matched gelatin standards to size and localize CeO2 in liver, spleen, lymph nodes and the kidney from rats was developed. Analyses were performed after 3 hours, 3 days and 3 weeks following a single intratracheal instillation of 0.6 mg CeO2 nanoparticles. Our data indicate increasing particle size over the time. Additionally, hardly any dissolution was observed, thus confirming the chemical stability of CeO2 nanoparticles in the organism after entering the body via the lung.

(ATOM-03.5) **New Ways of Chemical Imaging: Combining Selective Solute Sampling by Diffusive Gradients in Thin Films with High-Resolution LA-ICP-MS**

Stefan Wagner[1], Jakob Santner[2], Casey Doolette[3], Christoph Hoefer[4], Christina Hummel[4], Johanna Irrgeher[5], Enzo Lombi[3], Markus Puschenreiter[4], Erik Smolders[6], Walter Wenzel[4], Thomas Prohaska[5]; *[1]Department General, Analytical and Physical Chemistry, Chair of General and Analytical Chemistry, Montanuniversität Leoben, Austria, [2]Department of Crop Sciences, Institute of Agronomy, University of Natural Resources and Life Sciences Vienna, [3]Future Industries Institute, University of South Australia, [4]Department of Forest and Soil Sciences, Institute of Soil Research, University of Natural Resources and Life Sciences Vienna, [5]Department General, Analytical and Physical Chemistry, Chair of General and Analytical Chemistry, Montanuniversität Leoben, [6]Department of Earth and Environmental Sciences, Division of Water and Soil Management, KU Leuven*

Chemical imaging using the diffusive gradients in thin films (DGT) technique in combination with laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) has evolved as a unique analytical tool to assess solute dynamics at sub-mm spatial resolution. In DGT-based imaging, thin hydrogels with homogeneously distributed binding agents are deployed on the sample under study. The variety of binding agents allows for the preparation of gels which are selective for a multitude of analytes. When binding gels are stacked, specific analytes (species) can be sampled and separated simultaneously. The use of gels with known mass loadings allows for accurate quantification. Here, the potential of novel DGT LA-ICP-MS techniques is highlighted on selected applications in environmental and materials science. The first example presents the development of a non-invasive, stacked-gel DGT LA-ICP-MS approach for selective co-localization of labile As species (AsIII, AsV), P, and Mn distribution patterns alongside roots of As-hyperaccumulator plants grown in a natural As-rich soil. The second study highlights the capability of DGT for analyte preconcentration in the determination of nutrient and contaminant fluxes in soil at ultra-trace levels. In this work, the distribution of labile Cd and Zn was mapped at natural background levels in the rhizosphere of maize to elucidate the differential effects of soil liming on Cd and Zn uptake by plants. The third study is in the field of material sciences, where aqueous corrosion of Mg-based materials with biomedical relevance was assessed using DGT LA-ICP-MS in combination with pH imaging by planar optodes. This approach enabled time-resolved mapping of interfacial Mg dissolution and pH changes at the microscale, which makes it a promising tool to study localized corrosion processes and support the improvement of material design in technological engineering. In the most recent work, DGT was combined with synchrotron-based X-ray fluorescence microscopy (XFM) for the first time, allowing for mapping of Zn over large (150 cm2) gel areas without affecting downstream sample analysis. In conclusion, the presented methods provide unprecedented information on solute dynamics of multiple analytes across reactive (bio)interfaces, which either cannot be analyzed directly or are not stable at relevant spatiotemporal scales.

21CHEM01: Bringing it All Back Home: Data Integration Through Chemometrics

Chair: Federico Marini
On-site Chair: Peter Harrington

(CHEM-01.1) **Class-modeling for multi-block data: something borrowed, something new**

Federico Marini[1], Alessandra Biancolillo[2], Daniele Tanzilli[1], Claudia Scappaticci[1]; *[1]University of Rome La Sapienza, [2]University of L'Aquila*

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, whereas discriminant approaches are quite popular, class-modeling techniques, which on the other hand aim at describing one particular class at a time, are often underused, if not neglected, even if they are, in principle, best suited to deal with problems such as food authentication or process control. This is at least partly due to the availability of a larger number of efficient algorithms for discriminant classification, which results in a wider versatility especially as the complexity of the class boundary(-ies) as well as its(their) shape can be tuned depending on the problem at hand and the available training samples. These considerations become even more valid and diriment when multi-block data, i.e., multiple matrices of predictors, are involved.
To cope with these limitations, in this communication two different approaches for the analysis of multi-block data with the purpose of class-modeling will be described. One derives from the combination of soft independent modeling of class analogies (SIMCA [1]) with potential functions [2], and the other one is a "revival" of an old and unjustly neglected non-parametric class modeling tool originally designed to be able to deal with data of different nature (e.g., real and categorical) [3]. These approaches will be presented and the results of their application to real and simulated data sets will be discussed and compared.

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I.Broeckaert, A.Tassin, Anal. Chim. Acta 1981, 133, 215-224. [3] M.P. Derde, L. Kaufman, D.L. Masssart, J. Chemometr. 1989, 3, 375-395.

(CHEM-01.2) **MULTIEXPONENTIAL UNMIXING OF FLUORESCENCE MICROSCOPY DATA WITH MCR SLICING**

Cyril Ruckebusch[1], Raffaele Vitale, Dario cevoli, olivier devos, michel s.liwa; [1]*LASIR CNRS UNIV LILLE*

Data slicing consists of taking equally-sized subsets of a two-way data matrix of multiexponential curves at different time lags, reordering them into a three-way array and performing a trilinear decomposition of this array [1,2]. In this way, profiles obtained for the time mode are constrained to behave like mono-exponential functions. Alternatively, multiexponential resolution can also be achieved by performing a bilinear decomposition of the multiset data matrix yielded by the row-wise concatenation of the subsets sliced at different time lags using MCR-ALS [3] and a trilinearity constraint, an approach that we have called MCR slicing [4]. In principle, both approaches provide the same results but, since constraints can be implemented in a more flexible way in MCR-ALS, e.g. per profile, MCR slicing allows overcoming some limitations that may appear with single set trilinear slicing decompositions. Fluorescence imaging encompasses a set of non-invasive, highly specific and extremely sensitive analytical techniques, such as fluorescence lifetime imaging microscopy (FLIM) or time-lapse fluorescence bleach rate imaging, for which multiexponential fitting is usually exploited to extract characteristic lifetimes for the chemical species underlying the samples under study. In this presentation, we will show results obtained applying MCR slicing for tailored multiexponential curve resolution of biological cellular structures, bridging the gap between the aforementioned bilinear and trilinear approaches. Differently from multiexponential fitting approaches, trilinear decomposition algorithms do not require initial guesses of the parameters of the exponential functions to be estimated and their final solutions are unique. And differently from trilinear slicing, the constraints implemented in MCR slicing enable the decomposition of fluorescence data that are only partially describable by a purely exponential model.

(CHEM-01.3) **Limits of Detection Revisited**

Peter de Boves Harrington[1]; [1]*Ohio University*

Reported limit of detection (LoD) values are often favorably biased, unvalidated, and used to compare analytical methods. In most cases, the obsolete IUPAC procedure is used which albeit simple is ambiguous because the estimate of the baseline standard deviation is not strictly defined and may be calculated from a single observation or a subset of favorable measurements. For univariate calibrations, a statistical method is proposed that is based solely on estimates of the calibration line. This procedure is based on the ISO 11483 guidance that often is ignored. In addition, a method to provide a precision measure for the LoD in the form of a confidence interval is also given. Precision measures will allow the statistical comparison of published LoD values that has been missing from the chemical literature.

(CHEM-01.4) **Graph-based methods in multivariate calibration and calibration transfer**

Ramin Nikzad-Langerodi[1], Florian Sobieczky[1]; [1]*Software Competence Center Hagenberg*

In fifteen words or less, explain the significance of this contribution (Novel Aspect).: A method that allows to transfer calibrations between instruments based on arbitrary calibration standards.

Abstract Text: In contrast to classical, multivariate calibration techniques such as partial least squares (PLS) regression, graph-based or manifold learning approaches can take into consideration the geometric structure (i.e. the topology) of data in high-dimensional spaces. In the current contribution, we will show how to introduce and leverage topological information about data for multivariate calibration and calibration transfer (CT). To this end, we propose a manifold regularization scheme in order to derive latent variable (LV) models that preserve the topology of the data [1]. As will be shown, this topology can either be derived from, or imposed

onto, the data based on prior knowledge about the relationship between the samples in the calibration set. The former can be exploited for deriving semi-supervised calibrations, i.e. when reference values for some of the calibration samples are missing, while the latter turns out to be highly useful for removal of unwanted sources of variability (i.e. decluttering) in general and in CT problems in particular. In contrast to current state-of-the art pre-processing methods, such as generalized least squares weighting (GLSW) or piecewise direct standardization (PDS), the graph-based approach presented herein implicitly corrects for the difference in the instrumental response of a primary and a secondary device while constructing a LV space that is predictive with respect to the target property (e.g. analyte concentration). An important consequence is that, under appropriate conditions, calibration standards (e.g. NIST standards) and calibration samples (e.g. Corn samples) don't need to share the same spectral features in order to transfer calibrations between similar analytical instruments. Acknowledgements: The research reported in this work has been partly funded by BMVIT, BMDW, and the Province of Upper Austria in the frame of the COMET Program managed by the FFG. The COMET Centre CHASE is funded within the framework of COMET - Competence Centers for Excellent Technologies by BMVIT, BMDW, the Federal Provinces of Upper Austria and Vienna. The COMET program is run by FFG. [1] Nikzad-Langerodi, R, Sobieczky, F. Graph-based calibration transfer. Journal of Chemometrics. 2021;e3319. https://doi.org/10.1002/cem.3319

(CHEM-01.5) **Generative Adversarial Spectroscopy**

Garth Simpson[1]; [1]*Purdue*

In fifteen words or less, explain the significance of this contribution (Novel Aspect).: Generative adversarial linear algebra is developed and evaluated for addressing overfitting in chemometric analyses.

Abstract Text: A novel linear algebra formulation is proposed for generative adversarial linear analysis (GALA), specifically designed to address overfitting common in chemometric analyses. In brief, GALA consists of iterations between generation of spectral “attacks” designed to co-locate with genuine spectra in a reduced-dimensional space, followed by discriminatory “defenses” to identify directions in spectral-space that isolate and remove the generated decoy spectra. In contrast to more conventional feature extraction strategies common in chemometric analyses, GALA seeks to identify the regions in spectral space in which the genuine data aren’t, then exclude those locations from subsequent analyses. While inspired by generative adversarial deep convolutional networks (GANs), GALA relies on a wholly unique mathematical foundation based on straightforward linear algebra manipulations that are both intuitive and computational tractable for error propagation / uncertainty quantification. In an extreme example, GALA was applied for linear discriminant analysis (LDA) at full spectral dimension, for which the number of spectral-space parameters greatly exceeded the number of measured spectra (i.e., p>n). In this limit, LDA at full dimension is computationally nonsingular and exhibits extreme overfitting. Nevertheless, implementation of GALA enabled converged evaluation of LDA at full spectral dimension for both simulated and experimentally measured supervised Raman spectral datasets. The benefits of GALA were not limited to nonsingular chemometric analyses, also providing benefits to analyses based on dimension reductions combining principal component analysis (PCA) as a prelude to LDA (i.e., PCA+LDA) and “shrunken centroids” regularized LDA analyses. Results from GALA compared favorably with other commonly used simple linear analyses, including partial least-squares linear discriminant analysis (PLS-DA), PCA, regularized LDA, and PCA+LDA, generally yielding comparable inter-class resolution and lower overfitting than other supervised dimension reduction methods. Success in reducing overfitting across this suite of methods suggests the potential for broad applicability of GALA and related strategies for mitigating over-fitting propensities in numerous chemometric analyses.

21FORENS03: Forensic Analysis in the Lab and at the Crime Scene

Chair: Igor Lednev
On-site Chair: Igor Lednev

(FORENS-03.1) **National Institute of Justice: Novel Spectroscopic and Analytical Techniques Applied to Forensic Problems**

Gregory Dutton[1]; [1]*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 that spans a broad range from fundamental research with the potential for application to forensic science, to the development of prototype devices, to the evaluation of novel instruments and methods. Strengthening the forensic sciences through R&D 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 growth 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) **A New Mass Spectral Identification Algorithm to Discriminate Between Structurally Similar Fentanyl Analogs**

Glen P. Jackson[1], Alexandra Adeoye; [1]*West Virginia University*

This presentation describes a novel algorithm for the identification of compounds from their mass spectra. The algorithm uses common and robust statistical tools and, most importantly, is able to provide reliable measures of uncertainty in drug identifications. The Expert Algorithm for Substance Identification (EASI) is divided into three steps; 1) development of the spectral database, 2) building linear models to explain the variance between replicate spectra and predict ion abundances within each spectrum, and 3) using measures of similarity or dissimilarity between measured abundances and predicted abundances within a spectrum to make binary decisions, with error rates, about substance identification. The database of mass spectra is comprised of ~70 different fentanyl analogs from more than 9 different laboratories. We then extracted every spectrum across an eluting GC peak to provides more than 50,000 spectra. To conduct general linear modeling, we used the abundance of each fragment ion of a compound as a dependent variable and the remaining 19 abundances as "independent" variables. To conduct binary classification, we compared the use of measures of similarity (e.g., dot product) or dissimilarity (e.g., Euclidian distance) to compare the 20 predictions for each spectrum. The ground truth identity of each spectrum in the database is known, so the true positive rate and true negative rate could be assessed for different thresholds for decision-making. Finally, the effectiveness of each model is assessed through receiver operating characteristic (ROC) curves. In general, the multivariate linear modeling explained more than 90% of the variance that exists in the replicate spectra for each drug, and the ability to explain this spectral variance enables EASI to have superior identification rates than existing algorithms for making comparisons between laboratories. EASI is able to successfully resolve difficult-to-distinguish isomers

like crotonylfentanyl and cycloproylfentanyl. In theory, EASI is extendable to any substance and any fragmentation technique in mass spectrometry through which replicate spectra of standards can be acquired.

(FORENS-03.3) **Trace Detection of THC using Raman Spectroscopy**

Amanda J. Haes[1], Ryan Norton, Rose Schmitt, Timothy Brown; [1]*University of Iowa*

The overall goal of this study is to identify molecular fingerprints for THC and its metabolites using normal Raman and surface-enhanced Raman scattering (SERS) and to use this information for subsequent forensic purposes. To do so, participants are dosed with a randomized and blinded amount of THC under controlled conditions. A series of measurements are collected from bodily fluids, observation, responsivity, and perception. Normal Raman and SERS are used to quantify the amounts of THC and its metabolites. This information is then correlated to subjective and other quantifiable measures. These training data are expected to assist in the assessment of delayed impairment following medical or recreational use of these compounds.

(FORENS-03.4) **Differentiation Between Hemp-type and Marijuana-type Cannabis Using the Fast Blue BB and the 4-Aminophenol Color Tests**

Alexander G. Acosta[1], Ruthmara Corzo[2], Jose Almirall[1]; [1]*Florida International University,* [2]*National Institute of Standards and Technology*

The Agricultural Improvement Act of 2018 permits the legal cultivation and trade of hemp in the US. Under this law, hemp is defined as Cannabis Sativa and any part or derivative of the plant that has a tetrahydrocannabinol (THC) concentration below 0.3 % (w/w). Due to this change, there is now an urgent need to differentiate between hemp-type cannabis from marijuana-type cannabis (≥0.3% w/w THC). The Fast Blue BB (FBBB) color test has been shown to differentiate between the cannabinoids found in cannabis. Notably, FBBB forms a red chromophore in the presence of THC and an orange chromophore in the presence of CBD with additional differences in the fluorescence. We report, for the first time, a micro reaction (extraction of 10 mg of plant material with methanol) directly onto a proprietary substrate that has been preloaded with FBBB reagent and demonstrate the differentiation between THC-rich cannabis from THC-poor (CBD-rich) cannabis. Other cannabinoids such as CBN, extracts from various herbs and spices, and authentic cannabis samples were tested using the new miniaturized colorimetric test. RGB (Red, Green, Blue) codes were obtained for each color result for more objective reporting of the colors produced. The FBBB+THC chromophore is also reported to fluoresce under 480 nm irradiation, while FBBB+CBD does not fluoresce. The RGB scores combined with the fluorescence properties of the FBBB+THC chromophore enhances the selectivity of the FBBB test for marijuana-type cannabis. The same set of samples in this study including the same authentic cannabis samples of known THC and CBD concentrations were also evaluated using the 4-Aminophenol (4-AP) test to compare the FBBB results with the 4-AP results. Linear Discriminant Analysis (LDA) was employed to determine the classification performance for both the FBBB and the 4-AP tests for the cannabis samples as either hemp-type or marijuana-type. These models determined that the FBBB test can distinguish between marijuana-type (high THC:CBD ratio) and hemp-type cannabis, however marijuana-type cannabis (containing above 0.3 % (w/w) THC) with low THC:CBD ratios were not correctly classified. The results show that the new FBBB test can be used to differentiate between THC-rich cannabis and CBD-rich cannabis.

(FORENS-03.5) **Raman spectroscopy to tackle the analysis of bloodstains in crime scene conditions**

Alexis R. Weber[1], Alexis Barber, Igor K. Lednev[2]; [1]*SupreMEtric LLC,* [2]*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.

21MASS01: Rapid Hydrogen-Deuterium Exchange Mass Spectrometry for Structural and Mixture Analysis

Chair: Ian Webb
On-site Chair: Ian Webb

(MASS-01.1) **Real World Applications of Sub-second HDX for Drug Development in Cancer and Neurodegenerative Disease**

Derek J. Wilson[1], Cristina Lento, Mark Reed, Marcia Taylor, Shaolong Zhu; [1]*York University*

The conventional tools of structural biology - X-ray crystallography, structural NMR and CryoEM - have long been a critical part of the drug development process, used to provide detailed pictures of the binding modes of candidate molecules in exquisite detail. These images are mesmerizing because they are highly informative (and rather beautiful), but they also give the false impression that biology at the molecular level is static and binary (i.e., there is a singular 'unbound' configuration and 'bound' configuration for the protein). In fact, proteins - and the physicochemical processes of complexation - are inherently dynamic, requiring transient excursions to higher energy configurations that are often invisible to techniques, like those associated with classical structural biology, that rely on ensemble averaging. While the functional importance of these high energy configuration excursions (i.e., conformational dynamics) is increasingly recognized, the prevailing view remains that characterizing dynamics is an exercise in basic research and of little practical use to drug development. This talk will provide real-world examples of the incorporation of conformational dynamics into the drug development process. A prime example is in the area of neurodegenerative disease, where the pathogenic species are invariably intrinsically disordered proteins whose pathogenesis is linked to bias shifts in their conformational ensembles. Here, in collaboration with industrial partner Treventis, we applied the concept of 'dynamics-guided drug design' to enable the development of next-generation anti-amyloid drugs targeting all amyloidotic neurodegenerative diseases (including Alzheimer's, Parkinsons and ALS among many others). In cancer, we have used characterization of conformational dynamics to define the binding mode and allosteric effects associated with candidate drugs targeting STAT proteins. Ultimately, our work aims to unequivocally demonstrate that dynamics-resolved methods can play a critical role in drug development including Mechanism of Action (MoA) studies and off-target effects associated with allostery.

(MASS-01.2) **In-Electrospray H/D Exchange-Mass Spectrometry for Analyzing Carbohydrates**

Elyssia S. Gallagher[1], O. Tara Liyanage[1], H. Jamie Kim[1], Emvia Calixte[1], Ana Quintero[1], Jacob Hatvany[1], Emily Ziperman[1]; [1]*Baylor University*

Glycans are complex molecules with different carbohydrate subunits, linkage stereochemistries, and branching patterns; all of which play a role in their biological functions. Hydrogen / deuterium exchange–mass spectrometry (HDX-MS) has become a standard method for analyzing conformations and binding interactions

of solvated proteins. Carbohydrates, model systems for glycans, are susceptible to HDX since they contain labile hydrogens, primarily in the form of hydroxyls, which can be labeled with deuterium (D) upon exposure to deuterated solvents. However, compared to backbone amides, the functional group detected in traditional HDX-MS experiments for proteins, the exchange rate of glycan hydroxyls is two to eight orders of magnitude faster, depending on solution pH. This rapid exchange rate makes it unfeasible to monitor HDX of carbohydrate hydroxyls using traditional, bottom-up HDX methods. Herein, we describe our ongoing efforts to characterize solvated carbohydrates. We perform rapid HDX by introducing deuterating reagents (e.g. D2O) to carbohydrates during electrospray ionization (ESI). Our work illustrates that these rapid, in-ESI HDX methods characterize solvated carbohydrates rather than gas-phase structures. Furthermore, we have coupled our experimental work with molecular dynamics simulations to identify the mechanism of carbohydrate ionization during ESI. Experimentally, we have quantified how ESI source conditions alter the magnitude of HDX for carbohydrate model systems, developed an internal standard to control for daily humidity differences, and established methods to alter the HDX labeling time on the microsecond to millisecond timescale. Currently, we are applying these in-ESI HDX methods to characterize carbohydrate isomers. Together, this work provides a toolbox for analyzing carbohydrate structures in biologically relevant, solvated states.

(MASS-01.3) **Microfluidic Immunoassay for In Situ Amyloid-beta Mass Spectrometry Analysis from Microdissected Brain Cells**

Jorvani Cruz Villarreal[1], Ana Egatz-Gomez[1], George T. Noutsios[2], Todd R. Sandrin[2], Paul D. Coleman[3], Alexandra Ros[1]; *[1]Center for Applied Structural Discovery, The Biodesign Institute. School of Molecular Sciences. Arizona State University, [2]School of Mathematical and Natural Sciences. Julie Ann Wrigley Global Institute of Sustainability. Arizona State University, [3]Banner ASU Neurodegenerative Research Center, The Biodesign Institute. Arizona State University*

In fifteen words or less, explain the significance of this contribution (Novel Aspect).: Low abundance protein analysis from human tissue cells with all sample preparation steps on-chip.

Abstract Text: Soluble amyloid β (Aβ) oligomers are known to play a major role in Alzheimer's disease (AD) and considered responsible for neuronal dysfunction. However, details on their chemical composition have not been elucidated mainly due to a lack of analytical methods to access the proteomic cell content from post-mortem brains. Identification of AD-related Aβ species in brain cells and their environment could provide valuable insights into the disease pathology. To investigate Aβ from AD human brains, we developed a microfluidic immunoassay for in situ mass spectrometry (MS) detection of Aβ from microdissected cells. The microfluidic platform allows for sample preparation and immunocapture on-chip in tandem with MALDI-MS. The multi-layer PDMS manifold is reversibly bonded to a conductive substrate, which is used as the target for MALDI-MS analysis. Immunocapture was confirmed for synthetic Aβ using immunoglobulin G (IgG) 6E10, specific for Aβ peptides, isoforms, truncated species, and oligomers. Matrix solution was loaded for co-crystallization. The matrix-analyte crystals were accessed by peeling off the PDMS manifold for in situ MALDI-MS analysis. The limit of detection of Aβ in the current assay with an 8.75 nL well volume resulted in 35 nM using a Bruker Microflex instrument. Furthermore, the microfluidic platform was coupled with laser microdissection for direct cell loading with 70% capture efficiency. Initial tests using non-AD brain cortex neurons showed successful immunocapture of Aβ species. Additionally, synthetic Aβ monomers and oligomers were characterized by MALDI-MS. Oligomers up to 12-mers were observed, showing the capability of detecting a range of oligomeric states without any pre-treatment. Thus, sample loading and handling on-chip, as realized here, decrease sample losses and human error, increasing the sensitivity for AD-related Aβ species in brain cells. Although selectivity and sensitivity optimization are still necessary, here, we present the assay functionality using synthetic Aβ and microdissected brain cells. The assay implementation could overcome the current limitations of studying the protein content from human tissues and help to understand the role of Aβ species in AD. Detailed knowledge of the oligomeric state of AD-related Aβ species, truncations, and post-

translational modifications could advance the development of AD therapeutics and diagnosis tools.

(MASS-01.4) **Local and Global HDX of Nascent Gas-Phase Protein Ions**

Ian K. Webb[1], Ritu Chaturvedi; [1]*IUPUI*

Native mass spectrometry is a rapidly growing field using the sensitivity, specificity, and selectivity of mass spectrometry (MS) to measure protein structures. Native MS has been applied to the analysis of biopharmaceuticals, protein-protein complexes, and their assemblies, protein-ligand binding, and other important applications. Especially exciting is the use of ion mobility (IM) spectrometry to provide collision cross sections that inform on topology, new fragmentation techniques that do not disturb protein structure and/or allow for complex-down analysis, and the coupling of these experiments to high mass range and high-resolution mass spectrometers. Though these tools are in and of themselves incredibly useful, there is a lack of native mass spectrometry tools that directly probe folding and dynamics localized to specific amino acid residues. Therefore, new tools for gas-phase protein structural information are needed to fully use all of the advantages of MS for better native MS measurements. Recently, we have coupled in-source hydrogen deuterium exchange (HDX) with IM separations and electron capture dissociation (ECD). Gas-phase HDX immediately after ionization allows for rapid structural characterization without perturbations from gas-phase analysis (e.g., from collisional activation). Since fragmentation occurs after IM, IM separations allow the HDX data to be correlated to gas-phase collision cross sections, matching the data to specific conformer families. The use of ECD to provide localized exchange data prevents deuterium scrambling that can occur with fragmentation from slow-heating methods (e.g., collision induced dissocation) and yields high sequence coverage, and thus, better specificity in localizing exchange sites. In this presentation, I will present our work with this approach with globular proteins and intrinsically disordered proteins. These results will be compared with conventional solution HDX for the same proteins.

(MASS-01.5) **Standardizing gas-phase H/D exchange measurements across different platforms**

Miklos Guttman[1], Sunjit Uppal[1], Abhigya Mookherjee[1], Rick Harkewicz[1]; [1]*University of Washington*

Gas-phase hydrogen deuterium exchange (gHDX) has been utilized for several decades for identifying labile protons, resolving isomers, studying ion structures, and elucidating gas-phase reaction mechanisms. Despite its potential, the inevitable variability in instrumentation and exchange conditions have severely limited gHDX as a reproducible analytical tool. We have implemented and explored sources of variation in gHDX measurements on three MS platforms: Two different Q-TOF based instruments and a linear ion trap. By varying the exchange conditions and time it is possible to measure the exchange kinetics of nearly all labile protons. Furthermore, by incorporating a set of internal exchange standards with each measurement it is possible to standardize gHDX properties of various analytes.

21PAT01: PAT Biopharma

Chair: Edita Botonjic-Sehic
On-site Chair: Garth Simpson

(PAT-01.1) **Index of Refraction monitor as part of a PAT strategy for downstream processing**

Julio M. Huato[1], Julio M. Huato[1], Keith Gillette, Karl Rogler, Mark Schofield; [1]*Pall Corporation*

Measuring product concentration is imperative during bioprocessing as concentration can be both a critical process parameter and a critical quality attribute. With the trend toward quality by design and continuous processing, there is increased necessity for inline concentration measurement outside the linear range of commonly used inline methods, such as ultraviolet absorption (UV). Here we report the performance of the mPath index of refraction (IoR) sensor. We demonstrate that the sensor is scalable and can operate across a

comprehensive range of flow conditions expected during bioprocessing. The IoR sensor has a broad linear range, high accuracy and short response time. These attributes enable the sensor to be applied to tangential flow filtration (TFF) processes where we demonstrate successful monitoring of mAb concentration. This has the potential to eliminate the need for sampling. Additionally, as the IoR sensor has a broader dynamic range than UV, the IoR sensor enables measurement of the high concentrations typically required for mAb formulation. This ability to measure high concentrations may eliminate the requirement for over-concentration and/or offline monitoring often required for high concentration mAb formulations. In addition, we show how the IoR sensor can be applied to monitor and control chromatography processes. The concentration of the elution from a Protein A column can be accurately monitored with IoR, since it has a larger dynamic range than UV. The IoR sensor can also be used to monitor the breakthrough of mAb from Protein A columns enabling loading to a consistent 10% (w/w) breakthrough for single-column operation or to enable control of a multicolumn chromatography process.

(PAT-01.2) **Next Generation Sensors for Pharmaceutical Process Analysis**

Ray D. Reid[1], Rohit Bhartia Bhartia[1], Michael Reid[1], Kenneth Nguyen[1], Quoc Nguyen[1], Kripa Sijapati[1], Ray D. Reid[1]; [1]*Photon Systems, Inc.*

In fifteen words or less, explain the significance of this contribution (Novel Aspect).: Deep UV, Fluorescence free Raman, fluorescence, product quality, pharmaceutical, chemical, biological, manufacturing

Abstract Text: 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 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.

(PAT-01.3) **Investigation to Identify the Root Cause of Out of Specification Results for Color of a Topical Pour-On Drug Product – A Case Study**

Daoli Zhao[1], Lin Wang[1], rasangi wimalasinghe[2], Jingzhi Tian[2], Abu Rustum[2]; [1]*Boehringer Ingelheim,* [2]*Boehringer Ingelheim Animal Health*

In fifteen words or less, explain the significance of this contribution (Novel Aspect).: First report of color change in a liquid drug product caused by BHT's oxidation product

Abstract Text: One topical pour-on drug product formed a slight yellow color and continued to increase the intensity over the shelf life of the product. This caused out of specification (OOS) results for color of the drug product. Based on investigation experimental results using HPLC, HPLC-HRMS and color measurement spectrophotometer, the root cause of color formation in the OOS sample was identified and confirmed to be 3,3',5,5'-tetra-t-butyl-4,4'-stilbenequinone (2BHT-QM), an oxidative degradation product of t-butylated hydroxytoluene (BHT) which is used in this drug product as an anti-oxidant. Color formation and or increase of color intensity is observed more frequently than expected in aged liquid and semi-liquid pharmaceutical products. BHT is widely used in pharmaceutical products as an antioxidant to retain the stability of the active ingredient in drug products. Surprisingly, no previous report related to BHT for color change in drug product upon a thorough literature search by the authors. In this paper, we report for the first time of an investigation of color change in a liquid drug product. which was caused by one of BHT's oxidation products. The investigation approach and rationale of described in this paper should be helpful for similar investigations when BHT is used when BHT is used as an antioxidant in liquid or semi-liquid formulations.

(PAT-01.4) **Improving Long Term Stability of Process Raman Analyzer with Accurate Raman Shift Calibration**

jun zhao[1], Christopher Kautz[1]; [1]*B&W Tek, LLC, A Metrohm Group Company*

In fifteen words or less, explain the significance of this contribution (Novel Aspect).: novel Raman shift calibration method achieves high abscissa repeatability and model transferability

Abstract Text: The long term stability and precision of quantitative methods are important considerations for adopting spectroscopic analyzers for industrial process applications. We present a Raman analyzer designed for lab and control room settings with advanced features addressing these concerns. With an internal calibration reference and sensors, the condition of the analyzer is constantly monitored, and Raman shift accuracy and precision are improved by an order of magnitude over a wide operating temperature range, and is maintained to a level better than 0.05 cm-1. This is made possible by employing a novel calibration method that take into account of the temperature dependent nature of peak positions of the ASTM reference material. This also results in improved transferability of quantitative models. Automatic validation of the instrument performance ensured the data quality and compliance to industrial standards. With advanced laser technology, the lifetime of the laser is extended multiple times beyond the normally warranted 10,000 hours, which greatly reduces the long term operating cost. Our holistic approach to high throughput design of the entire optic train from the sample to the detector provides a high level of signal to noise ratio.

(PAT-01.5) **Real-Time Biophysical Characterization of Protein Higher Order Structures (HOS) with Novel Laser-Based Mid-IR Liquid Analyzer**

Craig Magee[1], Santosh Hodawaderkar[1], Jeremy Rowlette[1]; [1]*DRS Daylight Solutions*

In fifteen words or less, explain the significance of this contribution (Novel Aspect).: This is a new PAT tool for real-time protein concentration and higher order structure characterization.

Abstract Text: Current demands in the field of biologics and gene therapy pose technological challenges for the real-time quantitation and characterization of drug substances. Process analytical technology (PAT) has quickly gained importance in the biopharmaceutical industry for continuous monitoring and controlling critical process parameters in the manufacturing of biologics drugs. Widely accepted PAT has now become a regulatory initiative to characterize critical quality attributes to maintain the integrity of the final drug product, while improving the time throughput to meet global demand. Mid-IR spectrometry is a powerful and well-known

analytical technique that can be used to measure isolated analytes and complex mixtures in liquid phase. Mid-IR offers clear advantages by providing a high degree of selectivity to fingerprint chemical information and probe higher-ordered structure. Until now, mid-IR analysis has been precluded from the list of workhorse PAT solutions, because of the difficulty of providing sensitive measurements in real-time. Quantitative characterization of proteins and protein conjugates in aqueous environments has always posed a challenge in the fingerprint region of the mid-infrared spectrum, primarily due to the strong absorption of water in the regions of interest for characterizing chemical bonds. Our patented Quantum Cascade Laser (QCL) based liquid analyzer, Culpeo™, allows us to overcome this strong absorbance from water near 1638 cm-1 and acquire key spectral information from Amide II (1500-1600 cm-1) and Amide II (1600-1750 cm-1) regions. We will introduce an entirely new class of high-sensitivity, inline mid-IR liquid analyzers. Based on ultra-high-brightness tunable quantum cascade lasers (QCL), these analyzers are enabling routine, quantitative chemical analysis with analyte sensitivities going well-beyond the FTIR-ATR limit. This new platform technology also offers fast (10 Hz) scan rates, a large dynamic range, and an ability to easily measure small sample volumes (< 10 micro L). We will present the physical operating principles of these new analyzers and provide several application examples including the characterization of carbohydrates, polysaccharides, proteins, peptides, and amino acids.

21PMA02: Advanced Spectroscopy for Biopharmaceutical Characterisation: Using Multidimensional Fluorescence and Light Scattering Techniques for Protein Characterisation.

Chair: Alan Ryder
On-site Chair: Linda Kidder

(PMA-02.1) **In-line size determination of nanoparticulate biotherapeutics using spatially resolved dynamic light scattering**

Carl Schuurmans[1], Michiel Hermes, Jan-Piet Wijgergangs, Raquel Arribas Bueno, Ad Gerich, Michiel Damen, Rut Besseling; [1]*InProcess-LSP*

The development, production and quality control of biotherapeutics involve complex processes with many critical parameters to monitor. One essential monitoring parameter is the hydrodynamic diameter of the (formulated) biotherapeutic (typical size range: ~5-1000 nm). Until recently, methods to monitor particle size during processes were not available, with most sizing analysis being performed through analysis of samples taken during the process or on the end product. Often these samples have to be diluted or otherwise processed for measurement, potentially altering the therapeutic's observed particle size. These additional steps can result in impractical and/or incorrect measurements. Here, we introduce the NanoFlowSizer, an in-line measuring device that can determine the hydrodynamic size of nanoparticulate suspensions whilst in flow. The NanoFlowSizer is based on Spatially Resolved Dynamic Light Scattering (SR-DLS), a technique that combines low coherence interferometry with traditional dynamic light scattering. Through the use of SR-DLS, it becomes possible to simultaneously resolve both the diffusion speed and position of nanoparticles in suspension, allowing for sizing measurements to be done in flow. Additionally, SR-DLS confers the ability to differentiate between multiple and single scattered light, making particle size determination in highly turbid formulations possible. The ability to non-invasively measure particle size of biotherapeutics in flow allows for a myriad of options and opportunities in the analysis and control of biotherapeutical manufacturing processes. Several applications of the NanoFlowSizer in the real time in-line sizing of biotherapeutics will be discussed, specifically in situ process control measurements of lipid nanoparticles, emulsions, antibodies and contaminant protein aggregates will be shown. Finally, a short discussion on the future perspectives and further technological developments of SR-DLS in biotherapeutics formulation processing will be provided.

(PMA-02.2) **Accelerating AAV analysis - Viral titer and percentage full capsid analysis using OMNISEC**

Matt McGann[1], John Stenson[1]; [1]*Malvern Panalytical*

Viruses and their derivatives are increasingly being used as vectors for delivery of genetic material for gene therapy and vaccine applications. Recombinant Adeno-Associated Viruses (rAAV) are a class of viral vector that is being investigated intensively for the development of gene therapies, due to its mild immune response and ability to deliver the genetic payload to a wide range of host cells. Developing controlled and economical rAAV therapies requires the co-development of robust and reliable characterization tools to control and optimize a sample's critical quality attributes (CQAs). In this work, we will demonstrate the use of multi-detection SEC and Dynamic Light Scattering (DLS) for the extended characterization of different rAAV serotypes. We will show how these techniques allow the evaluation and comparison of viral titer, capsid empty/full ratios, impurity and aggregate profiling - all of which impact the final product's CQAs. Importantly, we will show how modern SEC systems, such as OMNISEC, do not require the use of dedicated and costly reference standards that frequently limit the application of other methodologies.

(PMA-02.4) **Tackling Low Concentration Samples - A-TEEM 3D Fluorescence Method Shines Where Raman and IR/NIR Fear to Tread**

Linda H. Kidder[1], Adam M. Gilmore[2], Karoly Csatorday[1], Karen E. Gall[2], Cary J. Davies[1]; [1]*HORIBA Scientific Instruments,* [2]*HORIBA Instruments Inc.*

In fifteen words or less, explain the significance of this contribution (Novel Aspect).: A-TEEM provides the means to characterize vaccines and other low concentration biopharmaceuticals with optical spectroscopy

Abstract Text: Low concentration samples, and particularly low concentration samples in complex mixtures, present an analytical challenge to standard optical spectroscopic methods such as Raman and NIR. Because optical methods have significant cost-per-measurement and speed advantages over chromatographic ones, a variety of industries are searching for a tool to fill this gap. Standard 2D fluorescence methods demonstrate high levels of sensitivity and specificity, and have contributed to a variety of basic research applications. However, a lack of repeatability and reproducibility has hampered its adoption as a robust analytical tool. Here we present the A-TEEM 3D fluorescence method that incorporates UV/Vis for a 2-in-1 measurement with reproducibility and repeatability metrics that meet or exceed standards required of a robust analytical tool. In addition to demonstrating performance against USP requirements, we will present applications showing robust classification and quantification of low concentration samples in complex matrices, including: differentiation and 100% classification of similar, multi-component vaccine formulations; quantification of polyphenols in wine; rapid determination of THC well below legal limits in hemp samples; and detection of adulterants in natural products used in nutraceuticals.

(PMA-02.5) **HTVS-Coupled Downstream Protein Purification**

Shamus Driver[1], Shaun J. Fraser[1], Mark S. Kemper[1]; [1]*Tornado Spectral Systems*

In fifteen words or less, explain the significance of this contribution (Novel Aspect).: Use of the Raman for monitoring results in faster quantification of protein elution profiles.

Abstract Text: Downstream processing refers to the recovery and the purification of biosynthetic pharmaceuticals from biological sources, such as a fermentation broth. Of particular interest is the production and purification of therapeutic monoclonal antibodies (mAb) proteins from mammalian cell lines in bioreactors. A multitude of sensors (pressure, temp, pH, and protein-specific sensors) are used to provide bulk chemical and process information and High Performance Liquid Chromatography (HPLC) used to provide detailed, but insufficiently infrequent, quantitative molecular information. Raman spectroscopy provides highly detailed molecular information that can be used to quantify multiple products and impurities simultaneously with measurement times taking seconds instead of minutes. Tornado Spectral Systems' HyperFlux™ PRO Plus (HFPP) Raman analyzer delivers considerable improvement in the Signal-to-Noise Ratio (SNR) compared to

conventional Raman spectrometers due to its patented (High Throughput Virtual Slit) HTVS™ technology. Using the model proteins Bovine Serum Albumin (BSA) and Cytochrome C (CytC), a fast protein liquid chromatography (FPLC) process was conducted with a coupled HFPP Raman spectrometer. The developed Partial Least Squares model estimated the concentrations of BSA and CytC from spectra collected in 45 second intervals with average prediction errors of 0.109 mg/mL and 0.070 mg/mL for BSA and CytC, respectively. Use of the HFPP as a monitoring tool results in faster and predictively specific quantification of protein elution profiles from an FPLC process.

21RAM09: Applications of Raman Microscopy

Chair: Tim Prusnick
On-site Chair: Tim Prusnick

(RAM-09.1) **Full Spectrum Raman Excitation Mapping: a Multidimensional Optical Hyperspectroscopy and its Application to Highly Purified Carbon Nanotube Dispersions**

Paul Finnie[1], Jianfu Ding[1], Jacques Lefebvre[1], Jianying Ouyang[1]; [1]*National Research Council Canada*

In fifteen words or less, explain the significance of this contribution (Novel Aspect).: The rapid acquisition of Raman spectra together with excitation profiles is demonstrated experimentally for nano-carbons.

Abstract Text: Raman spectroscopy (RS) and optical absorption (OA) are essential chemical analysis methods, with RS providing information about molecular vibrations, and OA providing information about electronic/excitonic level structure. Raman excitation mapping (REM), in which RS are taken at an essentially continuously variable range of laser excitation wavelengths, combines these two, with the advantage of much greater potential for specificity than either technique alone, however, with the disadvantage of being more difficult and costly to implement. The power of REM for the assessment of carbon nanotube dispersions is recognized for the identification of highly purified carbon nanotubes and their concentrations, however it is seldom used because it has been experimentally challenging to implement. Here we describe our current "full spectrum" REM approach, which uses supercontinuum white light in a custom optical setup to capture REM maps. This enables us to capture RS of essentially all the major Raman bands from purified carbon nanotubes of various types. We are able to simultaneously capture conventional RS and Raman excitation profiles (REPs) over wide ranges in real time. The combination of RS and REP - and the connection between them - makes this a very selective analytical technique, more than RS and OA alone would be. At the same time, taking full advantage of resonance results in sensitivity. This approach makes what can otherwise be a slow and challenging analytical technique much more practical. We will demonstrate how full spectrum REM can be used to evaluate highly purified carbon nanotubes and related materials intended for semiconductor applications, and why we believe it has the potential for wider applicability in chemical analysis.

(RAM-09.2) **Raman Microscopy without the Microscope**

Tim Prusnick[1], Tim Batten[2], Sarah C. Shidler[3], Lucy Grainger[4]; [1]*Renishaw Inc.,* [2]*Renishaw, Plc.,* [3]*Renishaw Inc,* [4]*Renishaw, Inc.*

In fifteen words or less, explain the significance of this contribution (Novel Aspect).: Novel Raman system enables microscopy and three-dimensional imaging of large samples with complex surfaces

Abstract Text: Raman spectroscopy has a proven history of being a valuable technique for the identification of microscopic samples across a wide variety of materials. However, identifying microscopic defects on, or imaging small areas of, large samples in situ is challenging or impossible due to the dimensions of the object to be studied. A large object, such as a turbine blade or work of art, will not fit under a conventional microscope.

Further, it is difficult to maintain focus on the sample surface as the object is unlikely to be flat on the microscopic scale. Here we will discuss recent advances in the design of a novel fibre optic system which enable micro-Raman spectroscopy of large, complex, and dynamic samples that are ill-suited to analysis with a conventional microscope. The need for a conventional microscope is obviated by the use of a novel fiber-optic probe system which allows samples of any size to be accommodated. The probe system moves relative to the sample, enabling mapping to be conducted on a large, stationary object. Focus is automatically maintained throughout the measurement in response to changes in the material's surface. Together these features enable the in situ measurement of large objects with complex and changing surface topographies. We will discuss the measurements of complex samples such as turbine blades, large semiconductor wafers, cultural heritage artifacts, and melting polymers with this fibre optic Raman system. In addition, the system allows for the measurement of bulk liquid samples. Using reaction monitoring software and a partial least squares (PLS) model, changes in concentration can be measured in real time.

(RAM-09.3) **Classification of senescent cells by Raman Microscopy and Machine Learning**

Carlo F. Morasso[1], Alessandro Caldarone[1], Sandra Altanta[1], Marta Truffi[1], Fabio Corsi[1], Carlo Gaetano[1]; [1]*Istituti Clinici Scientifici Maugeri IRCCS*

In fifteen words or less, explain the significance of this contribution (Novel Aspect).: The identification of subcellular regions by machine learning allows the classification of senescent cells correctly.

Abstract Text: Background: Cellular senescence is a process characterized by the permanent inability of cells to replicate and change their physiological function and morphological features. This phenomenon occurs in response to physiological aging and cellular stress, and it is a fundamental protection mechanism from the uncontrolled proliferation of cells. Noteworthy, senescent cells produce molecules that accumulate over time in some regions of the body, resulting in the onset of age-related diseases, such as Alzheimer's diseases and cancer. Nowadays, the detection of senescent cells is complex; developing a rapid tool for identifying senescent cells would be desirable. Raman microscopy might help in the study of senescent cells, although difficulties in its use remain since senescent cells are large and complex to map entirely. We used Raman microscopy and machine learning approaches to distinguish specifically the relevant subcellular regions to identify senescent cells. Method: 14 slides of IMR-90 cells, 6 with senescent cells and 8 with non-senescent cells, were prepared. A total of 222 cells were mapped by Raman imaging using a confocal Raman microscope equipped with a 532 nm laser. For each cell, 500 to 1000 Raman spectra were acquired. After preprocessing, a Louvain clustering algorithm was applied on each cell map. Each identified cluster was automatically assigned to one of these classes: cytoplasm, nucleus, out, and other. Then, the mean spectrum of cytoplasm and nucleus of each cell was extracted and used to classify cells as senescent or non-senescent. Results: The two datasets of nucleus and cytoplasm generated were used to implement a classification problem based on the SVM algorithm. The best results were obtained on the cytoplasm dataset. The model distinguished the two groups with an accuracy of 80% and a PPV and NPV, respectively, of 83% and 94%. In doing so, we also demonstrated that the information necessary to distinguish the two types of cells is contained in the cytoplasm.

(RAM-09.4) **Imaging of the Tomato Carotenoid Lycopene in Prostate Cancer Cells.**

Brian Scarpitti[1], Chureeporn Chitchumroonchokchai[1], Steven Clinton[1], Zachary Schultz[1]; [1]*The Ohio State University*

In fifteen words or less, explain the significance of this contribution (Novel Aspect).: Using Raman microscopy to examine delivery and subcellular distribution of lycopene in prostate cancer cells

Abstract Text: Lycopene is a carotenoid found in chloroplasts and chromoplasts of tomatoes, providing the

familiar red color. Epidemiologic and rodent studies support its anti-prostate cancer properties. Lycopene can quench singlet oxygen and such antioxidant properties may reduce DNA and macromolecule damage and inhibit carcinogenesis. However, the study of this and other putative mechanisms of action using in vitro cell culture have been limited by lycopene's highly lipophilic properties and challenges to mimic physiological uptake to cells. Understanding the localization of lycopene within the cancer cell can provide greater insight into lycopene's anti-cancer properties. Previous experiments focused upon fractionation of treated cells into subcellular components followed by quantification of lycopene by HPLC/MS. Raman spectroscopy has been used to understand the distribution of lycopene in tomatoes, and provides the potential for a non-destructive method of evaluating the subcellular lycopene distribution in living tissue culture. We have imaged lycopene treated PC-3 prostate cancer cells using Raman spectroscopy to show the resulting subcellular distribution of lycopene. The lycopene Raman signal is resonantly enhanced at the excitation wavelength used (532 nm), providing a convenient, sensitive, label-free technique to quantify lycopene in cells. Chemical information from Raman spectra acquired were used to plot the presence of lycopene throughout the cell volume. These chemical maps can be compared to darkfield images of the cells which show the cell membrane and other features for reference. Our initial studies of Raman maps show lycopene to accumulate at the plasma membrane of the imaged cells under these in vitro conditions. Additional studies to examine how different delivery systems impact cell distribution and anticancer properties are underway.

(RAM-09.5) **Overcoming the Limitations of Thiol-Maleimide 'Click' Chemistry for Surface-Immobilization of Biomolecules with Insight Gained from Confocal-Raman Microscopy**

Grant J. Myres[1], Joel M. Harris[1]; [1]*University of Utah*

In fifteen words or less, explain the significance of this contribution (Novel Aspect).: Raman characterization and application of aryl-maleimides at interfaces for long-term stabilization thiol-maleimide 'click' chemistry.

Abstract Text: Thiol-maleimide 'click' chemistry occurs under mild reaction conditions and is considered to be well suited for surface immobilization of thiol-containing molecules. The success of this surface-conjugation chemistry is limited, however, due to the spontaneous surface-dissociation as a consequence of the retro-Michael addition degradation pathway. To retain the attractive qualities of thiol-maleimide 'click' chemistry and address issues of poor stability, we describe an immobilization protocol for coupling thiol-containing molecules to surfaces presenting an aryl-maleimide linker. The enhanced stability described is a consequence of the aryl-succinimides susceptibility to a ring-opening hydrolysis which converts the thiosuccinimide to a stable succinamic acid thioether. To monitor these interfacial reactions in-situ, we perform the immobilization chemistry on nano-porous silica surfaces and characterize the internal composition of these materials with confocal-Raman microscopy. To terminate surfaces with an aryl-maleimide we react interfaces presenting immobilized thiols with N,N'-1,4-phenylene-dimaleimide. The Raman-based surface-characterization methodology allows us to monitor the chemistry at each step of the immobilization and reveals a decrease in the S-H stretch (2580 cm 1) accompanied with an increase in the maleimide/succinimide stretch (1770 cm-1) following the surface reaction. Aryl-maleimide terminated silica can then be reacted with an aliphatic-thiol conjugated to the molecule of interest under standard thiol-maleimide reaction conditions. Stabilization of the surface-immobilized molecule by hydrolysis and thioether formation was observed by a decrease in the succinimide stretch (1770 cm-1) and the addition of bands characteristic of an amide (1257, 1550, 1656 cm-1) revealing the formation of the succinamic acid thioether product. Over the course of the hydrolysis step,>90% of the surface-bound molecules were preserved at the surface with no detectable dissociation of the stabilized population after several weeks of storage in buffer. Future applications for investigations of biomolecule immobilized in porous silica surfaces will be discussed.

21AES03: Emerging Leaders in Electrophoresis, Electrokinetics, and Related Applications

Chair: Jason Dwyer
Co-Chair: Nicole Hill
On-site Chair: Jason Dwyer

(AES-03.1) **Significant heterogeneity in stem cell populations useful for transplantation**

Tayloria Adams[1], Anthony Tsai, Shubha Tiwari, Clarissa Ro, Andrew Yale, Lisa Flanagan; [1]*University of California, Irvine*

Stem cells are essential for cell replacement therapy because they differentiate into multiple distinct cell types, secrete bioactive molecules, and in some cases have positive immunomodulatory effects. Stem cell cultures are heterogeneous containing stem cells, partially differentiated progenitor cells, and fully differentiated cells, each with unique cell membrane features lending to their therapeutic diversity. However, stem cells' natural heterogeneity presents limitations in transplantation therapies and hinders our understanding of their basic biological functions. Sufficiently characterizing stem cells' functional behavior before using them in transplant therapy is essential to the development of reliable treatment options. In this work, two therapeutically relevant human stem cell populations, mesenchymal and neural, were screened using a multimodal profile consisting of cell size, cell proliferation, dielectrophoresis spectra, membrane capacitance, and cytoplasm conductivity. Additionally, differentiation gene expression and surface integrin expression were assessed for mesenchymal and neural stem cells, respectively. Using dielectrophoresis, a label-free cell analysis technique, we found that bone marrow-derived and adipose-derived mesenchymal stem cells have unique cytoplasm conductivity and similar membrane capacitance values. Through simulation, we identified the transient slope from the dielectrophoresis spectra as a metric to quantify the relative heterogeneity of mesenchymal stem cells. Two sets of the neural stem cells derived from GMP-grade human embryonic stem cells, Shef4 and Shef6, were assessed. We generated three batches of Shef4 (4-1, 4-2, 4-3), a batch of Shef6 sorted (6S), and unsorted (6U) cells. The 4-1, 4-2, and 4-3 cells have significant changes in size, proliferation, membrane capacitance, and integrin expression. The membrane capacitance depends on passage number of the 4-1 and 4-2 cells and alpha6 integrin correlated with the membrane capacitance of 4-2 cells. We assessed Shef6 cells because they were previously effective in a rodent spinal cord injury model. Our results show that 6S and 6U cells have significant changes in size, proliferation, membrane capacitance, and protein expression. The 6S cells were smaller and more proliferative than 6U cells and have a have higher membrane capacitance. Significant variability exists among mesenchymal and neural stem cells. Thus, careful screening to assess heterogeneity will be critical for the development of robust stem cell therapies.

(AES-03.2) **Thermal Gel Electrophoresis for Biological Analyses**

Tom Linz[1]; [1]*Wayne State University*

Gel electrophoresis is a ubiquitous bioanalytical technique used to determine the protein or nucleic acid contents within biological samples. Previous work has transitioned these electrophoretic separations from bulk slab gels into microfluidic systems, resulting in numerous benefits including faster analysis times and smaller sample volume requirements. The work presented here describes the development of a flexible system to further enhance the analytical performance of microfluidic gel separations. Thermally reversible polymer gels were employed as separation matrices because of their unique ability to change viscosity as a function of temperature. Our ability to incorporate a thermal dimension into the separation space provided an additional adjustable parameter to tune analytical performance. To demonstrate this concept, cells and small molecules were analyzed in a single device by maintaining distinct temperature regions to accommodate the analysis of each. Additionally, work was conducted to preconcentrate and separate nucleic acids and proteins in parallel. Whereas traditional gels require distinct regions for analyte stacking and separation, we demonstrated that a single thermal gel could be used to accomplish both simultaneously. Outcomes from our studies demonstrate robust analyte preconcentration (>10^3-fold enhancement) and high-efficiency separations (>10^6 plates/m) in

low-complexity microfluidic devices. The flexibility of our system to perform online preconcentration and separations of diverse analytes demonstrates its broad potential for bioanalytical measurements.

(AES-03.3) **AES Blue Fingers Award Winner**

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Please join AES in congratulating James Hagan, the winner of the 2021 AES Blue Fingers Award. To see James present his award-winning work, go to session 21AES04 on Tuesday, September 28, 2021 in meeting room 555. James's presentation is AES-04.3 - Electronic Single-Molecule Sensing for Glycomics and Genomics Using Chemically Tailored Nanopores from 2:10 PM – 2:30 PM. Congratulations to first runner up Jessica Torres presenting a poster on Tuesday: Tu-P35 Alternative solvents for the labelling of amino acids on extraterrestrial bodies Congratulations to second runner up Yu-Hsuan Cheng presenting a virtual poster which will be viewable in the on demand library after SciX: V-P9: "ASSURED" modular electrochemical sensor platform for rapid, sensitive, and selective detection of biomolecules

(AES-03.4) **Electromechanical lysis and rapid enzymatic assay of droplet encapsulated cells**

Robbyn K. Anand[1], Sungu Kim, Aparna Krishnamurthy, Baskar Ganapathysubramanian, Pooja Kasiviswanathan; [1]*Iowa State University*

Encapsulation of individual tumor cells in water-in-oil droplets supports assays that uncover cell-to-cell variations that drive disease progression and treatment outcomes. Obtaining a distribution of enzymatic activity among individual cells can identify subpopulations that are resistant to a chemotherapeutic agent or that are particularly invasive. Droplet microfluidics achieves many of the functions needed for cell analysis through merging, splitting, sorting, and thermal cycling as well as in-droplet mixing. Cell lysis is frequently accomplished by heat, which increases instrument complexity and can denature proteins, or by an added chemical agent, which can interfere with subsequent reactions. Similarly, while merging and mixing readily accomplish dilution, concentration enrichment is advantageous for many applications but is difficult to achieve once droplets are formed. In enzymatic assays, for instance, the reaction rate depends linearly on the concentration of the enzyme, and the limit of detection for the product is also concentration dependent. In this presentation, we describe an electrokinetic method to drive cell lysis and subsequent concentration enrichment (>15-fold) within droplets, thereby achieving increased assay speed and sensitivity. This approach can be applied to many droplets simultaneously and is readily integrated with existing droplet workflows. Using a coupled experimental and computational approach, we discriminate the role of the electric field, osmotic pressure, and shear force in cell lysis and quantify the influence of enrichment on reaction rate. We then leverage this method to determine the distribution in the activity of beta-galactosidase, a marker of cell senescence linked to drug efficacy, among a population of breast cancer cells. Our results demonstrate that electrokinetic manipulation of droplet contents have the potential for broad impact in cell analysis.

(AES-03.5) **Rapid Dielectrophoresis-based biodosimetry**

Erin A. Henslee[1], Danielle Cantoni[1], Christina Snyder[2], Juliana Hopper[1], Ravi Singh[2]; [1]*Wake Forest University,* [2]*Wake Forest School of Medicine*

In fifteen words or less, explain the significance of this contribution (Novel Aspect).: Dielectrophoresis can detect cellular changes due to ionizing radiation exposure within six hours.

Abstract Text: There is a present shortage of methods capable of rapidly determining the extent of accidental exposures of human beings to ionizing radiation (IR). In particular, early triage to distinguish exposed versus non-exposed individuals, as well as characterize the level of exposure is required. The dicentric chromosome assay is considered the gold standard for IR biodosimetry, however it is time consuming and requires sophisticated equipment and infrastructure. Peripheral blood has shown promise as an easily accessible sample,

where gene expression changes have been measured in mice models9-10, human blood irradiated ex vivo11-13, as well as blood from total-body irradiated individuals14-15. The most predictive of these methods, however, relies on total-body irradiation of humans, with most of these samples coming from cancer treatment patients, which could influence the transcriptional (genetic) profile. Whilst these methods are essential for fully characterizing IR biodosimetry, as an initial screening/triage method there remains a gap. Dielectrophoresis (DEP) is a technique in which non-uniform alternating (AC) electric fields induce cellular motion, dependent on both AC field frequency and the cellular electrical properties16. Analysis of the frequency dependence of cell movement enables determination of cellular electrophysiological parameters. Since DEP does not rely on biomarkers or other fluorescent labels, it has become an increasingly popular method of characterization and sorting5-7. In this work we use 3DEP, a DEP-based analysis system to elucidate, in real-time, cell parameters such as membrane conductance, membrane capacitance, and cytoplasmic conductivity. To our knowledge, DEP has not previously been used for radiation characterization, though it has been employed to quantify changes in RBC electrophysiology in various other applications. Here we present an assessment of DEP's ability by examine radiation-induced changes in a white blood cell model, Jurkats. This work demonstrates the timing and doses necessary to observe changes in Jurkat electrophysiologic parameters. It was found that only six hours post radiation exposure of 4GY was needed to yield changes in the DEP properties. Other standard measures required 24+ hours. With this, we postulate DEP as a viable technique of IR exposure detection, with future work aimed at the development of DEP as a rapid triaging tool.

21AWD07: SAS and Applied Spectroscopy William F. Meggers Award Symposium

Chair: Vartkess Apkarian
On-site Chair: Vartkess Apkarian

(AWD-07.1) **Quantitative analysis of surface-enhanced coherent anti-Stokes Raman scattering signals**

Eric O. Potma[1], Eric O. Potma[1], Shamsul Abedin; [1]*University of California, Irvine*

Coherent anti-Stokes Raman scattering (CARS) is a vibrational spectroscopy technique that, because of its coherent properties, generally offers stronger signals than obtained in spontaneous Raman scattering spectroscopy. The CARS signal strength can be further enhanced through the use of plasmonic resonances provided by nanostructured metal surfaces, even reaching the single-molecule limit. Recent advances in the surface-enhanced CARS (SE-CARS) field have been encouraging, yet, there are difficulties associated with the reproducibility of the observations and a lack of clarity on the experimental conditions under which SE-CARS signals can be reliably generated. In this contribution, we quantitatively analyze SE-CARS measurements for different plasmonic antennae obtained under a variety of illumination conditions. Using a classical dipole radiation model, the photon counts are compared with CARS signal levels in the absence of plasmonic enhancement. This comparison reveals that SE-CARS signals are substantially stronger that what can be expected from a classical radiation model, underlining the need for more advanced models for describing the light-matter interactions in plasmonic cavities.

(AWD-07.2) **Probing chemistry of surface-supported nanostructure at the angstrom-scale**

Nan Jiang[1], Nan Jiang[1]; [1]*University of Illinois at Chicago*

Fundamental understandings of chemistry and physical properties at the nanoscale enable the rational design of interface-based systems. However, the lack of a well-defined view of adsorbate-substrate interactions involving individual molecules presents a major barrier for controlling the properties of surface-supported nanostructures. To overcome these limitations, we are developing and applying scanning probe-based nanotechnology, including scanning tunneling microscopy (STM) and tip-enhanced Raman spectroscopy (TERS) to provide angstrom-scale mechanistic insights into complex chemical systems. Our research is at the interface of chemistry and materials science and provides the needed information about environmental heterogeneity in

complex chemical systems such as nanostructures on metal surfaces and the surfaces of two-dimensional (2D) materials. By using a plasmonically-active material for our scanning probe, the Raman signal at the tip-sample junction is incredibly enhanced, allowing for single-molecule probing. This method, further aided by the benefits of ultrahigh vacuum, is uniquely capable of obtaining (1) single molecules chemical identification; (2) adsorbate-substrate interactions determination; (3) the mechanism of chemical bond formation under near-surface conditions. By investigating the vibrational modes, we extract novel surface-chemistry information at an unprecedented spatial (< 1 nanometer) and energy (< 6 wavenumber) resolution. Our work provides new fundamental insights into the impact of changes in the chemical environment on the properties of nanostructures, and thereby lays the foundation in designing new atom- and energy-efficient materials and molecular assemblies with tailored properties.

(AWD-07.3) **Wiring pyridine through its Raman bonds: TERS driven by tunneling plasmons**

Joonhee Lee[1], Vartkess A. Apkarian[2]; [1]*University of Nevada, Reno,* [2]*University of California, Irvine*

We present tip-enhanced Raman spectro-microscopy of single pyridine molecules adsorbed on Cu(100). The observed hyperspectral data and intensity variation as a function of gap distance identify Raman scattering driven by tunneling of the atomically confined tip-apex plasmon. On strongly chemisorbed molecules at step edges, the optical images outlines the ball-and-stick extent of the upright molecules indicating the tip is reaching the Pauli repulsion regime. The coupling between the molecule and the tip antenna maps out the molecular contacts that provide continuity across the junction, through the tunneling current modulated by vibrations. In effect, we show the wiring of a single molecule to the plasmon and measure its static and dynamic conductivity.

(AWD-07.4) **Poincaré engineering of the nanofemto topology of surface plasmon polariton fields**

Hrvoje Petek[1]; [1]*University of Pittsburgh, Pittsburgh*

We perform ultrafast photoelectron emission microscopy of topological surface plasmon polariton fields. Evanescent fields at interfaces, such as surface plasmon polaritons, are chiral. By illuminating coupling structures rendered lithographically in silver films to define the geometrical charge with optical fields carrying designed polarization, we generate surface plasmon polariton wave packets with orbital angular momentum. As surface plasmon polariton wave packets propagate in space and time towards a focus, they undergo spin-orbit interaction to generate topologically nontrivial spin textures, which break the time-inversion symmetry on the excitation pulse 20 fs duration time, and nanometer spatial scales. By Poincaré engineering, we generate plasmonic fields with half-integer and integer topological charge that carry magnetic monopole spin textures homotopic to meron and Skyrmion-like magnetic quasiparticles, as well as their arrays. We record nanofemto movies by photoemission electron microscopy of the evolving surface plasmon fields by imaging the nonlinear two-photon photoemission that they excite from silver. The optical flow analysis of the fields reveals their spin textures on <50 nm spatial and <20 fs temporal scales. The Poincaré engineering of plasmonic fields provides the means for nanofemto manipulation of qubits in quantum computing.

(AWD-07.5) **Non-thiolated, non-resonant probe for SESORS detection in deep tissue**

Bhavya Sharma[1]; [1]*University of Tennessee*

Thiols are ubiquitous as self-assembled monolayers on gold substrates for biosensing modalities, including surface plasmon resonance (SPR), field-effect transistors (FET), electrochemistry, and surface-enhanced Raman spectroscopy (SERS). Thiols face several challenges including rapid degradation, aggregation, and chemical instability under harsh environmental conditions. Moreover, minute pH changes significantly affect SERS enhancement of thiolated gold surfaces. These challenges are more pronounced when considering biosensing in biofluids and tissue, particularly in vivo. Thus, there is a need for a suitable alternative to thiols for in vitro and in vivo biosensing. Here we present N-heterocyclic carbenes (NHCs) as an alternative to thiols for deep tissue

sensing using SERS combined with spatially-offset Raman spectroscopy (SESORS). NHC-functionalized gold nanoparticles (NHC-AuNPs) and thiol-AuNPs were injected into porcine tissue, as a human tissue mimic. We show the detection of NHC-functionalized AuNPs to greater depths than thiol-AuNPs and demonstrate the stability and durability of the NHCs over thiols. With appropriate probes, SESORS shows great promise for chemical sensing and imaging at tissue depths and time scales that are relevant for biomedical imaging.

21CHEM05: Current Applications of Chemometrics

Chair: Peter Harrington
On-site Chair: Peter Harrington

(CHEM-05.1) **Local modeling by classification of matrix effects**

John H. Kalivas[1], Robert Spiers[2]; [1]*Idaho State University,* [2]*Idaho State Univeristy*

In fifteen words or less, explain the significance of this contribution (Novel Aspect).: A calibration set matched to a predciton sample is selected from thousands of references samples.

Abstract Text: Local spectral multivariate calibration modeling attempts to learn the relationship (linear) between a sample spectral response and the analyte of interest. In order to accomplish this objective, a spectral library is required composed of samples similar to the target sample in terms of matrix effects. However, spectral libraries with accompanying analyte reference values often encompass matrix effects of vast diversity and hence the relationship between spectra and analyte amount is non-linear. Local modeling presumes there exists a subset of the library samples that predict the target sample more accurately than a global model (using all library samples). This subset (calibration set) should have nearly equivalent matrix effects and analyte content as the target sample and maintain a linear relationship. The presented approach, termed local adaptive fusion regression (LAFR), considers local modeling as a classification problem, where target samples are classified into respective calibration sets according to their matrix effects. Matrix effects, however, are hidden variables and thus are not directly accessible to the modeling process. Therefore, to classify a target sample to a calibration set, pseudo-labels must be created using unsupervised learning in LAFR. There are four stages to LAFR: (1) library searching, (2) linear clustering, (3) classification, and (4) regression. Library searching decimates a large library into a reasonably-sized subset that is spectrally similar to the target sample. Linear clustering generates pseudo-classes (calibration sets) using a novel method to cluster a library according to its hidden variables (matrix effects). Next, the target sample is classified into a calibration set using over a hundred similarity measures that are extended up to thousands using a novel cross-modeling technique. Finally, the selected calibration set is used to predict the target sample. Premier advantages over standard local modeling methods is that the LAFR hyperparameters are self-optimizing and the sample selection for forming and selecting calibration sets is holistic to all matrix effects. Results from multiple near IR datasets demonstrate strong performance in standard non-linear datasets, effective identification of hidden variables (such as instrument of origin), and great improvement over global models in difficult massive soil libraries.

(CHEM-05.2) **Saturated signals in spectroscopic imaging: why and how should we deal with this regularly observed phenomenon?**

Ludovic Duponchel[1], Alessandro Nardecchia[2], Vincent Motto-Ros[3]; [1]*University of Lille,* [2]*Université de Lille,* [3]*Institut Lumiere Matiere University of Lyon*

In fifteen words or less, explain the significance of this contribution (Novel Aspect).: Highlight the very strong impact of saturated signals on data analysis and propose solutions.

Abstract Text: We have all been confronted one day by saturated signals observed on acquired spectra, whatever the technique considered. A saturation, also known as clipping in signal processing, is a form of

distortion that limits a signal once it exceeds a threshold. As a consequence, clipped or saturated bands with their characteristic plateau present numerical values that do not correspond to the analytical reality of the analyzed sample. Of course, analysts know that they cannot consider these erroneous values and therefore reconsider either sample preparation or instrument settings. Unfortunately, there are many experiments today (and this is the case of spectroscopic imaging) for which we will not be able to fight against the saturation effect that will undeniably be observed on the acquired spectra. The aim of this article is first to show why it is important to correct these saturation effects at the risk of having a biased view of the sample and more specifically in the context of multivariate data analysis. In a second step, we will look at strategies for managing saturated bands. An original concept will then be presented by considering saturated values as missing ones. A statistical imputation strategy will then be implemented in order to recover the information lost during the measurement.

(CHEM-05.4) **Application of Fentanyl Analog Screening Kit Toward the Evaluation of Portable Gas Chromatograph - Mass Spectrometer for Field Use**

Rebecca Chan - Chao[1], Pauline Leary[2], Koby Kizzire[1], Brooke W. Kammrath[3]; *[1]University of New Haven, [2]Federal Resources, [3]Henry C. Lee College of Criminal Justice and Forensic Sciences, University of New Haven*

In fifteen words or less, explain the significance of this contribution (Novel Aspect).: Portable GC-MS evaluations and creation of fentanyl analog library used for identification in the field.

Abstract Text: The opioid epidemic is a growing global concern. In 2019, an estimated 10.1 million people aged 12 or older misused opioids in the previous year. An opioid of particular concern is fentanyl, which is 50 - 100 times more potent than morphine and has multiple analogs of variable potencies. In street samples it is primarily linked to illegally made fentanyl. To combat this growing crisis, suitable instrumentation that can be successfully deployed at the sample site is necessary. GC-MS has been used to provide confirmatory identification of drugs providing a highly specific result. For this reason, its potential value for the identification of a number of fentanyl analogs is significant. In this research, two portable gas chromatography-mass spectrometry (GC-MS) systems, one an ion trap and one a quadrupole, were evaluated to establish their abilities to detect a selection of fentanyl analogs. These GC-MS systems were used to analyze Cayman Chemical's fentanyl analog screening kits, which contain more than 210 fentanyl analogs, and results were evaluated to determine the identification capability for each system. Further, a fentanyl drug analog library was created for each system to enable automatic identification at the time of analysis. Both instruments have demonstrated the ability to detect fentanyl and its analogs and have value for use in the field for law enforcement and military personnel.

(CHEM-05.5) **Multivariate analysis of vibrational spectroscopic data for cannabis plant materials**

Ewelina M. Mistek-Morabito[1], Igor K. Lednev[2], Aaron Urbas[3]; *[1]University at Albany, SUNY, [2]University at Albany SUNY, [3]National Institute of Standards and Technology*

In fifteen words or less, explain the significance of this contribution (Novel Aspect).: Nondestructive and rapid identification and quantification of cannabis samples using vibrational spectroscopy and multivariate analysis.

Abstract Text: Identification and quantification of seized drugs are important aspects of current forensic practices. With the passage of the 2018 Farm Bill, the analysis of cannabis samples has become more challenging for forensic practitioners. There is an urgent need for rapid and reliable methods to differentiate

between legal hemp and illegal marijuana samples. There are no taxonomical differences between hemp and marijuana and current field tests to distinguish them are limited. The term hemp is used to refer to strains of cannabis that have low levels of tetrahydrocannabinol (THC). However, the new federal legislation defined legal hemp as containing 0.3% or less total-THC, estimated as decarboxylated-Δ9-THC, on a dry weight basis. This designation requires that forensic practitioners quantify the total-THC content in seized cannabis samples. In this work, we utilized vibrational spectroscopy (near infrared (NIR) and Raman spectroscopy) to analyze a wide variety of ground cannabis samples. The data sets were analyzed using multivariate methods to qualitatively discriminate between strains designated as hemp and marijuana as well as develop quantitative models for THC content. Classification models showed clear differentiation between hemp and marijuana strains. However, numerous hemp samples were found to contain levels of THC above 0.3%. Consequently, regression models were developed to quantify total-THC content to explore whether sufficient accuracy could be obtained for forensic or regulatory purposes. Vibrational spectroscopy coupled with multivariate analysis showed potential for the nondestructive and rapid identification and quantification of cannabis samples.

21CTP/EARLY01: We, the Scientists: Strategies to Support Diversity, Equity, and Inclusion

Chair: Karen Esmonde-White
On-site Chair: Karen Esmonde-White

(CTP-EARLY-01.1) **Don't Ask What a Society Can Do for Me but How I Can Enable the Society to Help the Broader Community**

Ellen V. Miseo[1], Jim Rydzak[2], Mary Carrabba[3], Mike George[4]; *[1]TeakOrigin, [2]Specere Consulting, [3]The Coblentz Society, [4]University of Nottingham*

Many times, when someone joins a professional society, the idea is how can the society help me. But professional societies are first communities of like-minded people. So, in addition to “What can the Society do for me?” someone should be asking “How will my participation in the Society help the broader community?” The Coblentz Society has a number of initiatives to address the broader community. They include help for early career scientist, education, mentoring, cooperation with other societies to broaden the reach of both organizations and stepping up to help when needed. The rewards from these efforts may be tangible, such as name recognition, but in most cases they are intangible. And you never know when what you did will come back to you ten-fold. This talk will briefly discuss some of the efforts that we have undertaken, some of the new efforts and how our members, and new members can assist in these efforts.

(CTP-EARLY-01.2) **See it. Believe it. BECOME It!**

Jeanita Pritchett[1]; *[1]JSP Coaching and Consulting, LLC*

Promoting a diverse and inclusive STEM ecosystem starts with creating environments where underrepresented people have role models, mentors, and educators to look to for inspiration. Representation matters and its impact becomes more evident when thinking about how to encourage more women and people of color to enter STEM fields. From offering guidance to providing insight from their personal experiences, mentors, educators, and role models help others grow and become more confident in navigating their educational and career paths. During this presentation, the critical role that each one plays in shaping the demographics of the STEM field as well as strategies for enhanced engagement will be explored.

(CTP-EARLY-01.3) **Contributions of Women to Science at an Analytical Instrument Engineering Company**

Fran Adar[1]; *[1]HORIBA Scientific*

Following my passion for exploring the mysteries of the universe I took an advanced degree in physics. My thesis advisor told me that he understood the problems of women in science, something that totally puzzled me until I had been working for him for a while. From there I went to a Biophysics Department, and again found that an opportunity for a stable job would be unusual. So when a position became available in an instrument company that was introducing the Raman microscope I took the job immediately. I knew that it would be in the company's interest for me to succeed, and my subsequent career has illustrated that both I succeeded in the recognition I received for my scientific efforts, and the increasing use and evolution of Raman microscopy produced success for the company. In fact, when I look around the company I see that many applications positions are staffed by women with Ph.D.'s in one of the sciences – usually physics or chemistry, but increasingly in the biochemical and pharmaceutical sciences. The job provides the stability that a woman needs who is raising a family. And the HORIBA website has devoted an entire section to their personnel policies that depend on their attitudes of diversity. Here is what Juichi Saito says: " The inclusion of diverse human resources forms a part of our management strategies at HORIBA, where management, HR, and field staff join hands together to promote diversity. Guided by our corporate motto, "Joy and Fun," we will continue to provide a great workplace where each and every employee is encouraged to fulfill his or her unique potential and have a sense of satisfaction with what he or she is doing, thus enhancing our corporate value." And because I am known in the field I am occasionally invited to help organize some program (actually organization is not something that I am good at.) I am now helping to recruit contributors to the Asian Journal of Physics' February 2022 publication entitled "Women in Science: Raman Spectroscopy".

(CTP-EARLY-01.3) **Optimizing Virtual Connectivity to Increase DEI Objectives**

Lori Ana Valentin, Lori Ana Valentin, Velda Iskandar[1], Thomas Bassindale; [1]*Teknor Apex Company*

While the COVID-19 pandemic prohibited most in-person programs, the transition to virtual programming provided the opportunity to network more broadly. The New York State Police shifted forensic outreach and internships to completely remote experiences. Outreach efforts targeted students in under-resourced school districts through virtual programming and customized workbooks for those without access to internet. The first-ever forensic virtual internship focused on topics beyond an introductory course - including chain of custody, accreditation, and courtroom testimony. Forensic experts from county, state, and federal laboratories in the United States provided lectures to nineteen students across the world. Sheffield Hallam University's novel approach using open-source simulators during its 2020 virtual forensic toxicology laboratory will also be explored.

(CTP-EARLY-01.4) **Enhancing Equity and Inclusion through Evidence-Based Approaches in Higher Education: Comprehensive Action at a Mid-Size State University**

Nathaniel V. Nucci[1]; [1]*Rowan University*

Historically, the field of physics has been one of the least diverse areas of science. Low retention of racial minorities and women has persisted despite many wide-spread initiatives across the physics community. In partnership with a new initiative at the American Physical Society, the Department of Physics and Astronomy at Rowan University, a mid-sized state university in southern New Jersey, is working change our local culture to promote retention and success of students from these groups. We are employing a multi-pronged approach to build more equitable classrooms, creating longitudinal tools for self-study, and building repositories of readings and best practices to educate our community. Active learning is a central theme of our efforts, and while the value of active learning in introductory courses is well-established, we are now working to understand how active learning in upper-level courses, especially using research-focused projects, influence student attitudes and influence equity.

() **Open Discussion**

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21MASS02: Ionization in Mass Spectrometry: Fundamentals and New Applications

Chair: Kaveh Jorabchi
On-site Chair: Kaveh Jorabchi

(MASS-02.1) **Vibrating Sharp Edge Nebulization: New Interfacing for Capillary Electrophoresis and Mass Spectrometry**

Lisa Holland[1]; [1]*West Virginia University*

A voltage free CE-MS interface is described based on vibrating sharp-edge spray ionization. This interface overcomes the challenges of decoupling applied voltages and improves the compatibility with separations performed at near-neutral pH. Acousto-mechanical energy from an inexpensive piezoelectric device is transferred at the tip of an electrophoresis capillary. This mode of ionization is cost effective as the price and piezoelectric device are less than $1 and the equipment to drive the source costs under $200. The vibrating sharp-edge spray ionization functions with flow rates from 70 to 200 nL/min and does not perturb the capillary electrophoresis electroosmotic flow as evidenced by the observation that migration times differ less than 7% (n = 3) across a lab-built system interfaced to mass spectrometry and a commercial system that utilizes absorbance detection. The interface was evaluated for different background electrolytes and with proteins, peptides, and small molecules (i.e. beta-blockers) analyzed at concentrations ranging from 10 nM to 5 □M, with an estimated detection limit of 2 nM. For cationic beta-blockers the theoretical plates achieved in the capillary electrophoresis-mass spectrometry setup were 80% to 95% of that observed with a commercial capillary electrophoresis-UV absorbance detection system.

(MASS-02.2) **Development of Novel Microwave Ionization Techniques for Mass Spectrometry**

Steven J. Ray[1], Maria Rivera, Caitlin Massimi, Khue Nguyen; [1]*The State University of New York at Buffalo*

Microwave radiation lies in region of the electromagnetic spectrum corresponding to low-energy (e.g. 10 μeV) spectroscopic transitions. However, the frequency range between 1GHz-300GHz is perhaps better recognized for its unique properties in heating applications, high-field communications, and as an inexpensive source of electromagnetic radiation. In this presentation, several new ionization techniques that exploit microwave fields generated by inexpensive and simple means are examined. In one example, microwaves fields with a frequency of 2.45 GHz are focused into a very small and controlled volume near the tip of an electrospray ionization (ESI) emitter for modulation of the ionization characteristics of ESI ionization for mass spectrometry. When the microwave radiation is absorbed, it induces very rapid evaporation through dielectric heating, modifying the features of the Taylor cone and effecting processes such as droplet generation, jet stability, ionization intensity and efficiency, and ion fragmentation. Because the microwave power can be amplitude modulated rapidly, these changes in ionization characteristics can be used to gain new chemical information and to improve the fidelity of certain analyses. In a second example, a novel ultra-low volume microwave heating reactor is evaluated as a means for the control of a wide range of chemical and biochemical reactions by exploitation of focused dielectric heating. In particular, a simple method for enhancing the kinetic rates of trypsin digestion of proteins by using a microwave microstrip heating cavity is evaluated for bottom-up proteomics applications. A microcapillary vessel exposed to a region of highly-focused microwave electric field is shown to reduce enzymatic digestion times by several hours, and to also permit modulation of digestion conditions when placed in-line before analysis by mass spectrometry. Finally, a novel microwave plasma developed as an alternative ionization source for inductively-coupled plasma mass spectrometry will be examined. This small, low-power ionization source is examined for the analysis of targeted halogen elements, and its analytical figures of merit presented.

(MASS-02.3) **Absolute Quantitation of Peptides and Proteins by Coulometric Mass Spectrometry (CMS)**

Hao Chen[1], Hao Chen[1]; [1]*New Jersey Institute of Technology*

Accurate quantification is essential in the fields of proteomics, clinical assay, and biomarker discovery. Popular methods for absolute protein quantitation by mass spectrometry (MS) involve the digestion of target protein and employ isotope labeled peptide internal standards to quantify chosen surrogate peptides. Although these methods have gained success, syntheses of isotope-labeled peptides are time-consuming and costly. To eliminate the need for using standards or calibration curves, herein we present a coulometric mass spectrometric (CMS) approach for absolute protein quantitation, based on the electrochemical oxidation of a surrogate peptide combined with mass spectrometric measurement of the oxidation yield. In our experiment, tyrosine-containing peptides were selected as surrogate peptides for quantitation, considering the oxidizable nature of tyrosine. Our data showed that the results for surrogate peptide quantity measured by our method and by traditional isotope dilution method are in excellent agreement, with the discrepancy of 0.3−3%, validating our CMS method for absolute quantitation. Furthermore, therapeutic monoclonal antibody (mAb) could be quantified by our method as well. Due to the high specificity and sensitivity of MS and no need to use isotope-labeled peptide standards, our CMS method would be of high value for the absolute proteomic quantification.

(MASS-02.4) **Enhanced Elemental Ionization for Quantitation Using Intrinsic Elemental Tags**

Kaveh Jorabchi[1], Samuel White[1], Frenio A. Redeker[1], Kunyu Zheng[1], Joseph E. Lesniewski[1]; [1]*Georgetown University*

Soft ionization techniques in mass spectrometry are widely used for analysis of complex samples but they encounter challenges in quantitation, particularly in nontargeted analyses. Widely different ionization efficiencies of compounds in soft ionization methods mandate use of compound-specific standards. Moreover, isotopically labeled standards may be needed to account for matrix effects. These shortcomings are exacerbated by rapidly growing nontargeted approaches where new compounds are detected in variety of matrices. Standards are often not readily available for newly detected compounds, limiting the quantitation to relative comparisons between similar samples in such approaches. Notably, absolute quantitation without compound-specific standards can be achieved using elemental ionization methods where ionization efficiencies of elements become independent of the chemical structures and are minimally affected by the sample matrix. This strategy has been successfully applied to compounds containing metals and via use of metal tags. However, extending this approach to intrinsic non-metal elements for label-free quantitation has been hampered by difficulties in efficient thermal ionization of non-metals and resolution of isobaric interferences. In this presentation we discuss a novel elemental ionization approach that addresses these shortcomings. Chromatographically separated analytes are introduced into a reactive plasma where intrinsic elements of compounds are quantitatively converted to stable polyatomic species. The plasma products are then ionized via ion-neutral reactions with reagent ions supplied by a nano-electrospray. The wide range of available reagent ions impart tunable chemical ionization for selective and sensitive detection. Mitigation of isobaric interferences is achieved by facile interfacing of this elemental ionization approach to a wide range of readily available atmospheric sampling instruments with advanced ion manipulation capabilities. Examples of metal-based reagent ions for detection of F, Cl, P, and quantitation of compounds without compound-specific standards are discussed using this new ionization technique.

(MASS-02.5) **Ionization Mechanisms of Alkanes**

Hilkka Kenttamaa[1]; [1]*Purdue University*

Mass spectrometric characterization of complex mixtures of large saturated hydrocarbons is critically important for numerous fields, including petroleomics and renewable transportation fuels, but challenging. Large alkanes are nonvolatile, difficult to ionize, and if ionization succeeds, they often fragment into small pieces. Therefore, many different evaporation/ionization approaches have been developed for the characterization of large alkanes. Atmospheric pressure chemical ionization (APCI) mass spectrometry based on hydrocarbon solvent/reagent has shown some promise in the analysis of saturated hydrocarbons. However, although being a relatively gentle ionization method, APCI still causes extensive fragmentation to saturated hydrocarbons, which impedes its

effectiveness. To prevent this fragmentation, its causes were examined via gas-phase ion-molecule reactions in vacuum in a linear quadrupole ion trap mass spectrometer. The results demonstrate that the mechanism proposed previously for ionization of saturated hydrocarbons upon APCI, hydride abstraction by carbocation reagent ions, is not correct. Instead, the fragmentation is caused by ionization of saturated hydrocarbons via exothermic proton transfer reactions involving highly acidic, protonated atmospheric molecules, such as protonated nitrogen and protonated water. Accordingly, the extent of fragmentation was found to correlate with the proton affinities of the atmospheric molecules studied. Remarkably, controlled experiments involving isolated atmospheric ions and neat saturated hydrocarbons in vacuum yielded almost identical mass spectra as APCI involving atmospheric pressure conditions, presence of many different chemicals, and an electrical discharge. As the proton affinities of large alkanes are unknown, mass spectrometric bracketing experiments were carried out to determine the proton affinities of several alkanes containing 5-10 carbons. Five different reference bases with proton affinities ranging from 171 to 181 kcal/mol were used. Surprisingly, the proton affinities of all the studied alkanes are very similar, ranging from 171 kcal/mol up to 173 kcal/mol. This finding suggests that even much larger alkanes are unlikely to have proton affinities much greater than 173 kcal/mol. Therefore, the reagent used to ionize large alkanes upon APCI should not have a proton affinity much greater than 175 kcal/mol in order to avoid excessive fragmentation.

21PMA03: Innovating PAT in Bioprocessing

Chair: Anja Mueller
Co-Chair: Geraldine Baekelandt
On-site Chair: Linda Kidder

(PMA-03.1) **Non-repetitive protein dynamics on microsecond to second time-scales monitored by mid-infrared dual comb spectroscopy**

Florian Eigenmann[1], Raphael Horvath[2], Carsten Koetting, Klaus Gerwert[3], Markus Mangold[1]; *[1]IRsweep AG, [2]IRSWEEP, [3]Ruhr University Bochum, Center for Protein Diagnostics (PRODI) & Department of Biophysics*

Spectroscopic reaction monitoring is a proven tool in R&D and production environments, where it is used to gain insight into chemical reactions. In this presentation we showcase how dual-comb spectroscopy (DCS) can be used to monitor protein dynamics on a microsecond to second time-scale. Kinetics of irreversible protein reactions require an analytical technique that provides time-dependent infrared spectra in a single shot. First results have been validated with the recording of transients of the photoactivated proton pump bacteriorhodopsin with DCS and stepScan FTIR [1]. Structural model of G□i·RGS and 3D-plot of the absorbance changes observed during its GTPase reaction [2]. FTIR with time resolutions faster than 10 ms is only possible with photoactivatable proteins, where the reactions can be repeated hundreds of times because they undergo a photocycle. Many relevant protein reactions are non-repetitive. In many cases reactions can be induced by caged compounds [2]. As an example, we investigate the inhibiting Gi protein and the larger protein-protein complex of G□i with its cognate regulator of G-protein signaling (RGS). We compare caged compound induced reactions monitored by FTIR and DCS. With DCS we observe good data quality with 4 µs time resolution, four orders of magnitude faster than the FTIR measurement. DCS allows for infrared spectroscopic studies in the so-far unresolvable microsecond time regime for non-repetitive biological systems including GTPases and ATPases. Additionally, we will present how dual-comb spectroscopy can be used to monitor fast kinetic reactions in combination with the stopped-flow technique, which was previously used more with UV-VIS or fluoresce because of lack of speed of currently available FTIR spectrometers. Mid-IR spectroscopy is however much more sensitive and specific compared to these other techniques and will pave the way to new research possibilities for kinetic studies. [1] Klocke, Mangold, Allmendinger, Hugi, Geiser, Jouy, Faist, Kottke, (2018) Anal. Chem. 90, 17, 10494–10500. Single-Shot Sub-microsecond Mid-infrared Spectroscopy on Protein Reactions with Quantum Cascade Laser Frequency Combs [2] Norahan, Horvath, Woitzik, Jouy, Eigenmann, Gerwert, Kötting (2021) Anal. Chem. 93, 17, 6779-6783 Microsecond resolved

infrared spectroscopy on non-repetitive protein reactions by applying caged-compounds and quantum cascade laser frequency combs

(PMA-03.2) **PAT for Real-Time Monitoring of mRNA Production**

Elliott Schmitt[1]; [1]*Moderna*

The clinical utility of mRNA-based medicines has been highlighted by COVID-19. Process analytical technology (PAT) has played a critical role in the industry for improving process knowledge, consistency, and product quality of mRNA production. In-line analytical techniques can readily be used as a means for early process fault detection and real-time monitoring and/or release. This talk will discuss two potential in-line analytical technologies that could be used for real-time monitoring of typical mRNA production processes. The first discusses a case study for using Raman spectroscopy to quantify mRNA process raw material. The second case study outlines use of UV slope spectroscopy to monitor a tangential flow filtration (TFF) step commonly used for downstream production.

(PMA-03.4) **High-sensitivity mid-IR spectroscopy for proteins in water by solvent absorption compensation**

Young Jong Lee[1], Bonghwan Chon[1], Shuyu Xu[1]; [1]*National Institute of Standards and Technology*

In fifteen words or less, explain the significance of this contribution (Novel Aspect).: This simple optical technique can enhance the protein concentration sensitivity without changing the detection system.

Abstract Text: Infrared (IR) absorption spectroscopy is a powerful tool that can quantify complex biomolecules 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 very short path length and a limited 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. Absorption spectra without any post-processing show good linearity from 100 mg/mL to 0.1 mg/mL protein concentration, allowing a >100 times enhanced signal-to-noise ratio in the amide I band compared to the non-SAC results. We apply this method to in-situ investigate the isothermal kinetics of insulin fibrillation at two clinical concentrations at an elevated temperature (74 °C) for 18 hours. Simultaneous monitoring of both reactants (native forms) and products (fibrils) allows quantitative discussion of the detailed fibrillation mechanisms, which are not accessible with other single modality measurements. This simple optical technique can be applied to other absorption spectroscopies of analytes in strongly absorbing solvents, allowing for enhanced sensitivity without changing the detection system.

(PMA-03.5) **Cell Therapy Product Characterization by Raman Spectroscopy**

Lifu Xiao[1]; [1]*Janssen Research & Development LLC*

In fifteen words or less, explain the significance of this contribution (Novel Aspect).: This paper demonstrates the potential of Raman characterization of cell therapy product attributes.

Abstract Text: Cell therapy therapeutics are complex products that require sophisticated product characterization methods during cell therapy product and manufacturing process development. Conventional bioassays are often based on specific labeling of cellular markers to report cell viability and density, cell identity and health, as well as specific biomolecules. These methods are highly informative but tend to be time-consuming and costly. Raman spectroscopy can be a fast, noninvasive, and information-rich complementary technique to the conventional bioassays for cell therapy product characterization. Here we report two proof-of-

concept studies using Raman spectroscopy for cell therapy drug product characterization. In the first study, we correlated the acquired cellular Raman signals with the established flow cytometry markers associated with apoptosis of T cells detected during drug product cryopreservation. In the second study, we demonstrated the capability of Raman to measure viable cell density and cell viability of T cells directly in product solutions without sampling. Our results demonstrate the potential of Raman spectroscopy for label-free and non-invasive measurements of T cell characteristics relevant to cell therapy product design and process control.

21RAM06: Spatially Offset Raman Spectroscopy

Chair: Sara Mosca
Co-Chair: Fay Nicolson
On-site Chair: Fay Nicolson

(RAM-06.1) **SORS – How Deep?**

Pavel Matousek[1], Sara Mosca[2], Priyanka Dey[3], Marzieh Salimi[4], Ben Gardner[5], Francesca Palombo[5], Nick Stone[6]; *[1]STFC Rutherford Appleton Laboratory, [2]CLF, RAL, STFC, [3]Teesside University, [4]Exeter University, [5]University of Exeter, [6]Biomedical Physics, School of Physics and Astronomy, Uni of Exeter, UK*

This presentation will discuss a fundamental question relevant to a wide range of SORS studies: How deep SORS probes for any specific spatial offset. Or in turn, what magnitude of spatial offset one should select to probe a specific depth. This issue is addressed by using Monte Carlo simulations, under the assumption of negligible absorption, which establishes that the key parameter governing the extent of the probed zone for a point-like illumination and point-like collection SORS geometry is the reduced scattering coefficient of the medium. This can either be deduced from literature data or directly estimated from a SORS measurement by evaluating the Raman intensity profile from multiple spatial offsets. Once this is known, the extent of the probed zone can be determined for any specific SORS spatial offset using the results of Monte Carlo simulation. The proposed method was tested using experimental data on stratified samples by analyzing the signal detected from a thin layer moved through a stack of layers using both non-absorbing and absorbing samples. The simple methodology provides important additional information on SORS measurements with direct relevance to a wide range of SORS applications including biomedical, pharmaceutical, security, forensics, and cultural heritage.

(RAM-06.3) **Facing challenges of Cultural Heritage materials using micro-SORS**

Claudia Conti[1], Alessandra Botteon[2], Christopher Corden[3], Ioan Notingher[4], Pavel Matousek[5]; *[1]National Research Council, Institute of Heritage Science, [2]CNR, Institute of Heritage Science, [3]School f Physics and Astronomy, University of Nottingham, [4]University of Nottingham, [5]STFC Rutherford Appleton Laboratory*

Cultural Heritage materials are intrinsically complex and their non-invasive investigation at micrometre scales is a major challenge for conservation scientists; in the present talk, the recent advances in Raman spectroscopy that are paving ways to novel analytical approaches for facing challenges in Cultural Heritage materials and improving the knowledge and conservation of art objects will be presented. Micro Spatially Offset Raman Spectroscopy (Micro-SORS) has been proposed as an effective tool for investigating non-invasively compounds located below the surface, for instance, in a hidden painted layer, in the preparation layer or in the substrate (i.e. plaster) [1]. Its ability to "read" hidden texts through turbid layers and to suppress the fluorescence of the surface layer recovering the subsurface Raman signal have been demonstrated with benchtop instruments. Interestingly, non-invasively and in-situ analyses have been carried out with a portable micro-SORS prototype, enabling the possibility to analyze the artworks where they are located (museum collections, archaeological sites etc.) [1]. The diffusion process of an agent into a matrix is one of the latest research streams, which is crucial in Cultural Heritage in situations when conservation or decay products diffuse into a plaster or stone. Preliminary results demonstrate the micro-SORS capability to monitor the absorption and diffusion processes

non-invasively, providing essential information about the penetration depth of a product [2]. The diffusion process is also monitored within thin layers (on the order of 10-15 μm), in case of a dye used in dyed sensitized solar cells, paving the way to useful applications also outside art. The results of a recent study carried out in collaboration with the University of Nottingham will be also shown, where micro-SORS has been coupled with Time-Gated Raman Spectral Multiplexing, enabling fast sub-millimeter resolution molecular depth Raman mapping of fluorescing and non-fluorescing samples [3]. [1] S. Mosca, C. Conti, N. Stone, P. Matousek, Nat Rev Methods Primers 2021, 1, 21. [2] A. Botteon, J. Yiming, S. Prati, G. Sciutto, M. Realini, C. Colombo, C. Castiglioni, P. Matousek, C. Conti, Talanta, 2020, 218, 121078. [3] C. Corden, P. Matousek , C. Conti, I. Notingher, Appl Spectrosc 2021, 75, 156.

(RAM-06.3) **Physicochemical analysis of chemicals and biological tissues using spatially offset Raman spectroscopy**

Hyung Min Kim[1]; [1]*Kookmin University*

The physicochemical properties of analytes can be achieved when spatial and spectral information are comprehensively correlated. Raman spectroscopy has been known as a versatile tool for chemical analysis by offering molecular fingerprints and two-dimensional (superficial) information of analytes can be achieved by combining various scanning or mapping methods. And it has been reported that spatially offset Raman spectroscopy (SORS) provides the depth-resolved chemical information. Therefore, we applied the SORS methods to physical (morphological) and chemical analysis of pharmaceutical and biological samples. For rapid SORS measurements, we developed a hyperspectral system offering hundreds of offset-dependent spectra at once using a multi-line image sensor. The coating thickness of pharmaceutical tables were recorded in a single-shot hyperspectral SORS measurement and their thickness confirmed by confocal Raman spectroscopy. The particle-size dependence of SORS results were also investigated for polyethylene powder models envisioning pharmaceutical application. In addition, we developed a fiber-based method to extend the adjustable range of offset distance. We applied the long offset measurement to analyze the fat thickness of pork mainly composed of muscle and fat tissues. Consequently, we propose that the physical and chemical information can be achieved rapidly with our SORS system.

21ATOM06: ICP-MS/MS and advanced applications using ICP-MS

Chair: Jenny Nelson
On-site Chair: Jenny Nelson

(ATOM-06-1) **What Advantages does ICP-MS/MS offer? Current and Future applications of ICP-MS/MS**

Jenny Nelson[1]; [1]*Agilent*

In fifteen words or less, explain the significance of this contribution (Novel Aspect).: ICP-MS/MS has changed what is possible in an elemental analysis laboratory

Abstract Text: Since the introduction of the first commercially available ICP-MS/MS in 2012, hundreds of users have publishing outstanding research and studies in many fields. Including Clinical Research, Consumer Products, Energy and Fuel, Environmental, Food testing and Agriculture, Geochemistry and Geology, Life Science and Biomedical, Materials, Nanomaterials, Nuclear, Pharmaceuticals, and others. In this talk, we will take a closer look at many different triple quad applications and how this powerful technique has made significant contributions to analytical labs in all of the above industries. We will review current and future applications for many of these fields.

(ATOM-06-2) **Single Cell ICP-MS Analysis Of Solid Tissues Using Pneumatic Nebulization: The Analytical Challenge**

Roberto Álvarez-Fernández García[1], Jörg Bettmer[1], María Montes-Bayón[1]; [1]*University of Oviedo*

In fifteen words or less, explain the significance of this contribution (Novel Aspect).: Isolation of cell suspensions from solid tissues for SC-ICP-MS using pneumatic nebulization without needing LA-ICP-MS

Abstract Text: Due to the improvements in detection sensitivity, inductively coupled plasma-mass spectrometry (ICP-MS) has become a powerful tool for the analysis of small objects such as single cells and nanoparticles. Developments in single cell ICP-MS (SC-ICP-MS) have increased significantly essentially through the development of sample introduction systems based on pneumatic nebulization for the introduction of cell samples to the ionization source with maximum transport efficiency. In this sense, SC-ICP-MS is nowadays an exceptional strategy for the analysis of cell suspensions in a great variety of applications. However, the application of this strategy to in vivo studies would involve the use of solid tissues, not compatible with the downstream SC-ICP-MS using pneumatic nebulization. An alternative is the use of solid sampling introduction techniques such as laser ablation (LA)-ICP-MS in the so called "imaging" strategies. However, the need for complex instrumentation, the requirement of suitable calibration standards and the ablation of larger areas than this of an "individual" cell are current limitations hampering the routine use of this strategy.1 Here we propose an alternative, without the need of laser ablation, consisting in the isolation of cell suspensions from animal tissues that can be directly transferred into the SC-ICP-MS system through pneumatic nebulization. For this aim, adequate sample treatments have to be conducted in order to minimize the samples tissues required (to be used in clinical biopsies) and the sample preparation. Optimization of the enzymatic digestion procedures are followed to degrade the extracellular matrix of the solid tissues, break cell-cell junctions and prevent cell aggregation. Initial experiments including mice liver and spleen reveal the suitability of the developed strategy using pneumatic nebulization with high-efficiency sample introduction systems and SC-ICP-MS. 1 M. Corte-Rodríguez, R. Alvarez-Fernández, P. García-Cancela, M. Montes-Bayón, J. Bettmer. Trends in Analytical Chemistry 132 (2020) 116042.

(ATOM-06-3) **Speciation Analysis as a Tool to Investigate the Wash-Out Effect of Intravenous Iron by Perioperative Cell Salvage**

Marcel Macke[1], Jennifer-Christin Müller[1], Roman M. R. Olivier[2], Andrea U. Steinbicker[2], Uwe Karst[3]; [1]*University of Münster, Institute of Inorganic and Analytical Chemistry,* [2]*University of Münster, University Hospital of Münster, Department of Anesthesiology, Intensive Care and Pain Medicine,* [3]*Institute of Inorganic and Analytical Chemistry, University of Münster*

Iron deficiency anemia is one of the most common medical disorders worldwide, and affected individuals who undergo major surgery have an increased risk of postoperative morbidity and mortality. Therefore, supplementation of intravenous iron (IVI) preparations up to one day before surgical intervention is strongly recommended for these patients. Frequently administered iron supplements, such as ferric carboxymaltose (FCM), consist of a nanoparticulate Fe(III)-oxyhydroxide core surrounded by a carbohydrate shell and allow controlled delivery of iron. If high blood loss is expected, additional perioperative cell salvage is employed: blood from the surgical field is collected, washed, and the patient's own red blood cells are re-transfused. However, there is no data on the impact of this practice on short-term IVI supplementation, and a wash-out of high molecular iron compounds could be considered minimizing the usage of such medication.

In order to assess the wash-out effect of IVI by perioperative cell salvage, sensitive and quantitative techniques of speciation analysis are required. In this work, we present the utilization of complementary approaches to determine FCM in the highly complex matrix of serum samples. A method based on size-exclusion chromatography (SEC) was developed, enabling a fast on-line separation of FCM from the Fe-containing blood

protein fraction of holo-transferrin in less than 10 min. In combination with quadrupole-based inductively coupled plasma-mass spectrometry (ICP-MS), quantification with species-specific detection limits in the low ng/mL range was achieved. Furthermore, molecular mass spectrometry was employed to provide detailed structural information about the compounds of interest.

The presented SEC-ICP-MS method has proven to be a valuable tool for the rapid monitoring of IVI in serum samples. It was finally applied in the context of a medical trial that included a total of 23 patients who underwent elective cardiac surgery with perioperative cell salvage. Quantitative results for blood samples that were obtained before, during, and after surgical procedures indicate a wash-out effect of previously administered FCM. Hence, the current strategy of IVI supplementation shortly prior to major surgery might have to be re-evaluated.

(ATOM-06-4) **Single-cell ICP-MS Analysis of Essential Element Distributions in Chlamydomonas Reinhardtii Algae with Nutritional Iron Deficiency**

Matthias Elinkmann[1], Sarah Reuter[2], C. Derrick Quarles[3], Michael Sperling[1], Michael Hippler[2], Uwe Karst[1]; *[1]Institute of Inorganic and Analytical Chemistry, University of Münster, [2]Institute of Plant Biochemistry and Biotechnology, University of Münster, [3]Elemental Scientific, Inc.*

In fifteen words or less, explain the significance of this contribution (Novel Aspect).: First single-cell ICP-MS study of iron deficient algae using novel sample introduction system and software.

Abstract Text: Inductively coupled plasma-mass spectrometry (ICP-MS)-based approaches to analyze nanomaterials and biological cells have experienced a decade of fast development and performance improvement. Through the combination of specialized sample introduction systems and data processing algorithms, the investigation of trace elements in single cells directly from a dilute suspension is becoming increasingly accessible. Recent publications highlighted the capabilities of highly sensitive single-cell ICP-MS methods to study the uptake of different elements after exposure to both metallopharmaceuticals and environmental toxins. In this work, a novel cell introduction system was used to map the endogenous elemental composition in Chlamydomonas reinhardtii green algae that were cultivated under physiological and iron depleted medium conditions. The setup included an on-axis total consumption spray chamber equipped with a microliter low-flow nebulizer. To minimize polyatomic interferences and background contribution from the cell medium matrix, a dilute ammonium acetate solution was used to wash the cells prior to analysis and for matrix-matched calibration. Particular focus was directed to the nebulizer argon gas flow to ensure a mild aerosol generation with minimum cell damage. Further plasma parameters were adjusted for highest signal intensities. In addition, both kinetic energy discrimination with helium collision gas (KED) and triple quadrupole experiments with oxygen as a reaction gas (TQ) were compared to achieve best signal-to-background-ratios. Data analysis was carried out using a software tool written in java that employs an iterative baseline search and a two step event detection including a split cell event correction. Single cell-based distributions for six naturally occurring elements were separated from the background for both medium conditions. Importantly, cell count rates revealed a highly efficient transport that was independent from the respective element. Furthermore, the observed cellular iron content showed significant differences reflecting the culture media composition. To investigate the effect of a potentially growth-limiting iron deficiency on the metabolic status of the algae, magnesium and manganese were quantified. These key trace elements play an important role in the light absorbing pigment chlorophyll and the water-splitting complex of the photosynthetic reaction center. A great advantage of this methodology is that no error-prone cell counting was needed in advance.

21AWD06: AES Mid-Career Award Symposium Honoring Nathan Swami

Chair: Nathan Swami
On-site Chair: Nathan Swami

(AWD-06.1) **Selective capture and analysis of melanoma cells at an array of wireless interdigitated bipolar electrodes**

Robbyn K. Anand[1], Janis Borchers, Morgan Clark, Savanah Van Scoy, Claire Campbell; [1]*Iowa State University*

An array of bipolar electrodes (BPEs) can be controlled by a single pair of driving electrodes yet allows for multiplexed analysis of many single cells at once. To read out the signal from these wireless electrodes, the current flowing through each BPE is reported by an electrochemiluminescent reaction, which obviates the need for fluorescence imaging equipment. However, the sensitivity of BPEs is poor. To address this shortcoming, we recently described signal amplification by redox cycling accomplished by interdigitation of each BPE in an array with a shared driving electrode. Using this approach, the BPEs behaved as independent, equivalent sensors, while the limit of detection was improved by an order of magnitude. The signal increases exponentially as the dimensions of the gaps between the BPEs and the shared driving electrode are decreased, therefore, this approach renders BPEs relevant for a wide range of biosensing applications. Here, we implement these interdigitated BPEs for dielectrophoretic capture of melanoma cells at the wireless BPE array for subsequent electrochemical analysis. To demonstrate this principle, we evaluate the expression of melanoma-associated cell surface antigens. This integration of selective capture of tumor cells with an array of wireless electrochemical sensors allows for rapid and low-cost profiling to obtain diagnostic information relevant to treatment decisions.

(AWD-06.2) **Stem Cell and Cancer Cell Phenotypes Revealed by Biophysical Properties**

Lisa A. Flanagan[1]; [1]*Departments of Neurology, Biomedical Engineering, Anatomy & Neurobiology, University of California Irvine*

Cells are dynamic and can shift phenotype and function in response to external cues. Methods to detect the phenotypes of living cells are limited and often rely on minimal numbers of cell surface markers that are not sufficiently specific. Dielectrophoresis (DEP) is a unique method for the analysis and separation of living cells that does not require labels or markers, but instead measures inherent composite biophysical properties. The biophysical properties detected by DEP are sufficient to identify subtle differences in cell phenotype, leading to new areas of study. Two fields where this approach has significantly contributed to the understanding of biology are stem cells and cancer. Neural stem/progenitor cells differentiate into neurons, astrocytes, and oligodendrocytes, but distinguishing progenitor cells that form each differentiated cell type is challenging. This creates problems for understanding brain development and for stem cell transplants to treat neurological diseases and injuries. We found cells that are similar in size and marker expression but vary in ability to form differentiated cell types can be separated by DEP because they differ in the biophysical property whole cell membrane capacitance. Our data show that membrane capacitance is sensitive to the composition of the plasma membrane, specifically cell surface glycosylation. We identified glycosylation pathways that regulate neural stem/progenitor cell fate, impacting the formation of differentiated cells in culture and in the brain. Recently, we discovered that similar approaches can be used to determine the phenotypes of brain cancer cells. Glioblastoma is the deadliest brain cancer and a significant problem for treatment is the presence of cells resistant to the most commonly used glioblastoma chemotherapeutic. Resistant cells are difficult to identify using markers, making development of novel therapeutics challenging. Our new studies show that the chemotherapeutic resistance of glioblastoma cells is predicted by membrane capacitance. We found resistant cells can be enriched by DEP, paving the way for characterization of these critical cells and identification of effective treatments. In summary, cell biophysical properties provide a novel way to investigate cell identity and discover new determinants of important cellular functions such as differentiation and chemotherapeutic resistance.

(AWD-06.3) **Building The Electrome: Rewriting The Interaction of The Cell's Electric Properties**

Michael Pycraft Hughes[1]; [1]*University of Surrey.*

There are many ways to measure the electrical properties of cells. Classical electrophysiology relates the electrical potential across the membrane to the diffusion of ions due to the relationship between extracellular and intracellular ions. Dielectrophoresis uses the Clausius-Mossotti factor to determine the conductivity and permittivity of the membrane and cytoplasm. Surface scientists can use electrophoresis to measure the zeta potential, caused by the charge on the membrane surface. All three measures are regarded to be discrete and independent. In order to assess this, we conducted an extensive review of the properties of red blood cells, measured using dielectrophoresis, electrophoresis and a proton ionophore method of measuring Vm. Measurements were taken at three medium conductivities (with ions gradually replaced by sugar to maintain osmolarity) and subject to four combinations of drug treatments as well as in a native state. Results were analysed to identify connections between the variables. Analysis of the results suggest a high degree of interconnectedness across all of these measures. Changes in the membrane potential can be detected in the zeta potential by a measure related to the capacitance of the slip plane; whilst the surface potentials of the outer and inner membrane leaflets play significant roles in both the membrane potential and cytoplasm conductivity. We term this combination of all of the electrical parameters – fundamentally, the organisation of electrical charge, both fixed (at the surface) and mobile (ions), and the associated capacitances and potentials as the cellular electrome. The connection between the membrane and zeta potentials is particularly of note, since this suggests that changing the membrane potential can potentially alter the ion concentration outside the membrane, with the effect of mimicking the function of voltage-activated ion channels; It may have further-reaching effects regarding attraction or repulsion of proteins or even cells at short range.

(AWD-06.4) **A Neural-Network-based Microfluidic Approach for Online Single-Cell Dielectric Spectroscopy**

Federica Caselli[1]; [1]*University of Rome Tor Vergata*

Due to their label-free nature, electrical sensing techniques are tremendously attractive for the analysis of particles and cells. Microfabrication technology and microfluidics have enabled single-cell assay technologies, which are widely used for the characterization and phenotypic analysis of mammalian cells, yeast cells, plant cells and bacteria. In particular, multifrequency impedance measurements have been used to determine the complete intrinsic electrical properties of thousands of single cells flowing at high throughput (200 cells/s) in a microfluidic impedance cytometer. Besides high acquisition throughput (i.e., the number of single-cell measurement events per unit time), applications such as active particle sorting or selective enrichment also require high processing throughput (i.e., the number of analysed single-particle signals per unit time). This calls for tailored signal-processing approaches that are capable of working in real time. To tackle this need, we explore the use of neural networks for fast processing of impedance cytometry data streams. The whole processing workflow is considered, from cell-passage detection to cell electrical properties determination, as briefly described in the following. Individual flowing cells interact with the electric field established in the electrical sensing zone by a multifrequency voltage stimulation. This results in a variation of the measured electrical current. Event-signals associated to the passage of a cell are regarded as voice activity in a sound recording. A recurrent neural network based on a bidirectional Long Short-Term Memory (Bi-LSTM) layer is used to achieve real-time cell-passage detection. Each event-signal is composed of multiple complex traces (one for each frequency). The information is reshaped as a time-frequency image with two colours (real part and imaginary part). A Convolutional Neural Network (CNN) with three convolutional layers is trained to predict cell features (i.e., radius, intracellular conductivity and permittivity, membrane capacitance). Preliminary results show an accuracy in cell-passage detection higher than 95% and a normalized root-mean-squared-error lower than 4% for features prediction. Unitary prediction times were in the order of fractions of ms, for both detection and characterization. Overall, these results strongly encourage the use of neural networks towards impedance-based label-free online phenotyping and sorting.

(AWD-06.5) **Molecular and cellular analysis in nanoconfined geometries**

Chia-Fu Chou[1], Chia-Fu Chou[1]; [1]*Institute of Physics, Academia Sinica*

Simple geometric nanostructures, in the forms of nanoslits, nanochannels, nanoconstrictions, and plasmonic nanogaps, offer unique platforms for the study of molecular and cellular characteristics, with the potential for bioanalytical applications. In this talk, we will review various nanostructure-enabled platforms developed in house for the manipulation and analysis of DNA, proteins, and bacteria.

21CHEM04: Chemometrics for Food and Drug Analysis

Chair: Mengliang Zhang
On-site Chair: Mengliang Zhang

(CHEM-04.1) **Metabolite Ratio Rule-Based Method for Automated Metabolite Profiling and Species Differentiation of Cinnamon Samples**

Mengliang Zhang[1], Yifei Wang, Roderick Moore[1], Pei Chen[2]; [1]*Middle Tennessee State University,* [2]*Food Composition and Methods Development Laboratory, BHNRC, ARS, USDA*

Cinnamon is a spice that has been commonly used as ingredients in food and herbal medicines. Recent studies have reported various health promoting activities of cinnamon owing to the signature phytochemical contents in cinnamon such as coumarin, cinnamaldehyde, and proanthocyanidins (PACs). However the phytochemical compositions in cinnamon were found to be species dependent, therefore it is important to authenticate the cinnamon species before the evaluation of their health benefit potentials and use in food and dietary supplements. In this study, a metabolite ratio rule-based classification method was developed to classify the cinnamon species. The computer program combines the high resolution mass spectrometric data processing for tentative identification of major metabolites such as PACs coumarin, and cinnamaldehyde in cinnamon extracts and the stepwise classification strategy, which allows the high throughput analysis of highly dimensional and complex liquid chromatography high resolution mass spectrometric data. The proposed method was also compared with a metabolomic approach with Partial Least-Squares Discriminant Analysis models and has shown a more accurate and reliable classification of cinnamon species from both cross-validation evaluations and the prediction of new cinnamon samples. This study offers a valuable tool for the researchers to perform cinnamon sample authentication and differentiation and the cinnamon phytochemicals investigation under an automated fashion, which is critical to understand and evaluate the health beneficial effects of cinnamon materials.

(CHEM-04.2) **Machine Learning Enabled Nondestructive Paper Chromogenic Array Detection of Multiplexed Viable Pathogens on Food**

Boce Zhang[1]; [1]*University of Massachusetts Lowell*

Fast and simultaneous identification of multiple viable pathogens on food products is critical to public health. Low-cost colorimetric arrays using volatile organic compounds (VOCs) as biomarkers have recently received significant attention as a nondestructive pathogen detection platform. However, technology development using such a strategy has only achieved limited success due to several major technical barriers. First, the use of conventional multivariate data analyses (e.g. principal component analysis) to extract information from colorimetric data requires prior attribution and correlation of pre-selected chromogenic responses with specific bacteria. Second, background VOC signals emitted by food matrices and other non-target microorganisms confound the test results and render the technology unsuitable for real-world food products. Taking advantage of automated feature extraction and noise suppression afforded by machine learning, we have developed a VOC-based nondestructive multiplex detection system for foodborne pathogens using a paper chromogenic array (PCA). The system exhibits distinguishable color changes and pattern shifts in response to pathogen VOCs. These color changes are further digitized and used to train a neural network (NN), which does not depend on predetermined features to attribute digitized color datasets. An advanced NN with a learning rate

schedule, L2 regularization, and shortcut connections was also developed to provide exceptional performance in samples with high background microflora and signal/data complexity. The trained PCA-NN system can accurately identify single pathogens or multiple pathogens in the presence of background microflora on food, such as Romaine lettuce, cantaloupe, cod, and salmon. This approach has the potential to advance nondestructive pathogen detection and identification on food without enrichment, culturing, incubation, or another sample preparation.

(CHEM-04.3) **Detecting Seafood Decomposition with Portable Devices and Chemometric Modeling**

Betsy Jean Yakes[1], Zachary Ellsworth[2], Sanjeewa Karunathilaka[2], Eric Crump[1]; *[1]U.S. Food and Drug Administration, Center for Food Safety and Applied Nutrition, 5001 Campus Drive, College Park, MD 20740, [2]U.S. Food and Drug Administration*

To support National Seafood Sensory Expert (NSSE) organoleptic screening of seafood decomposition, portable instrumentation is being researched for field use. Previous studies employing vibrational spectroscopy, bioelectrical impedance analysis, and electronic nose instruments (e-nose) have shown potential success of these analytical techniques in evaluating freshness of seafood. However, the smaller sample sizes and limited sample diversity combined with not having the fish graded by sensory experts could limit the greater validity of these techniques. To build on these works, two parallel investigations were performed in our lab using NSSE graded, naturally diverse sample sets to determine the broader robustness of each device. Our first study focused on direct analysis (i.e., no sample preparation) and four devices (two micro-miniaturized near-infrared (NIR) devices, one portable Raman spectrometer, and one bioelectrical impedance analysis (BIA) device) for analysis of two imported fish species (mahi-mahi and red snapper) that are commonly suspected of decomposition. Results indicated that while the techniques initially appeared promising, when more diversity was added into the models (e.g., natural variation (protein, water, fat), fillet thickness (8 to 30 mm)) the instruments lost the ability to consistently identify fresh from spoiled fish. In our second study, a portable e-nose combined with chemometric modelling was used to evaluate NSSE graded mahi-mahi, croaker, red snapper, and weakfish. This device required sample preparation: a 10 g fillet portion was heated in a sealed jar for approx. 40 min at 30 °C to allow trapping of volatiles in the headspace. By analyzing these gases and using eight informative metal oxide sensors with support vector machine (SVM) models, correct classification rates in a calibration-independent test set of samples were 93-100% for sensory pass versus sensory fail samples. With further development (e.g., preparation/sampling improvement, additional species evaluation), the e-nose measurements coupled with SVM models may be capable of predicting the spoilage of seafood in field applications.

(CHEM-04.4) **Investigation of Material-Sparing Calibration Approaches for Monitoring Pharmaceutical Powder Blends in a Feed Frame**

Adam J. Rish[1], Samuel Henson[1], Md. Anik Alam[2], Yang Liu[3], James K. Drennen[4], Carl A. Anderson[1]; *[1]Duquesne University, [2]Pfizer, [3]Duke University, [4]Duquesne University Graduate School of Pharmaceutical Sciences, Duquesne Center for Pharmaceutical Technology*

In fifteen words or less, explain the significance of this contribution (Novel Aspect).: Use of pure component spectral approaches for modeling drug content in pharmaceutical powder blends

Abstract Text: The application of near-infrared spectroscopy (NIR) for monitoring pharmaceutical powder blend uniformity and content has gained widespread acceptance in the pharmaceutical industry. However, proper implementation of NIR traditionally requires material and time intensive calibrations to capture the breadth of expected variation. The burden is especially emphasized when formulation adjustments are made during development of new pharmaceutical products, requiring new calibration. This has promoted research into the development of alternative NIR methods that require a minimal quantity of calibration samples. Utilization of modeling approaches that rely on pure component spectra offer advantages over other approaches.

There is a reduced burden in the collection of pure component spectra calibration compared to traditional calibration methods. By generating a library of pure component spectra, new calibrations can be implemented efficiently from the pre-existing data before product production begins (prospective calibration). To demonstrate the development of a pure component spectra NIR method, a pre-existing component library combined with appropriate modeling was employed to generate a prospective calibration for monitoring drug content in a pharmaceutical powder blend on a direct compression feed frame. The primary modeling approaches considered were classical least squares (CLS) based models and iterative optimization technology (IOT) algorithms. Both approaches can be extended to account for non-chemical interferences and variations. Altogether, five separate modeling approaches were considered and contrasted with a partial least squares (PLS) model developed using a D-optimal calibration design. The pure component spectra library was generated by scanning static powder beds of separate formulation components with a SentroPAT reflectance NIR probe from 1100 nm to 2100 nm. The modelling approaches were compared by testing with NIR data generated on feed frames at different manufacturing sites. The CLS models and IOT algorithms showed comparable performance to the PLS models.

(CHEM-04.5) **Development of an Approach to Efficient Detection of Insect-infested Flour using DART-HRMS and Efficient Data Reduction-Multivariate Curve Resolution (EDR-MCR)**

Samira Beyramysoltan[1], Amy M. Osborne[2], Rabi Ann A. Musah[2]; *[1]Department of Chemistry,Universityat Albany, State University of New York, Albany, New York12222, United States, [2]Department of Chemistry, Universityat Albany, State University of New York, Albany, New York12222, United States*

In fifteen words or less, explain the significance of this contribution (Novel Aspect).: Development of a novel approach combining DART-HRMS and EDR-MCR to efficiently detect flour infestation

Abstract Text: Infestation of agricultural products such as wheat flour, remains a major challenge to food security. Pests not only cause billions of dollars in food losses, but also transmit harmful micro-organisms such as fungi and bacteria that affect human health. The U.S. Food and Drug Administration (FDA) has established flour infestation limits for the milling industry. The conventional methods for infestation testing are time-consuming, expensive and labor intensive. Here, the capabilities of direct analysis in real time – high-resolution mass spectrometry (DART-HRMS) and efficient data reduction-multivariate curve resolution (EDR-MCR) were exploited to develop a method for rapid identification of infestation and its markers in wheat flour. Species-specific compounds can be used to determine insect species, and their levels can be correlated to the insect population present. Flour samples were deliberately infested with the red flour beetle (T. castaneum) over a period of 6 weeks. Samples were prepared in replicates of 5 using 5 batches of flour with different lot numbers. In each case, 25 insects were added to 50 g of flour, and the samples were incubated at 30 oC. Control replicates comprised of un-infested flour were incubated at the same temperature and over the same time period. Chemical profiles of infested and un-infested flour were collected using DART-HRMS in less than minute with no sample pretreatment required. Multi-block (multiset) analysis can reveal the common and distinct information present in the data blocks (i.e. derived from analysis of infested versus un-infested flour samples) which are manifested over time. EDR-MCR, a recently devised approach for processing of chemical data, was applied for multi-block discrimination of infested and control flour in order to reveal chemical markers of infestation. The predictions were then compared with the output of multi-block partial least square discriminant analysis. The results for both were comparable and revealed masses that enabled the differentiation of infested and non-infested flour, but EDR-MCR conferred the advantage of more rapidly and efficiently revealing discriminating m/z values and resolving their importance weights. One of these masses was m/z 137, which GC-MS confirmed to be 2-ethyl-1,4-benzoquinone, a molecule known to be produced by red flour beetles.

21IR08: Advances in Gas Sensing

Chair: Mike George
On-site Chair: Robert Lascola

(IR-08.1) **Time-resolved FTIR Gas-phase Analysis of Photolysis Using a Long Path Cell: UV Photolysis of Methyl Iodide Gas in Air**

Kendall Hughey[1], Russ Tonkyn[1], Valerie Young[2], Tanya L. Myers[1], Warren Harper[1], Timothy J. Johnson[1];
[1]Pacific Northwest National Laboratory, [2]The Ohio University

In fifteen words or less, explain the significance of this contribution (Novel Aspect).: The paper reports first identification of CHI several photolysis products using gas-phase FTIR

Abstract Text: Halogen-bearing compounds are known to photolyze under UV radiation; analysis of the various decay products is of interest for environmental considerations as to their fate and transport. Time-resolved Fourier transform infrared spectroscopy was used in the laboratory to study the 260 nm-induced photolysis of methyl iodide. Photolysis products iodine (I2), methanol (CH3OH) and formaldehyde (HCHO) have been clearly identified, the last two in a time-resolved manner. Preliminary kinetic models predict the formation of both CH3OH and HCHO. In this paper report results as to time scales as well as both the carbon and iodine reaction pathways.

(IR-08.2) **Methane isotope analysis: land, sea, and air**

Jason M. Kriesel[1], Andrew Fahrland[2]; *[1]Opto-Knowledge Systems, Inc. (OKSI), [2]Opto-Knowledge Systems, Inc.*

In fifteen words or less, explain the significance of this contribution (Novel Aspect).: Demonstration of novel field isotope analyzer enabling better characterization of sources and sinks of methane.

Abstract Text: Better understanding the sources and sinks of methane has economic, biological, and environmental applications. Stable isotope analysis of methane, e.g., the ratio of 13CH4 / 12CH4, provides additional information that can be used to attribute sources and better understand biogeochemical processes. Field measurements are vitally important in these studies; however, isotope analysis is typically performed by large-scale instruments in a laboratory setting. We describe recent field work on land, sea, and air in which methane isotope ratios were measured with an innovative spectroscopy sensing platform that utilizes mid infrared laser absorption spectroscopy in a capillary absorption spectrometer (CAS). The CAS uses a hollow fiber optic waveguide with a reflective inner coating and a small internal volume on the order of 10 ml. The hollow fiber both guides the laser light from source to detector and contains the gas sample at reduced pressure. Near unity overlap between the laser beam and sample enables sensitive analysis with ultra-small sample size. Land: A methane-isotope CAS system was utilized for field analysis in a remote wetland region. A custom front-end system was developed to enable both injection of gas samples, as well as direct injection of discrete water samples. The resulting data is being used to study processes in the terrestrial-aquatic interface, which is an important, yet poorly characterized global driver of greenhouse gases. Sea: A modified version of the custom water sampler was developed and packaged together with a CAS in a pressure housing capable of depths down to 3000 m under the surface of the ocean. The system is being used to study seeps and vents in the ocean floor. These studies have both environmental and energy related applications. Air: One version of the methane-isotope CAS was developed for drone-borne operation. The system size is < 0.05 m3, weight < 7 kG, and power < 40 W. The system has been deployed on a remote-controlled quad copter. In addition, a related version of a drone-borne CAS was used to measure CO/CO2 ratios over prescribed burns in the Sierra-Nevada mountains.

(IR-08.3) **High-resolution spectroscopy of gases using a quantum cascade laser dual-comb spectrometer**

Markus Mangold[1], Pitt Allmendinger[1], Florian Eigenmann[1], Jakob Hayden[1], Andreas Hugi[1]; [1]*IRsweep AG*

In fifteen words or less, explain the significance of this contribution (Novel Aspect).: We present techniques to overcome the sparse-sampling limitation of QCL combs for gas measurements

Abstract Text: Optical frequency comb spectroscopy has proven a very useful tool for high resolution molecular spectroscopy of gaseous samples. Frequency combs based on quantum cascade lasers (QCL) offer the possibility to easily explore the mid-infrared spectral range (4-12 μm), but suffer from very large repetition frequencies (~10 GHz) which make them seemingly unsuitable for high resolution gas measurements. Here, we present techniques to overcome this limitation, which we call the rapid-sweep and step-sweep technologies. In rapid-sweep, full spectra can be measured in only 6ms, but the wavelength information has to be inferred from the measurement. The step-sweep technique guarantees the accurate knowledge of the frequency axis in a record time of several minutes. We have employed both techniques to measure absorption spectra of gases with narrow absorption lines with high resolution and sensitivity. The measured spectra cover a range of more than 60 cm-1 and the narrowest observed lines have a full width at half maximum of 15 MHz (0.0005 cm-1). The broadband coverage is useful for the analysis of complex gas mixtures. The low measurement noise makes the technique the ideal tool for studying gas concentration in environmental or industrial applications.

(IR-08.4) **Photothermal Spectroscopy for Sensitive Gas and Liquid Sensing**

Benedikt Schwarz[1]; [1]*TU Wien*

(IR-08.5) **Remote Raman Sensing Using Modified Monolithic Spatial Heterodyne Raman Spectroscopy - Candidate For Planetary Exploration**

Evan M. Kelly[1], Shiv K. Sharma[2], Stanley M. Angel[3]; [1]*University of Hawaii at Manoa, Hawaii Institute of Geophysics and Planetology,* [2]*Hawaii,* [3]*The University of South Carolina, Department of Chemistry and Biochemistry*

In fifteen words or less, explain the significance of this contribution (Novel Aspect).: Evaluates a compact, robust, and high-resolution Raman spectrometer as a candidate for Planetary Exploration

Abstract Text: The SHRS is a modification of a Michelson interferometer, which uses a grating in place of the mirrors to provide high spectral resolution as compared to traditional single slit spectrometers. A new free-space variation recently created (2017) called the modified SHRS (mSHRS) replaces one grating with a mirror increasing the number of photons reaching the detector and spectral bandwidth, but with a reduction in the spectral resolution as compared to the two grating SHRS. We investigated an iteration of the mSHRS, which compacts the beam-splitter, grating, and mirror into a single, compact monolithic device, reducing the number of adjustable components, allowing for easier alignment. The size of the monolith is 25x15x15 mm3, much smaller than the smallest grating–based Raman spectrometers. To test this device, remote Raman spectra at 3m were collected using a 532-nm pulsed laser to illuminate liquid/solid samples with a telescope to collect the scattered light and direct it into the monolith, where the light is recombined and sent through a collecting lens, to an intensity charged-coupled device (ICCD) camera. The data collected with mmSHRS on inorganic, organic, and mineral samples were processed using a Fast Fourier transform. The use of a monolith greatly reduced the installation and alignment time and gave the instrument a large bandwidth coverage by slightly tilting the grating. The use of two bandpass filters (550/49 and 625/50, central wavelength/bandwidth both in nm) allowed the Raman spectra consisting of ranges 0-1500 cm-1 and 1500-3500 cm-1 regions to be collected without antialiasing. The results of Raman measurements with the modified mSHRS will be presented and discussed.

21LIBS07: LIBS a versatile analytical tool

On-site Chair: Matthieu Baudelet

(LIBS-07.1) **Microwave-Enhanced Laser-Induced Breakdown Spectroscopy Using a Low-Power High-Repetition Rate Laser**

Kelsey L. Williams[1], Steven J. Ray[1]; [1]*The State University of New York at Buffalo*

In fifteen words or less, explain the significance of this contribution (Novel Aspect).: A novel microstrip resonator to enhance LIBS signal using a low-energy high-repeition rate laser

Abstract Text: Laser-induced breakdown spectroscopy (LIBS) is an attractive technique due to its simple experiment set-up, lack of sample preparation, and stand-off capabilities. One remaining drawback of some LIBS experiments is the relatively low duty cycle of the laser sources used, which decreases sample throughput, lengthens imaging analysis times, and compromises signal averaging. Low-power, high-repetition rate lasers have been previously used in LIBS experiments and have been shown to increase analysis duty cycle and speed of imaging applications. However, the high-repetition rate of the laser comes with an associated cost. Since the laser power increases directly with the pulse repetition rate, high-repetition rate lasers must either be of much greater power (and cost) as compared to conventional systems, or provide much lower pulse energies for each LIBS experiment. When high-repetition rate, low-power lasers are used, atomic emission yield from each individual LIBS event decreases as a consequence. Our laboratory has recently investigated the use of microwave enhancement in order to improve atomic emission observed from LIBS experiments using high-repetition rate, low-power lasers. Here, a novel microwave resonator is used to couple microwave power into the LIBS plasma, providing a secondary source of energy for excitation. Here, we examine the use of a high-repetition rate laser (>100 kHz) coupled with a novel microstrip resonator to simultaneously improve the LIBS duty cycle and emission signal intensity. Analytical performance, design specifications, and a prognosis for LIBS imaging analysis will be presented.

(LIBS-07.2) **Quantitative fluoride imaging of teeth using CaF emission by laser induced breakdown spectroscopy.**

Mauro Martinez[1], Christine Austin[1], Manish Arora[1]; [1]*Icahn School of Medicine at Mount Sinai*

In fifteen words or less, explain the significance of this contribution (Novel Aspect).: Fluoride analysis present a high impact on epidemiology studies and LIBS could bring new answers

Abstract Text: Fluoride exposure has been associated with neuro and renal toxicity. An important aspect to studying the health effects associated with fluoride exposure is capturing the timing of exposure. Teeth are an excellent matrix to study fluoride exposure as they grow incrementally and fluoride has a high affinity with calcium. However, longitudinal measures of fluoride in teeth, is challenging due to high detection limits for most imaging methods. In this work is propose a new method to quantify fluoride in teeth using CaF molecular emission by laser induced breakdown spectroscopy (LIBS). The major mineral component of teeth and bones is hydroxyapatite, Ca5(PO4)3OH, which is substituted with fluoride, carbonate and other ions. When analyzed by a basic LIBS setup with optimized plasma conditions, the high Ca content reacts to form the CaF molecule with a signature emission around 530 (orange) and 600 nm (green). Furthermore, to obtain a quantitative image of fluoride in teeth, a series of matrix-matched reference materials is required. As no such standard series is available commercially, we applied a new method to generate matrix-match standards of hydroxyapatite with known concentrations of fluoride. This new material reproduce the chemical composition of teeth and similar optical conditions under laser ablation process, enabling the optimization of LIBS acquisition parameters for high sensitivity and linearity at fluoride concentrations in teeth between 0 and 750 µg·g-1. To validate this approach, we measured fluoride in teeth from rats exposed to fluoride at varying levels and a control group. We then compared results to that obtained by ion selective electrode analysis. This method can be used to

reconstruct a history of early life fluoride exposure through quantitative mapping of fluoride in human teeth.

(LIBS-07.3) **Combined LIBS and Raman spectroscopy with advanced data analysis for investigation of forensic samples**

Lutz T. Pfeifer[1], Virginia Merk[1], Sven Merk[1], Saskia Damaske[1], Leonhard Lenz[1], Wolfgang Werncke[1]; *[1]LTB Lasertechnik Berlin GmbH*

In fifteen words or less, explain the significance of this contribution (Novel Aspect).: Combined LIBS-Raman-spectroscopy with microscopic spatial resolution and advanced data analysis of forensic samples

Abstract Text: The combination of laser-induced breakdown spectroscopy (LIBS) with Raman spectroscopy allows to gain information on the elemental as well as the molecular composition of a sample. Here we will show with two examples, namely the analysis of glass and automotive paint samples, the benefits of the combination of both techniques with advanced data analysis for forensic investigations. The measurements were subsequentially performed at the same microscopic sample spot by using a two-wing Echelle spectrometer and joined excitation and observation optics for both techniques. This allows us to complementary obtain elemental and molecular information and analyze them with high level data fusion. Very good discrimination (up to 99 %) of sixteen different glass samples was achieved with conjoint LIBS and Raman and high-level data fusion. The discrimination is based primarily on different contents of trace elements detected by LIBS and differences in photoluminescence detected by Raman. The results were compared to results obtained with the single methods (LIBS and Raman). This showed that LIBS and conjoint LIBS and Raman have similar discrimination power, but the combination and fusion of LIBS and Raman leads to greater distances between the data in the principal component space due to the additional information added by Raman. This should result in a lower misclassification rate of unknown samples. Automotive paint samples consist of different layers with varying composition. Layer and sublayer-specific chemical information with high spatial resolution were obtained by measuring cross sections as well as subsequent LIBS and Raman measurements from top (drill-down). The results of both methods were compared and will be presented here. The elemental profiles together with the characteristic Raman bands turned out to improve the distinguishability of visually very similar samples. [1] Merk et al, Discrimination of automotive glass by conjoint Raman and laser-induced breakdown spectroscopy and multivariate data analysis, Spectrochim Acta B, 180 (2021), 106198

(LIBS-07.4) **A Comparison of Handheld Field Chemical Sensors for Soil Characterization with a Focus on Laser Induced Breakdown Spectroscopy**

Jay L. Clausen[1], Richard R. Hark[2], Russell S. Harmon[3], John R. Plumer[4], Sam A. Beal[1]; *[1]USACE ERDC-CRREL, [2]Yale University, Institute for the Preservation of Cultural Heritage, [3]Department of Marine, Earth and Atmospheric Sciences, North Carolina State University, [4]JR Plumer Assoc. LLC*

In fifteen words or less, explain the significance of this contribution (Novel Aspect).: Performance comparison of LIBS for quantifying elemental soil concentrations with standard accepted techniques.

Abstract Text: Commercially available handheld chemical analyzers for forensic applications have been available for over a decade. Portable systems from multiple vendors can perform X-ray fluorescence (XRF) spectroscopy, Raman spectroscopy, Fourier transform infrared (FTIR) spectroscopy, and recently laser-induced breakdown spectroscopy (LIBS). Together, we have been exploring the development and potential applications of a multisensor system consisting of XRF, Raman, and LIBS for environmental characterization with a focus on soils from military ranges. Handheld sensors offer the potential to substantially increase sample throughput through the elimination of transport of samples back to the laboratory and labor-intensive sample preparation

procedures. Further, these technologies have the capability for extremely rapid analysis, e.g. analysis time on the order of tens of seconds or less. We have compared and evaluated results from the analysis of several hundred soil samples using conventional laboratory bench top inductively coupled plasma atomic emission spectroscopy (ICP-AES) for metals evaluation and high-performance liquid chromatography (HPLC) and Raman spectroscopy for detection and characterization of energetic materials against handheld XRF, LIBS, and Raman analyzers. The soil samples contained antimony, copper, lead, tungsten, and zinc as well as energetic compounds such as 2,4,6-trinitrotoluene (TNT), hexahydro-1,3,5-triazine (RDX), nitroglycerine (NG), and dinitrotoluene isomers (DNT). Precision, accuracy, and sensitivity of the handheld field sensor technologies were compared against conventional laboratory instrumentation to determine their suitability for field characterization leading to decisional outcomes.

(LIBS-07.5) **Monitoring biomarkers and nanoparticle labels in soft tissues using laser-induced breakdown spectroscopy**

Pavel Pořízka[1], Pavlina Modlitbova[1], Karel Novotny[2], Zdenek Farka[3], Petr Skladal[3], Jozef Kaiser[1]; *[1]CEITEC Brno University of Technology, [2]Masaryk University, [3]CEITEC Masaryk University*

In fifteen words or less, explain the significance of this contribution (Novel Aspect).: Monitoring biomarkers within soft tissues through nanoparticle labels and multi-elemental laser-induced breakdown spectroscopy

Abstract Text: Immunohistochemistry (IHC) and immunocytochemistry (ICC) are widely used to identify cancerous cells within tissues and cell cultures. Even though the optical microscopy evaluation is considered as the gold standard, the limited range of useful labels and narrow multiplexing capabilities create an imminent need for alternative readout techniques. Laser-Induced Breakdown Spectroscopy (LIBS) enables a large-scale multi-elemental analysis of the surface of biological samples (e.g., thin section or cell pellet) and is, therefore, a potential alternative for IHC and ICC readout of various labels or tags (Tag-LIBS approach). Here, we introduce Tag-LIBS as a method for a specific determination of HER2 biomarker. The cell pellets were labeled with streptavidin-conjugated upconversion nanoparticles (UCNP) through a primary anti-HER2 antibody and a biotinylated secondary antibody. The LIBS scanning enabled to detect the characteristic elemental signature of yttrium as a principal constituent of UCNPs, thus indirectly provided a reliable way to differentiate between HER2-positive BT-474 cells and HER2-negative MDA-MB-231 cells. The comparison of results with upconversion optical microscopy and luminescence intensity scanning confirmed that LIBS is a promising alternative for the IHC and ICC readout.

21PAT04: Advances in On-Line Process Analysis

Chair: Alison Nordon
On-site Chair: Mark Rickard

(PAT-04.1) **Thermal Infrared Hyperspectral Imaging for Gas Detection, Identification, and Quantification**

Benjamin Saute[1], Mathieu Gagnon[1], Jacob Thibodeau[1], Marc Duval[1], Jean-Philippe Gagnon[1], Martin Larivière-Bastien[1]; *[1]Telops*

Detection, identification, and quantification of gas emissions is essential to ensure compliance with regulatory guidelines and mitigate damage associated with anthropogenic climate change. Passive infrared hyperspectral imaging technology is among the solutions that can detect, identify and quantify multiple gases simultaneously. The Telops Hyper-Cam is an established system for aerial and ground-based thermal infrared hyperspectral measurements for gas survey applications. In support of the Hyper-Cam, Telops has developed a suite of

hyperspectral imaging data processing algorithms that allow for gas detection, identification, and quantification analysis in real-time. This work presents the principles underlying gas detection, identification, and quantification from hyperspectral imaging data. Quantitative results from recent measurement campaigns are also presented to demonstrate the utility of hyperspectral imaging for gas emissions analysis in diverse applications and environments.

(PAT-04.3) **Improving NIR moisture analysis through a novel synchronized, automatic calibration data collector**

Elena Hagemann[1], Frank Koch[1], Scott Segro[1], Adam J. Hopkins[1]; [1]*Metrohm USA*

NIR spectroscopy can be implemented for real-time moisture analysis, but due to sample handling challenges during method development, the limits of detection are typically around 500 ppm. To reliably predict levels of moisture below 100ppm, users need to calibrate to around 30 ppm or less. This is virtually impossible with traditional manual sample transfer and analysis practices. We have developed an automated solution for creating robust models for moisture analysis that extends the range of NIR moisture analysis well below the commonly used limits of detection. This presentation describes an automatic calibration data collector that combines a coulometric Karl Fischer titrator with a Process NIR system. This system eliminates the challenges associated with manual sample handling and ensures that the NIR data is provided with high-accuracy primary data for model development. We demonstrate the capability of this calibration system on an example system of water in propylene oxide, with reliable calibration below 30 ppm and a target moisture level of 80 ppm.

(PAT-04.4) **Spectroscopic Quantification of Target Species in a Complex Mixture using Blind Source Separation and Partial Least-Squares Regression: A Case Study on Hanford Waste**

Stefani Kocevska[1], Giovanni M. Maggioni[1], Ronald W. Rousseau[1], Martha A. Grover[1]; [1]*Georgia Institute of Technology*

In fifteen words or less, explain the significance of this contribution (Novel Aspect).: This work combines blind source separation with PLS for spectroscopic analysis of nuclear waste simulants.

Abstract Text: One of the challenges associated with multicomponent mixture analysis using chemometrics models, such as partial least-squares regression (PLSR), is collecting calibration data. Depending upon the number of constituents, the size of the calibration set can be quite large. For example, nuclear waste at the Hanford site contains a large number of radioactive and non-radioactive species, which complicates remediation efforts, but only the concentrations of a few target species must be quantified in real-time to facilitate operation of the clean-up process. In this work, we introduce a preprocessing procedure that reduces the need for extensive model calibration. The preprocessing framework uses blind source separation (BSS) to identify the independent components in the mixture, which is followed by a correlation to classify them as either target species (part of the critical quality attributes that need to be measured during waste processing) or non-target species. The classification is used to preprocess the original mixture data: the signals of the target components are retained, while those of the non-target components are removed. Since the preprocessed spectra only contain the target components, the spectra-to-concentration regression model can be trained with a smaller calibration set. The approach is tested for Raman and infrared spectroscopy using simulated and experimental data sets of nuclear waste. Combining the BSS preprocessing with a partial least-squares regression (PLSR) model resulted in greater accuracy in concentration predictions for both spectroscopic techniques, in simulations and in experiments. One of the main advantages of using BSS to preprocess the data is removing the dependence of the PLSR model on the non-target species. Therefore, even if the number and concentrations of non-target species fluctuates throughout the process, the training data set for the PLSR does not need to be updated. While the BSS-PLSR framework was tested on Raman and IR spectra, it can theoretically be applied to any other spectroscopic technique.

(PAT-04.5) **Hey, Keep the Noise Down! The Importance of Photon Statistics in Process Raman Measurements**

Bradford Behr[1]; [1]*Tornado Spectral Systems*

In fifteen words or less, explain the significance of this contribution (Novel Aspect).: We present an accessible framework for Raman PAT users to achieve optimum sensitivity and precision.

Abstract Text: Signal-to-Noise Ratio is a fundamental concept in measurement science, but its relevance for quantitative analytical methods like Raman spectroscopy is not always well understood. We present a framework for translating photon shot noise and other instrumental noise contributions into application-specific metrics like limit of detection, relative standard deviation, and quantitative resolution. Using several different case studies, we demonstrate how superior photon management leads directly to better chemometric precision and greater confidence in process Raman measurements.

21PMA06: Small Molecule and Metabolic Screening

Chair: Katherine Hollywood
Co-Chair: Roy Goodacre
On-site Chair: Nicolas Morato

(PMA-06.1) **Triboelectric Nanogenerator Ion Mobility-Mass Spectrometry for In-Depth Lipid Annotation**

Facundo Fernandez[1], Marcos Bouza[2], Yafeng Li[2], Zhong Lin Wang[3]; [1]*Georgia Institute of Technology,* [2]*School of Chemistry and Biochemistry. Georgia Institute of Technology.,* [3]*Material Science and Engineering. Georgia Institute of Technology.*

Lipids play a critical role in cell membrane integrity, signaling and energy storage. However, in-depth structural characterization of lipids is still challenging, and not routinely possible in lipidomics experiments. Techniques such as collision induced dissociation (CID) tandem mass spectrometry (MS/MS), ion mobility (IM) spectrometry, and ultrahigh performance liquid chromatography are not yet capable of fully characterizing double bond and sn-chain position of lipids in a high throughput fashion. Herein, we report on the ability to structurally characterize lipids by using large-area triboelectric nanogenerators (TENG) coupled with time-aligned parallel (TAP) fragmentation IM-MS analysis. Gas phase lipid epoxidation during TENG ionization, coupled to mobility-resolved MS3 via TAP IM-MS, enabled the acquisition of detailed information on the presence and position of lipid C=C double bonds, the fatty acyl sn-chain position and composition, and the cis/trans geometrical C=C isomerism. The proposed methodology proved useful for the shotgun lipidomics analysis of lipid extracts from biological samples, enabling the detailed annotation of numerous lipid isobars.

(PMA-06.2) **Metabo-endotyping as a route to precision medicine**

Rachel S. Kelly[1], Kevin Mendez[2], Mengna Huang[2], Clary Clish[3], Robert Gerszten[4], Craig Wheelock[5], Juan Celedon[6], Nicole Prince[2], Scott Weiss[2], Jessica Lasky-Su[2]; [1]*Channing Division of Network Medicine, Brigham and Womens Hospital, Harvard,* [2]*Channing Division of Network Medicine,* [3]*Broad Institute,* [4]*Beth Israel Deaconess Medical Center,* [5]*Karolinska Institute,* [6]*Children's Hospital of Pittsburgh*

A majority of complex chronic diseases are heterogenous in etiology, pathology and phenotypic manifestation. This heterogeneity is rarely taken into account in clinical care leading to suboptimal management in certain subgroups. Where subtyping is applied, it tends to rely on grouping patients based on their clinical characteristics which does not always translate into improved care. More detailed molecular classification of individuals with a given disease could instead tailor therapeutic strategies towards the underlying mechanisms of that individual's disease. Given its unique position on the central biological dogma, as the "ome" closest to phenotype reflecting genetics, environmental factors and their interactions, metabolomics represents a novel and

compelling approach to accurately identify endotypes, i.e., subtypes defined by their functional or pathobiological mechanisms. Through the integration of untargeted metabolomic profiling and machine-learning approaches we have demonstrated that we can derive and validate metabolomic driven endotypes, or "metabo-endotypes", of common complex disorders including asthma. In a cohort of children with asthma we identified five metabo-endotypes with differing asthma-relevant clinical characteristics including lung function. We were then able to replicate these differences when we recapitulated the five metabo-endotypes in an independent asthma cohort. Interrogation of the metabolomic drivers of the metabo-endotypes, revealed an enrichment of metabolites involved in the regulation of pulmonary surfactant homeostasis; a critical mechanism for lung function. Furthermore, we were able to demonstrate that individuals in the most severe metabo-endotype were more likely to carry variants in key pulmonary surfactant regulation genes, providing support for our metabolomic results and helping us to hone in on the specific pathways of interest. These findings, and our work in other complex disorders including Autism Spectrum Disorders, demonstrate that clinically meaningful endotypes can be derived and validated using metabolomic data. Crucially, they also show that interrogating the drivers of these metabo-endotypes can help us understand their pathophysiology and identify therapeutic targets specific to each metabo-endotype. In this way metabo-endotypes can pave the way for more personalized approaches to the management and treatment of complex disease and a new era of precision medicine.

(PMA-06.3) **Machine Learning and Chemical Imaging to Elucidate Enzyme Immobilization for Biocatalysis**

Nicole Ralbovsky[1], Joseph P. Smith[1]; [1]*Merck*

In fifteen words or less, explain the significance of this contribution (Novel Aspect).: Applying multivariate analysis for understanding and optimizing an enzyme immobilization process

Abstract Text: Biocatalysis has rapidly become an essential tool in the scientific and industrial communities for development of efficient, safe, and sustainable chemical syntheses. Immobilization of the biocatalyst, typically an engineered enzyme, offers significant advantages, including increased enzyme stability, resistance to environmental change, control of the enzyme, and enhanced reusability. Determination and optimization of the spatial and chemical distribution of enzymes immobilized to inert resins is critical for proper functionality; however, analytical methods currently employed for doing so are inadequate. Machine learning, in the form of multivariate curve resolution, applied to Raman hyperspectral imaging is presented herein as a potential method for investigating the spatial and chemical distribution of evolved pantothenate kinase immobilized onto diverse, microporous resins. An exhaustive analysis indicates this method is able to successfully resolve, both spatially and spectrally, the chemical species involved in enzyme immobilization, including the enzyme, both resins, and other key components. Optimal analytical parameters for analyzing these data sets were determined through evaluation of two different excitation wavelengths and quantitative estimation of spatial coverage of enzyme immobilization, a key parameter used for process development. Lastly, an exploratory chemometric approach, principal component analysis, was also utilized to investigate the chemical species within the data sets and their relationships. The totality of this information can be utilized for understanding and optimizing the enzyme immobilization process and will allow for further implementation of these biocatalytic reactions to improve pharmaceutical product development.

(PMA-06.4) **High-Throughput Label-Free Enzymatic Assays Using Automated Desorption Electrospray Ionization Mass Spectrometry**

Nicolas M. Morato[1], Samadhi Kulathunga[1], Christina R. Ferreira[1], Dylan T. Holden[1], Andrew D. Mesecar[1], Graham Cooks[1]; [1]*Purdue University*

In fifteen words or less, explain the significance of this contribution (Novel Aspect).: High-throughput (>1 sample/s) mass spectrometry platform capable of label-free enzymatic assays directly from reaction mixtures

Abstract Text: High-throughput (HT) screening is integral to current drug discovery workflows. Both the synthesis of large compound libraries and the bioactivity assessment of those molecules against particular biological targets rely greatly on HT experimentation. With regards to bioassays, traditional screening methods are based almost exclusively on optical or radiometric detection techniques, which are fast and sensitive, but require the use of labels or coupled reactions to generate a detectable output. This alters the native conditions of the system under study, makes the assays susceptible to interferences, slows method development, and limits flexibility, increasing at the same time costs and safety concerns. On the other hand, more recent mass spectrometry (MS) based platforms have become a fast alternative to traditional HT bioactivity screening, providing high versatility and molecular specificity for label-free assays. However, in spite of these advantages, some sample work-up is still required prior to analysis in many cases. Here we present an automated HT platform based on desorption electrospray ionization (DESI), an ambient ionization technique that allows for direct analysis from complex samples, such as buffers with detergent and high salt concentrations, removing completely the need for any sample work-up. This HT DESI-MS system was initially developed for organic reaction screening and has been recently extended to perform enzymatic assays. Sub-second analysis times (effective analysis time: 300 ms), low sample consumption (50 nL), great matrix tolerance, and excellent quantitative performance (RSDs < 10%), make this HT DESI-MS system a powerful tool in label-free enzymology and drug discovery, as it has been proven in several enzymatic systems. In particular, the cases of acetylcholinesterase (AChE), angiotensin-converting enzyme II (ACE2), and cholesterol sulfotransferase (SULT2B1b) will be discussed. Diverse applications such as the rapid kinetic study of the enzymatic systems with various native substrates, the identification and characterization of inhibitors and reactivators, and enzyme determinations directly from tissue extracts, have been demonstrated and will be presented. Special focus will be given to specific examples where DESI-MS label-free assays outperform traditional approaches: oximolysis of substrate in AChE assays, and SULT2B1b inhibitor discovery.

(PMA-06.5) **Development and Validation of a Stability-Indicating Reversed-Phase HPLC Method for Assay and Estimation of Related Substances of Ivermectin In an Oral Paste**

Nilusha Padivitage[1], Jingzhi Tian[2], Lin Wang[1], Jinyou Zhuang[1], Andrew McAdoo[1], Daoli Zhao[1], Abu Rustum[2]; *[1]Boehringer Ingelheim, [2]Boehringer Ingelheim Animal Health*

In fifteen words or less, explain the significance of this contribution (Novel Aspect).: A stability-indicating reversed-phase HPLC method for Ivermectin oral paste has been developed and validated.

Abstract Text: Ivermectin is a potent semi-synthetic antiparasitic drug used in veterinary medicine. A reversed-phase high performance liquid chromatography (RP-HPLC) method has been developed and validated for the identification and assay of Ivermectin, including the identification and estimation of its related impurities in an oral paste. Analytes were separated using a gradient elution at a flow rate of 1.5 mL/min on a Zorbax Extend-C18 column (150 mm x 4.6 mm i.d., 3.5 µm particle size) maintained at 30 °C. The mobile phase was composed of water as mobile phase-A and acetonitrile/methanol (85/15, v/v) as mobile phase-B. UV detection at 245 nm was employed to monitor the analytes. LOQ and LOD of the method are 0.6 µg/mL and 0.2 µg/mL, respectively. The validation results demonstrated excellent linearity of the method in the range of 0.1 to 150% of the analytical concentration (0.6 mg/mL) of the method. The stability-indicating capability of the method has been demonstrated by adequately separating the degradation products from the stress degraded samples of the oral paste as per method validation requirements prescribed in the current ICH guidelines.

21RAM01: Emerging Raman Spectroscopy Breakthroughs

Chair: Pavel Matousek

(RAM-01.1) **Noninvasive glucose monitoring using NIR Raman spectroscopy**

Jeon Woong Kang[1], Luis Galindo, Ramachandra Dasari, Peter So; [1]*Massachusetts Institute of Technology*

The importance of monitoring blood glucose cannot be overemphasized considering the increasing population of diabetics worldwide and the associated costs. However, the painful lancing process of obtaining blood drops by finger-stick hinders people from actively monitoring blood glucose levels. Noninvasive glucose monitoring has been a technology in high demand to provide people in need with pain-free, convenient, and continuous or as frequent measurements as necessary. Over the past decades, a variety of technologies have pursued this long quest. Among many, Raman spectroscopy has been recognized as a promising method. Raman spectra have distinctive spectral features, specific for target molecules. Quantitative analysis for diagnostic feasibility has been reported using various biological samples such as serum, blood, tissue, and skin. For in vivo transdermal Raman spectroscopy, acquired Raman spectra contain information on glucose molecules from the interstitial fluid underneath the epidermis. High-throughput Raman spectroscopic instruments have been developed and validated with small-scale clinical trials of human oral glucose tolerance test or animal glucose clamping test. Although these reports have claimed the diagnostic capability of the Raman system optimized for transcutaneous measurement, the absence of the characteristic Raman peaks and true prospective prediction has been a contradiction to the original motivation of using Raman spectroscopy for glucose sensing. Furthermore, glucose-specific peaks in in vivo Raman spectra are very weak, subdued by strong and time-varying skin autofluorescence and associated shot noise, which make it difficult to construct good prediction models and may lead to misinterpretation of experimental results depending on the choice of the validation method. Here, we present experimental data that may finalize the long debate of whether real glucose Raman peaks can be measured in vivo. We present the results of direct observation of glucose-specific Raman peaks in three swine glucose clamping experiments. The clamped levels of the glucose concentration were carefully controlled by infusing dextrose solution and insulin into the swine subjects. From the measured spectra, we confirm the presence of the glucose signal and linearity between intensities of the glucose Raman peaks and the reference glucose concentrations. Prospective prediction of glucose is achieved by tracking glucose peak intensities.

(RAM-01.2) **Raman spectroscopy and imaging of single cells in flow**

Kotaro Hiramatsu[1], Keisuke Goda[1]; [1]*University of Tokyo*

Raman spectral imaging is a powerful tool for label-free visualization of biological molecules such as proteins, nucleic acids, and various metabolites at a single-cell level. Although numerous biological phenomena that cannot be monitored by fluorescence microscopy have been studied by Raman imaging, the number of cells that could be interrogated by Raman imaging in a realistic timescale is typically up to a few to dozens of cells even with the sensitivity boost by the coherent Raman processes. To understand biological systems more statistically, a method to realize larger-scale label-free cell measurements is needed. Flow cytometry, in which many cells in a flow stream are rapidly interrogated optically or electronically, is an ideal method for realizing such a large-scale single-cell analysis. In the conventional fluorescence-based flow cytometry, however, target molecules are limited to those stainable by fluorescent probes, which exclude single-cell quantification of many small molecules such as metabolites. Combining flow cytometry with Raman spectroscopy/imaging is a promising approach for overcoming these existing issues but hindered by small Raman scattering cross-section. Here we present the developments of high-throughput Raman-based spectroscopic/imaging flow cytometers realized by integrating state-of-the-art coherent Raman techniques and acoustofluidic devices for cell manipulation in a flow stream. With the spectroscopic Raman flow cytometer, which is based on rapid-scan Fourier-transform coherent anti-Stokes Raman scattering, we can acquire broadband (400-1600 cm-1) Raman spectra of flowing single cells at a throughput higher than >2,000 cells/s. We applied this method to large-scale single-cell analyses of various bioproducts such as paramylon, starch, and astaxanthin in microalgae, Haematococcus lacustris and Euglena gracilis. With the imaging Raman flow cytometer, which is based on multi-color stimulated Raman scattering, we can acquire 4-color (2899, 2954, 3006, and 3034 cm−1) Raman images of flowing cells at > 80 cells/s, which is followed by cell sorting based on the obtained Raman images. We

performed label-free cell sorting of 3T3-L1-derived adipocyte cells and Chlamydomonas sp. mutants based on the localization of lipid droplets in each cell. We also demonstrated sorting of E. gracilis cells based on their metabolic activity by combining this method with stable isotope probing.

(RAM-01.3) **Swept-source Raman spectroscopy for low-power, sensitive and high spectral resolution molecular fingerprinting**

Amir H. Atabaki[1], William F. Herrington[1], Rajeev J. Ram[1]; [1]*Massachusetts Institute of Technology*

In fifteen words or less, explain the significance of this contribution (Novel Aspect).: Overcoming the limitations of dispersive Raman spectrometers with a swept-source Raman spectroscopy approach

Abstract Text: Dispersive Raman spectrometers are prevalently used in many industries for molecular fingerprinting. However, these systems face a fundamental tradeoff between size, sensitivity and spectral resolution. As a result, compact Raman spectrometers have lower spectral resolution and sensitivity, and oftentimes use high-power lasers to compensate for their low optical throughput. Here, we demonstrate a swept-source Raman spectrometer architecture that does not have the tradeoffs of dispersive spectrometers. This Raman spectrometer uses a chip-scale tunable laser, along with a high-throughput, high spectral resolution, compact optical probe for molecular fingerprinting. The optical throughput of the probe is about 1000 times greater than handheld dispersive Raman spectrometers while having a comparable size and spectral resolution. By leveraging the high throughput of this architecture, we lowered the excitation power to eye-safe levels (1.5mW, about 100x lower than most handheld systems) and used only a single uncooled silicon photodiode instead of cooled CCDs. Despite lowering the excitation power and using a low-cost uncooled detector, we demonstrated a high detection sensitivity (<1% by weight or volume) for identifying micronutrients in plants or contaminants in drinking alcohol. We also acquired the Raman spectrum of various analgesic tablets and were able to distinguish different types from each other. Besides providing opportunities for lowering the excitation power and use of low-cost, uncooled components, the demonstrated swept-source approach is a powerful tool for detailed analysis of individual vibrational states. While in dispersive Raman spectrometers the achievable spectral resolution for sampling the Raman spectrum is determined by the pixel pitch of the CCD (typically around 1cm-1), in the demonstrated swept-source approach, the Raman shift resolution is limited by the accuracy of the wavemeter used for monitoring the wavelength of the excitation laser. This enables orders of magnitude higher spectral resolution in sweeping the Raman spectrum and provides unique opportunities for studying individual vibrational states in detail. Here, we use this capability to study small changes in the C-O stretching vibration of methanol when mixed with drinking alcohol. Therefore, the demonstrated swept-source Raman spectrometer provides complementary capabilities to the dispersive approach, while overcoming some of its tradeoffs between size, spectral resolution and sensitivity.

September 30, 2021

21ART01: Student Research in Archaeological Chemistry

Chair: Alex Bertacchi
On-site Chair: Alex Bertacchi

(ART01.1) **Modern Methods on Old Mandibles: New Analysis of Caprines from Tepe Yahya**

Melina Seabrook[1]; [1]*Department of Anthropology, Harvard University*

Caprine husbandry supported the development of urban centers in Southwestern Asia with meat and secondary products. One site known for its extensive use of both sheep and goats is Tepe Yahya, a site in present-day Iran, which was occupied from 5,600 BCE to 1000 CE. Previous studies of the caprine faunal remains found a preponderance of goats compared to sheep. While many skeletal elements of sheep and goats are

distinguishable to the genus and species levels, mandibles, maxillae, and teeth provide a particular challenge for zooarchaeologists. These elements cannot be confidently speciated using morphological characteristics. This speciation problem is particularly apparent when investigating kill-off patterns. Kill off patterns are calculated using the fusion status of post-cranial elements and mandibles or maxillae containing in-situ teeth. Existing kill-off patterns lack species-specific nuance. While frequently herded together, sheep and goats were not always used in the same way, especially with respect to secondary products. Three prominent patterns of animal use include focus on meat, milk, or wool. Kill-off patterns for meat might be less affected, whereas wool patterns are skewed by the inclusion of goats and milk patterns skewed by the mixture of male and female animals. To answer questions of differential sheep and goat management and utilization, we must separate the two species and sexes. To this end, I apply three methods to caprine mandibles from Tepe Yahya for a diachronic comparison of sheep and goat husbandry. Each method provides information about a bone fragment beyond its morphological identification. I employ zooarchaeology mass spectrometry (zooMS), amelogenin enamel detection, and stable isotope analysis. ZooMS uses collagen peptide fingerprinting to identify peptides unique to a species. Amelogenins are a group of sexually dimorphic proteins in enamel that can be used to determine sex. The resulting information allows kill-off patterns to be calculated for sheep and goats separately. Finally, table isotope analysis of carbon, nitrogen, and oxygen elucidate diet, movement, and seasonality. The combination of results from the three analyses and the age stages could reveal other unseen patterns. This work offers a new perspective on animal husbandry from the 6th to 3rd millennium BCE.

(ART01.2) **Interpreting geochemical data in archaeological context; a case for interdisciplinary methodology in site formation research**

Elena Skosey-LaLonde[1], Gideon Hartman; [1]*Department of Anthropology, University of Connecticut*

Bones are invaluable archives of the life history and behavior of fauna and hominins in the archaeological record. As a composite material made of tightly bound collagen fibrils, non-collagenous proteins, and biomineralized carbonate-apatite micro-crystals, bones contain a wealth of isotopic information which is believed to be affected by diagenesis. Studies involving bone diagenesis are often centered in the chemical and structural aspects of the process, measuring element and isotope exchanges and spectral changes. Still, little is known about the diagenetic process that occurs on the nano-scale and how those tie to spectral changes and isotopic measurements. The current experimental study is designed to bridge this gap by studying the effect of diagenesis on single bone micro-crystals (>100nm scale) and correlate those with the widely applied infrared spectral analyses. Carbonate-apatite crystals of modern fresh bone, modern calcined bone, and archaeological bone from two different environments and time-periods, are isolated following the methods of Weiner and Price (1986). Prepared samples are analyzed using Scanning Transmission Electron Microscopy (S-TEM), providing images and elemental mapping of individual crystals, and the more accessible and cost-effective Fourier Transform Infrared analysis (FT-IR). Using S-TEM analysis, EDS Elemental Mapping, and FT-IR, this study seeks to investigate the potential of carbonated apatite crystals to retain biogenic elemental concentrations by (1) testing the assumption that the degree of crystallinity, or Infrared splitting factor, is directly correlated with crystal size; (2) investigating whether diagenetic alteration involves a coating process on existing biogenic crystals or alternatively creates a full dissolution and reprecipitation of authigenic and biogenic minerals; and (3) test the hypothesis that bone calcination increases carbonated apatite crystallinity irreversibly thus providing better preservation of biogenic minerals in archaeological calcined bone samples. Acknowledgements: Thermo Fisher Scientific Center for Advanced Microscopy and Material Analysis at the University of Connecticut for access to their analytical facilities. References: Steve Weiner and Paul A. Price (1986) "Laboratory investigations: Disaggregation of Bone into Crystals" Calcif Tissues Int (39):365-375.

(ART01.3) **Removing the Unwanted: A Systematic Comparison of Decontaminating Protocols for Ancient Dental Calculus Research**

Sterling Wright[1], Andrew Farrer, Laura Weyrich, Keith Dobney, Emily Skelly, Raphael Eisenhofer;
[1]*Department of Anthropology, Pennsylvania State University*

Ancient DNA analysis of human oral microbial communities within calcified dental plaque (calculus) has revealed key insights into human health, paleodemography, and cultural behaviors. However, contamination imposes a major concern for paleomicrobiological samples due to their low endogenous DNA content and exposure to environmental sources, calling into question some published results. Decontamination protocols (e.g. an ethylenediaminetetraacetic acid (EDTA) pre-digestion or ultraviolet radiation (UV) and bleach immersion treatments) aim to minimize the exogenous content of the outer surface of ancient calculus samples prior to DNA extraction. While these protocols are efficient, no one has systematically compared them. Here, we compare untreated dental calculus samples to four previously published decontamination protocols: a UV only treatment; a 5% sodium hypochlorite (bleach) immersion treatment; a pre-digestion in EDTA treatment; and a treatment with a combined UV irradiation and 5% bleach immersion (UVB) treatment. We examine their efficacy in ancient oral microbiota recovery by applying 16S rRNA gene amplicon and shotgun sequencing to ancient calculus samples from a single archaeological site. We identify ancient oral microbiota, as well as soil and skin contaminants. Overall, both the EDTA and UVB treatments are effective at reducing the proportion of environmental taxa and increasing oral taxa in relation to untreated samples.

(ART01.4) **Establishing Provenience of a Miocene Fossil Collection from Kenya - A Standardless pXRF Method Proof of Concept**

Kimberly Foecke[1], Ashley Hammond[2], Jay Kelley[3]; [1]*Department of Anthropology, The George Washington University,* [2]*American Museum of Natural History,* [3]*Arizona State University*

Questionable provenience is a significant issue in museum and university fossil/skeletal collections, from both ethical and research standpoints. This project presents a proof-of-concept study using portable X-ray fluorescence spectroscopy (pXRF) to test hypotheses about the provenience of a set of Miocene primate fossils held in the collections of the National Museums of Kenya. These fossils were collected during multiple excavations over the last 50 years. Their historically attributed provenience is in question, either due to poor labeling or suspicions raised by their skeletal morphology. Our study presents a method whereby the provenience of these specimens could be tested. We used pXRF to analyze other fossil specimens from the relevant group of sites in Kenya to create a reference database, and developed a standardless data processing protocol that uses cluster algorithms and linear discriminant analyses to assign the questionable specimens to their most likely site of origin based on the diagenetic chemical fingerprint left in fossils at each site. Our results revealed incorrect provenience attributions in 4 significant Miocene fossil specimens, which has implications for both previous studies of these fossils and ongoing research. This protocol has the potential to be applied to a wide variety of collections, and represents a low cost, non-destructive, and rapid way to approach questions of provenience in these materials.

(ART01.5) **Using concentrations of trace elements to detect diagenesis in archaeological hard tissues**

Alex Bertacchi[1], Andrew Zipkin[2]; [1]*Department of Anthropology, Yale University,* [2]*School of Human Evolution and Social Change, Arizona State University*

The measurement of traditional heavy (e.g., 87Sr/86Sr) and non-traditional isotope ratios in biological hard tissues from archaeological contexts is a powerful and increasingly common approach to investigating ancient diet, mobility, and physiology. This endeavor assumes that certain tissues preserve biogenic signals and are resistant to post-depositional contamination (diagenesis). With the exception of human dental enamel, this is seldom tested and a handful of studies have shown that hard tissues are susceptible to diagenesis affecting isotope systems of archaeological interest. Here, we expand on previous work using Maximum Threshold Concentrations (MTCs) to detect diagenesis in archaeological biomaterials. The premise of the MTC approach is that modern, unaltered hard tissues will exhibit characteristic concentration ranges of trace elements. When

these concentrations are exceeded in archaeological tissues, the specimens are likely contaminated and unsuitable for isotope ratio analysis. We analyzed 56 enamel samples from modern Kenyan mammals and 34 modern ostrich eggshells from South Africa, Namibia, and the United States by ICP-MS. We calculated MTCs using two different methods, one taken from the literature and a more conservative one developed for this project. We observe that our "conservative" MTCs are about three times lower than "traditional" MTCs for most elements, but at this time we remain agnostic as to whether these differences will lead to interpretive changes in real world studies. We propose that our MTCs can serve as reference concentrations to detect diagenesis in African faunal hard tissues. We tested this proposition using a sample of 51 archaeological enamel samples from sites in Malawi spanning the last 30,000 years. We found that ~20% of the sample is strongly altered and should be considered contaminated, ~40% is weakly altered and should preserve biogenic ratios of Sr, and the remaining ~40% is completely unaltered. Consistent with previous studies, alteration does not have a linear relationship with time spent in the burial environment. Critically, strongly altered and unaltered samples coexist in the same deposits and even at different loci on the same tooth, underscoring the importance of testing each and every sample for diagenetic alteration.

21AWD02: Spectroscopy's Emerging Leader in Molecular Spectroscopy Award Symposium Honoring Bhavya Sharma

Chair: Bhavya Sharma
On-site Chair: Bhavya Sharma

(AWD02.1) **Recent Advances in Remote Raman Spectroscopy and LIBS for Planetary Exploration**

Shiv K. Sharma[1], John N. Porter, Tayro Acosta-Maeda; [1]*Hawaii*

The SuperCam instrument onboard Mars 2020 Rover, Perseverance, is exploring Mars surface mineralogy and chemistry of rocks and soils with a combination of Remote Raman and Laser-induced Breakdown Spectroscopy (LIBS) since its landing on Mars at the Jezero crater on February 18, 2021,. The SuperCam's Raman and LIBS system can measure the spectra at a distance of < 10 m. For DoD applications of the remote Raman and LIBS systems have been designed with large telescopes and high power lasers for detecting hazardus chemical at >100 m distances. At the University of Hawaii, we have been exploring possibility of extending the range of both these techniques for planetary exploration and DoD applications. We have successfully extended the range of remote Raman and LIBS technique to >100 m using a low pulse energy (<5 mJ/pulse) of a 532-nm pulsed laser and a compact Raman-LIBS system with ICCD detector developed at the University of Hawaii. The combined LIBS and Raman instrument is mounted on a stationary platform and a small remotely operated rover carries a 45-degree mirror and a focusing lens for exciting the LIBS and Raman spectra of samples at > 100 m distance. These advances in the combined LIBS and Raman instrumentation will be very useful for future planetary exploration including the frozen volatiles (e.g., water-ice, gas hydrated, minerals, etc.) in the permanently shadowed region of the Moon and even Europa with a lander accompanying a small rover. These technological advances will allow detection of both molecular structures the elemental compositions of rocks, minerals and soils for terrestrial mineral explorations. These advances will also be useful in detecting hazardous chemicals used in home-made explosive devices (HMEs) from a safe distance. Potential applications of the remote Raman and LIBS system for planetary exploration, mining, homeland security, and defense will be discussed.

(AWD02.2) **Bringing Spectroscopy back to Raman Microscopy to Visualize Structural Dynamics and Molecular Interactions in Biological Systems**

David Punihaole[1], David Punihaole[1]; [1]*University of Vermont*

Conventional microscopy techniques probe biological processes by imaging the location of molecules in time and space. These techniques, however, lack the ability to reveal the physiochemical nature of how these molecules dynamically interact with their local environment. This knowledge is crucial for not only

understanding biological functions, but also establishing the molecular basis for human diseases. To gain a molecular understanding of biological processes, my group is developing a technique that we call Raman Chemical Imaging. This technique combines the chemical specificity of Raman spectroscopy, which probes environmentally sensitive molecular vibrations, with sophisticated coherent microscopy techniques. This method enables us to image the structural dynamics and interactions of biomolecules in their native cellular environment. We are interested in broadly applying Raman Chemical Imaging to investigate how living cells regulate protein folding, how protein aggregates cause neurodegeneration, and how polymer-based nanoparticles deliver nucleic acids for use in gene therapies.

(AWD02.3) **Taking a Closer Look at Art: Applied Spectroscopy at the Museum**

Stephanie Zaleski[1]; [1]*California State University East Bay*

Spectroscopy has become an indispensable tool for studying objects of cultural significance. In particular, Raman spectroscopy and surface-enhanced Raman spectroscopy (SERS) provides highly sensitive chemical information, often in a minimally invasive or non-invasive fashion, to inform art historical questions, conservation treatments, and preservation strategies. The first half of this talk will highlight the development and application of Raman spectroscopy and SERS to study a diverse range of artists' materials, including Japanese woodblock prints, glass objects, textiles, and organic lake pigments. The second half of the talk will focus on future directions of the novel application of SERS as a degradation monitoring tool and the establishment of a student research-focused university-museum partnership.

(AWD02.4) **Factors Impacting Standoff Ultraviolet Resonance Raman Trace Detection**

Sergei V. Bykov[1], Ryan Roppel, Sanford A. Asher[1]; [1]*University of Pittsburgh*

Raman spectroscopy is one of the few spectroscopic methods used for standoff measurements; laser light probes a distant object, and the scattered light is collected by a telescope and dispersed into a spectrum. The Raman spectrum serves as a sensitive and specific fingerprint that can be used to determine the chemical composition of an illuminated object. UV resonance Raman spectroscopy (UVRRS), in turn, is uniquely suitable for trace detection of enhanced species due to its high sensitivity and selectivity. Detection of trace quantities at a distance, however, is challenging and can be affected by sample illumination geometry, analyte morphology, analyte photochemistry, interferences from the substrate, UV luminescence, etc. We discuss several factors that impact standoff UVRR trace detection and potential ways to improve sensitivity.

(AWD02.5) **Antibody Approvals hit 100 in USA! The role of FTIR, Raman and ROA in the Biologics Industry**

Rina K. Dukor[1]; [1]*BioTools, Inc.*

Biopharmaceutical Industry celebrated a milestone in April 2021 as FDA approved its 100th commercial antibody based therapeutic. When biologics first immersed on the scene in 1986, with a 2nd approval in 1994, scientists from all fields of analytical chemistry rushed to determine and understand what available technologies would be useful during development / formulation, characterization, and quality control. Vibrational Spectroscopy entered the young field via seminal work of Drs. Steve Prestrelski and John Carpenter and their collaborators on a pharma / academic sides (1) and BioTools on the commercial front with an introduction of a dedicated solution known as Prota – an FTIR based protein analyzer. FT-IR turned out to be an ideal tool for studying monoclonal antibodies (mAbs) for several reasons. First and foremost, it allowed scientists to compare structure of mAbs in liquid and lyophilized states and screen many different formulations in a fast and easy way. Compared to Circular Dichroism (CD), samples could be studied at any concentration. And as a field moved to higher concentration formulations, FT-IR allowed measurements without any dilutions. Different application of FT-IR quickly followed including comparability, methionine degradation, detection of aggregation and silicon. But as new antibody formats are being developed, such as antibody-drug conjugates

(ADC's), bispecifics, fragments, and antibody-protein fusion, in addition to new modalities such as mRNA, oligonucleotides and carbohydrate-based drugs, more sensitive techniques are needed. Raman and Raman Optical Activity (ROA) offer a deep insight into Higher Order Structure (HOS) of antibodies due to its ability to detect not only the amide bands (and thus the secondary structure), but also all aromatic sidechains and disulfide-bonds providing information on tertiary structure. Specifically - ROA, combining the fingerprint region of vibrational spectrum and chiroptical sensitivity of CD, has proven to be one of the most sensitive structural techniques for all types of biologically relevant molecules. In this presentation we will demonstrate the utility of vibrational spectroscopy in characterization of all kinds of bio-based therapeutics. 1. S. J. Prestrelski, T. Arakawa, JF Carpenter "Separation of Freezing- and Drying-Induced Denaturation of Lyophilized Proteins Using Stress-Specific Stabilization", Archives of Biochemistry and Biophysics 303(2):465-73

21CHEM03: Chemometric Opportunities in the Forensic Sciences

Chair: Brooke Kammrath
On-site Chair: Brooke Kammrath

(CHEM-03.1) **Probabilistic Reporting of Glass Evidence Comparisons from Elemental Data Acquired by LA-ICP-MS**

Jose Almirall[1], Anuradha Akmeemana; [1]*Florida International University*

A standard test method for the chemical analysis of glass evidence using LA-ICP-MS (ASTM E2927-16a) describes a consensus-based approach to sampling, sample preparation, multivariate quantitative elemental analysis and also suggests a "match" criterion for the comparison of glass evidence. The result of the application of this method is a binary decision of either finding a difference in the elemental composition (exclusion) or a failure to exclude, based on elemental composition. This presentation aims to improve on this conclusion by demonstrating the utility of likelihood ratio (LR) calculations from comparisons of glass samples of known manufacturing history. LRs were calculated using a multivariate kernel density (MVK) followed by calibration with pool adjacent violators (PAV) using a method previously reported by the authors. Three different test datasets derived from the analysis of glass from known manufacturing origin (> 400 samples) using the ASTM analytical method for data collection, are evaluated using LR calculations. Two (2) different background databases are used to calculate the LRs; elemental data from ~ 600 different authentic vehicle glass samples and ~ 430 casework samples from different sources provided by the Bundeskriminalamt (BKA) forensic laboratory in Germany. The LRs calculated from comparing glass manufactured at three different plants over short periods (over 2-6 weeks) result in a range of calibrated LR values from very low (LR~10-3) when the glass are manufactured at different plants or manufactured weeks-months apart in the same plant to very high (LR~103) when the glass samples either originate from the same source or were manufactured on the same day and in the same plant. Although some of the glass samples being compared may not originate from the same broken window source, they exhibit chemical similarity within these lower and upper bounds and the LRs presented here facilitate the correlation between chemical relatedness to manufacturing history, specifically the time interval between production. The overall aim of this research is to improve on the opinion statement provided to the court for the significance of finding matching glass evidence in a particular case.

(CHEM-03.2) **Spatial Domain Forensic Applications of the Fourier Transform**

Jacqueline Speir[1]; [1]*West Virginia University*

Fourier transform (FT) is a common image processing technique that can convert a spatial domain image into its frequency components. Once accomplished, an infinite number of forensic applications are readily available. In this presentation, three specific applications will be highlighted. First, forensic analysts can use frequency filtering as a mathematical transform to reduce background interference in fingerprint images. The process effectively increases fingerprint ridge clarity, and in turn, enhances the ability of an examiner to extract

minutiae relevant to pairwise comparisons. Results indicate that frequency filtering has a low probability of creating false positive associations, that 90% of post-filtered images result in a normalized gain in match score, and that filtering can double the probability of obtaining 10 or more matching minutiae when comparing same source prints. The second application that will be illustrated is the automated classification of questioned footwear impressions. Outsoles take on a variety of geometric configurations; these patterns can be driven by fashion trends, trademarking goals, and intended end-use. Moreover, configurations tend to dynamically evolve over time as manufacturing trends and needs change, presenting a classification challenge for footwear examiners confronted with questioned impressions at crime scenes. Various approaches to this database search-and-retrieval problem have been explored in the literature, but results indicate that phase-only correlation (POC) outperforms other approaches, and that this superiority is statistically significant across a wide variety of image comparison scenarios, including mixed media (blood and dust), transfer mechanisms (gel lifters), enhancement techniques (digital and chemical) and print substrates (ceramic tiles, vinyl tiles and paper). Finally, the third illustration of FT that will be highlighted is image deconvolution to remove license plate blur. The degraded image of the forensically important motor vehicle information is considered the convolution of an ideal image with a point spread function (PSF) based on linear motion blur. Consequently, image restoration becomes a deconvolution problem which can be readily solved after estimating the motion induced PSF, thereby allowing for enhanced plate readability. In conclusion, it is hoped that attendees will come to appreciate the power and diversity of FT as applied to forensic problems in the spatial domain.

(CHEM-03.3) **Data analysis strategies for the elemental analysis of tire evidence**

Matthieu Baudelet[1], John Lucchi, Daniel Gluck, Larry Tang; [1]*University of Central Florida*

Tire mark evidence is often overlooked in today's forensics while often being found at crime or accident scenes. Traffic accidents represent a large portion of incidents in the world, covering property damage, injuries, and/or fatalities. In the United States between 2004 and 2018, 5% of traffic cases were fatal hit and runs, where knowledge about the car tire could sometimes be the only information available. While the pattern of the tire skid mark has been used before to link a tire or car to a scene, the widespread use of anti-lock braking systems makes this an almost impossible and often abandoned route of analysis. With this is mind, using the chemical profile of a tire has potential to link a car or tire back to a scene in which its trace material is found. Most current research into this topic involves looking at the molecular signature of the tire through pyrolysis-gas chromatography mass spectroscopy. However, there is concern that the conditions of skid mark trace evidence formation will obscure the molecular signal of the tire and will be hard to replicate exactly through analytical methods, possibly making classification impossible. A route to avoid this issue is to instead look at the elemental profile of the tires, which is less likely to be different between tire and skid mark. Looking at the elemental profile is an accepted technique in the current forensics field, which can be obtained in many ways from EDX to (LA-)ICP-MS to LIBS. Thirty-two tire samples (# brands) provided by the Florida Department of Law Enforcement were analyzed using LIBS under argon atmosphere. These samples were taken from the surfaces of tire treads and analyzed using a UV-LIBS unit (J200, Applied Spectra). Data were analyzed through several data analysis approaches such as Naïve Bayes, Linear Discriminant Analysis (LDA), Quadratic Discriminant Analysis (QDA), Support Vector Machines (SVM), and k-Nearest Neighbors. These first results will be discussed and show a promising path to the use of tires and their residues as a forensic evidence that has been neglected so far.

(CHEM-03.4) **Species Identification of Endangered Macaws Using Direct Analysis in Real Time – Mass Spectrometry and Statistical Analysis**

Rabi Ann A. Musah[1], Samira Beyramysoltan[2], Meghan Appley[3], Pepper Trail[4]; [1]*Department of Chemistry, Universityat Albany, State University of New York, Albany, New York12222, United States,* [2]*Department of*

Chemistry,Universityat Albany, State University of New York, Albany, New York12222, United States, [3]State University of New York at Albany, [4]U.S. Fish and Wildlife Service Forensic Laboratory

In fifteen words or less, explain the significance of this contribution (Novel Aspect).: New approach for the rapid and accurate forensic identification of endangered macaw species

Abstract Text: Macaws (giant new world parrots—Family: Psittacidae), which include multiple endangered species, are trafficked for use as pets, and for jewelry, ceremonial garments, and ritual practices. The unrelenting trade in both macaw's and macaw-derived products have resulted in a steady decline in the worldwide wild macaw population. The tracking and interruption of trade in these species hinges on the ability to distinguish between them, which is extremely challenging even for highly trained ornithologists, because of the visual similarities of the bird plumage. To overcome the challenges associated with forensic identification of illegally traded species, the application of direct analysis in real time–high-resolution mass spectrometry (DART-HRMS) and multivariate analysis to feather barbs as a means to accomplish species identification was investigated. Multiple feathers from several individuals representative of the following species were analyzed: scarlet (SC), hyacinth (HY), military (MI), red-and-green (RG), blue-and-yellow (BY) and greater-green (GG) macaws. The spectral data were aligned, explored for potential outliers and treated for class imbalances. The logarithmically transformed data for SC, HY, MI, RG and BY were subjected to sparse partial least square-discriminant analysis (sPLS-DA) to determine the discriminative variables. Then, soft-output classifiers including principal component analysis-linear discriminant analysis; PLS-DA; and support vector machine were fused based on Bayesian model averaging to enable discrimination of macaw species based on feather barb chemical profiles. The external validation samples, which were examined for validation of the resulting fused classifier, were comprised of two types of samples: those that were members of the training set (which included 154 BY, 12 RG, and 8 SC), and those that were not used to train the model (54 GG feathers). The performance analysis of the method showed 99% accuracy for identification of the species that were present in the trained model, and a 78% rejection rate for the sample that are not featured in the model. The results demonstrate a powerful technique for the rapid forensic identification of macaw species, which not only correctly identifies test samples that are represented in the training set, but also rejects samples of species that are not present in the trained data.

(CHEM-03.5) **Sand analysis by micro-XRF and geographic profiling.**

Sergey Mamedov[1]; [1]*HORIBA Scientific*

In fifteen words or less, explain the significance of this contribution (Novel Aspect).: A new approach to differentiate sands from different geographic locations will be presented.

Abstract Text: X-Ray Fluorescence spectroscopy is commonly used to quantify substances and confirm their identity. This technology requires no sample preparation. New capabilities of XRF analytical microscope (micro-XRF) equipped with X-ray optics enable the recording spectra of small particles, in addition to a hyperspectral image of objects with high spatial resolution. A hyperspectral image is a set of data containing information about the point's position along with the full XRF spectrum. Multivariate analysis can produce material classification based on several techniques such as Principal Component Analysis (PCA), Partial Least Square Discriminative Analysis (PLS-DA), or Gradient Boosted Tree Discriminant Analysis (XGBoostDA). For example, analysis of micro-XRF data can differentiate sands taken from different locations based on similarities or differences in spectra. The XGT-900 XRF analytical microscope was used in this study. This desktop unit utilizes a portable 50W X-ray Rh X-ray tube, Silicon Drift Detector (SDD), up to four programmable X-ray optics with spot size from 10 microns to 1.2 mm, and the capability to work in a vacuum, in a partial vacuum, in He-environment, and under ambient conditions. Spectra of sands from different locations from the United States, Italy, and Israel were collected and analyzed in the range of 1.00-40.96 keV.

Fundamental Parameters Method was used to calculate the concentration of oxides in the samples. Statistics of the measurements will be presented and discussed. For the classification of sands, several classification techniques were applied to the spectra and concentration profiles. The results show that statistical methods allow differentiating samples, which have very similar spectra features (concentration profiles), and this approach is helpful for forensic investigation.

21PAT05: TBD

Chair: John Wasylyk
On-site Chair: John Wasylyk

(PAT-05.1) **Fluorescence A-TEEM for Protein Concentration and Aggregation State: A Technique Comparison**

Karen E. Gall[1], Eunah Lee[1], Michelle Sestak[1], Jeffrey Bodycomb[1], Linda H. Kidder[2]; [1]*HORIBA Instruments Inc.,* [2]*HORIBA Scientific Instruments*

In fifteen words or less, explain the significance of this contribution (Novel Aspect).: Clarifying method limitations and exploration of the benefit of fluorescence absorbance-transmittance excitation emission matrix techniques

Abstract Text: Protein concentration and aggregation state in solution are two critical criteria for biochemical analysis of protein formulations and function. Biomolecule concentration determines a protein's native state or aggregated state, and high temperature can also cause dissociation of the protein structure. The range of protein concentrations of interest for a specific biomolecule or formulation is also very broad, depending on the protein size and structure. From ng/mL to mg/mL concentrations, the optimal protein structure and aggregation condition can vary. Particle size analysis and Raman spectroscopy present challenges for biomolecule analysis at very low concentrations and protein molecular weights, and more sensitive techniques, such as fluorescence spectroscopy, present challenges at the other end, where concentrations of protein are high. We present the Absorbance-Transmittance Excitation Emission Matrix (A-TEEM) technique for expanding the concentration range of protein analysis enabled by fluorescence and how that compares to measurements of the same proteins using Raman and Particle Size methods. Three proteins of varying molecular weight are analyzed with the A-TEEM method, as well as Raman spectroscopy and Particle Size images. As all three methods have their benefits for working with different molecular weight and concentration ranges, there are also limits to each. Overlapping ranges enable us to give an overview of the preferred solutions for both concentration and molecular weight, which are critical to all protein analysis.

(PAT-05.2) **Sensitive detection of halogens and sulfur in pharmaceutical materials by combustion ion chromatography**

Qiang Tu[1], Wendy Zhong[1], Douglas Richardson[1]; [1]*Merck & Co., Inc.*

In fifteen words or less, explain the significance of this contribution (Novel Aspect).: Applications of an automated analytical method for halogens and sulfur in pharmaceutical samples

Abstract Text: Determination of halogens and sulfur in pharmaceutical materials has been a difficult task. Ion-selective electrodes or ion chromatography can be used for the analysis, but many samples are difficult to dissolve in aqueous solutions and the analytes need to be present in ionic forms. Alternatively, ICP-MS can be used for the quantitation of a specific element (except fluorine). Unfortunately, the detection suffers from high ionization potentials of these elements and severe spectral interferences. Tedious sample preparation procedures are often needed for ICP-MS, and high detection limits are expected for samples that are difficult to dissolve.

We developed a new approach for sensitive detection of F, Cl, Br and S in pharmaceutical materials by combustion ion chromatography (CIC). The CIC technique provides a fully automated method of determining individual halogen and sulfur in liquid or solid samples without the need for sample preparation. Special effort has been made on selecting an appropriate means for calibration and method validation. The method has been successfully applied to the analysis of in-process pharmaceutical samples. The CIC demonstrates a superior sensitivity to halogens and sulfur over ICP-MS and can be used as a universal method for fast screening of mutagenic impurities (MIs), and for structural elucidation of various pharmaceutical materials.

(PAT-05.3) **Low Mass Analyte Monitoring for Fusion Reactor Processes using Multi-turn Time-of-Flight Mass Spectrometry**

Alicia Fessler[1], Nicholas Groden[1], Willis B. Jones[1], Louis McNamara[1], Randall Achey[1], Matthew Wellons[1]; [1]*Savannah River National Laboratory*

In fifteen words or less, explain the significance of this contribution (Novel Aspect).: Application of multi-turn time-of-flight to monitor Fusion reactor byproducts/waste streams at high resolution

Abstract Text: ITER is the international campaign to advance fusion science and develop a fusion reactor for energy. Savannah River National Laboratory (SRNL) is part of the nation's research centers that supports the ITER mission. The reactor process byproducts/waste streams contain a variety of low mass analytes, such as hydrogen, ammonia, and methane, which need to be monitored to provide information on the process, as well as the content of the waste stream for disposal purposes. Direct gas analysis mass spectrometry is a useful technique for process monitoring. Due to the similar mass-to-charge values some of the analytes share, a high resolution system is required. A team at SRNL has investigated the use of a multi-turn time-of-flight mass spectrometer, JEOL InfiTOF, for the detection of these low mass analytes. The JEOL InfiTOF offers tunable high resolution, direct gas analysis in a compact system. Using the tunable capability of the instrument, hard to resolve compounds, like CO/N2, can be resolved by increasing the number of times the ions travel through the mass analyzer. The instrument has been evaluated and optimized for hydrogen limit-of-detection and dynamic range. The presentation will highlight the application of high resolution low mass mass spectrometry using the JEOL InfiTOF to detect and quantify the gas phase analytes related to the fusion process.

(PAT-05.4) **Examining the Quality and Stability of Reagents**

John M. Wasylyk[1], Thomas LaCruz[1], Ming Huang[1], Robert Wethman[2]; [1]*Bristol Myers Squibb,* [2]*Bristol Myers Squibb Co.*

In fifteen words or less, explain the significance of this contribution (Novel Aspect).: Presentation covers multi-techniques used to understand the quality and stability of common and critical reagents

Abstract Text: The demand for common as well as novel, customized and even sustainable reagents has grown over the last two decades. This has put pressure on the suppliers of reagents to meet the specific needs of their customers. The 'just in time' approach to manufacturing the reagents, the complexity of supplying bulk quantities along with quantities designed for laboratory exploratory work has compounded the challenge faced by the suppliers. Not to be overlooked is the how the reagent is packaged, an understanding of the short versus long term stability and shipping methods. As a result, we have increased our analysis capabilities to ensure that critical reagents are of the quality we demand. In addition, in-house stability studies have allowed us to gain an increased understanding of variables in our handling of the reagents. Analytical analyses include turbidity measurements, mass spectrometry, along with near Infrared, infrared, and Raman spectroscopies. Recently we have added open-access spectroscopy-based instruments for the rapid analyses of common and unique reagents to aid chemists and engineers in determining the quality of their reagents prior to utilization in laboratory and

plant settings. In addition, we are in the process of evaluating the stability of common catalysts with respect to temperature, moisture, and solvent type to provide guidance during route scouting in the development cycle of pharmaceuticals.

(PAT-05.5) **Size, Chemistry, and More: Raman and Laser Diffraction for Pharma Particle Analysis**

Eunah Lee[1], Jeffrey Bodycomb[1], Julie Nguyen[1]; [1]*HORIBA Instruments Inc.*

In fifteen words or less, explain the significance of this contribution (Novel Aspect).: Collaborative and complementary analysis of pharmaceutical particles using Raman microscopy and laser diffraction.

Abstract Text: Pharma particles range from suspension droplets in a liquid formulation to dry particles used in solid dosage forms. The effectiveness of a formulation depends on both the size distribution and chemical composition of these particles. Raman microscopy and laser diffraction can be collaborative with and complementary to each other in analyzing pharmaceutical particles: Raman microscopy to probe particle composition and particle size, and laser diffraction for high throughput and high precision analysis of particle size and particle size distribution. This paper will present examples of particle analysis using particle correlated Raman spectroscopy (PCRS), laser diffraction, and both.

21PMA05: Industrial Applications of Vibrational Spectroscopy

Chair: Patrick Wray
Co-Chair: Andrew Chan
On-site Chair: John Wasylyk

(PMA-05.1) **Label-free study of intracellular glycogen level in Metformin and Resveratrol treated insulin-resistant HepG2 cells by live-cell FTIR spectroscopy**

Anchisa Poonprasartporn[1], Andrew Chan[1]; [1]*King's College London*

Fourier-transform infrared (FTIR) spectroscopy is a non-destructive, label-free, sensitive, and low-cost technique that is recently found to be suitable for studying cellular changes in diabetes metabolism. This study has demonstrated that live-cell FTIR can be applied to study the differences in glucose metabolism in cells treated in either normal or high glucose medium with or without insulin and the reinstatement of insulin sensitivity of the insulin-resistance cells by Metformin and Resveratrol. Principal component analysis was used to highlight any possible correlated changes for the first two hours of treatments with results confirmed by traditional glycogen assays. Cells treated in high glucose and insulin have shown insulin resistance with little spectral change than the cells in normal condition when treated in 100 nM insulin. Adding 2 mM metformin or 50 μM Resveratrol have shown a significant reinstatement of insulin sensitivity compared to control ($p<0.01$) with an increase of glycogen spectral peaks (1150, 1080, 1020 cm-1) from the 1st and 2nd hour after treatment. The increase of glycogen for the drug-treated cells was confirmed by the traditional glycogen assay. In conclusion, live-cell FTIR provided information regarding insulin resistance, insulin sensitivity, and drug efficacy for insulin resistance treatment within one approach. It can be a low-cost complementary method for the studies of metabolic changes in insulin-resistance diabetic cells

(PMA-05.2) **Subcellular FTIR imaging with novel ZnS hemispheres for studying phospholipidosis in live macrophages**

Ohood Alshareef[1], Andrew Chan[2]; [1]*Institute of Pharmaceutical Science, King's College London,* [2]*King's College London*

Live cell FTIR imaging system has the power to be used as an analytical tool to study foamy macrophages and provide valuable information on biochemical changes inside them at a single cell/subcellular level. To overcome the small size of macrophages with respect to the wavelength of IR light, this project will introduce the novel spatial resolution enhancement technique by the use of a ZnS hemisphere as the sampling unit that can magnify the image to capture intracellular components includes vacuoles. We demonstrate the capability of FTIR micro-spectroscopy to detect phospholipids accumulation in single living cells after treatment with amiodarone using a label-free non-distractive method. This can be used to shed light on the two roles of adaptive and adverse response to xenobiotics in macrophage, which is not yet been established.

(PMA-05.3) **Vibrational spectroscopy to assess degradation of purification resins used in the downstream processing of monoclonal antibodies**

James W. Beattie[1], Richard Kucia-Tran, Monika Farys[2], Ruth Rowland-Jones, Bernadette Byrne[1], Sergei Kazarian[1]; [1]*Imperial College London,* [2]*GSK Biopharm Process Research*

Biotherapeutics represent a major and growing area of pharmaceuticals. The majority of approved biotherapeutics are monoclonal antibodies (mAbs) used to treat a range of both cancerous and non-cancerous diseases, including lymphocytic leukaemia, arthritis and more recently Covid-19. Although effective, mAbs are expensive with treatments costing ~$100,000 per year per patient on average. Currently, most mAbs are produced recombinantly using cultured Chinese Hamster Ovary cells, meaning a robust downstream process must be in place to successfully isolate the mAbs. Of particular importance, is the reduction of potentially immunogenic contaminants from the mAb samples to under 100 pg per dose. Protein A affinity chromatography (PrAc) removes 98% of the contaminants and accounts for the large majority of downstream processing costs. Protein A ligand leaching and protein fouling reduce resin lifetime, contributing to mAb production costs. Currently, PrAc resins are monitored by methods such as static binding capacity (SBC) assays which assess both the affinity of a mAb for the Protein A ligand and the maximum binding capacity of the resin. This approach, whilst useful, does not provide any chemical information on why changes in resin binding capacity occur. Confocal Raman microscopy is a label-free method providing information on the secondary structure of proteins present in a sample. Here, we utilised confocal Raman microscopy combined with SBC analysis to explore mAb binding to resin beads from different locations within an industrially used MabSelect Sure Protein A column. This work builds upon ATR-FTIR spectroscopic analysis of the ability of resin from different parts of an industrially used pilot-scale column. ATR-FTIR spectroscopy allowed for measuring ~2-6 μm into the bead which are between 40-120 μm in diameter. mAb concentration was successfully quantified within a thin surface layer these samples. These resin samples were further analysed by exploiting confocal Raman microscopy's ability to probe binding events inside the resin beads. Our results indicate a reduction in mAb adsorbed to the Protein A ligand within the resin beads compared to the bead surface.

(PMA-05.4) **Quantum cascade laser-based IR spectroscopy for sensitive analysis of proteins**

Andreas Schwaighofer[1], Christopher Akhgar[1], Bernhard Lendl[1]; [1]*TU Wien*

In fifteen words or less, explain the significance of this contribution (Novel Aspect).: Laser based IR spectroscopy enables detection limits for protein analysis in the ppm range.

Abstract Text: Mid-IR spectroscopy is capable to provide both qualitative and quantitative information on proteins in a fast, non-destructive and label-free manner by probing the strong, fundamental vibrations of molecules. In protein analysis determination of the secondary structure (a-helix, b-sheet, random coil, etc. …) of a given protein is the most relevant qualitative information accessible by mid-IR spectroscopy. Protein analysis in aqueous solutions by conventional Fourier transform infrared (FTIR) spectroscopy is limited due to the strong water absorption overlapping with the information-rich protein amide I band. Consequently, only short path-lengths (<10 μm) can be used for measurements as otherwise all light would be absorbed by the aqueous sample matrix. The significant progress made in mid-IR lasers has recently changed this situation. High spectral

power densities and broad tuning ranges, as made possible by modern external cavity quantum cascade lasers (EC-QCL), now allow for pathlengths of 30 µm and more, even for analysis of the amide I band. We present a broadband EC-QCL based IR transmission setup with balanced detection covering the amide I+II bands for highly sensitive protein sensing that outperforms commercially available IR spectrometers by almost an order of magnitude. Furthermore, we report on application of laser-based IR spectroscopy on monitoring protein conformational changes after external perturbation (chemical, temperature, pH).

21RAM08: Biomedical and Bioanalytical Raman Spectroscopy

Chair: Santosh Paidi
On-site Chair: Samuel Mabbott

(RAM-08.1) **Developing immune-SRS microscopy: profiling galectin expression for cancer diagnosis**
Marie B. Gjika[1], Duncan Graham[1], William J. Tipping[1], Karen Faulds[1], Kev Dhaliwal[2]; [1]*University of Strathclyde,* [2]*the university of Edinburgh*

In fifteen words or less, explain the significance of this contribution (Novel Aspect).: Imaging of specific cellular biomolecules with fast image acquisition speeds, high spatial and temporal resolution

Abstract Text: A variety of imaging techniques have been widely used in imaging biological samples to understand the underlying biochemistry of cells and tissues. Immunofluorescence, which relies on the ability of target-specific antibodies against antigens, is the most commonly used method for detecting and monitoring biomolecules and studying biological pathways at the cellular and subcellular level. Despite its widespread use, fluorescence microscopy requires fluorophores, which suffer from photobleaching and present limited multiplexed capability. Alternatively, Stimulated Raman scattering (SRS) is a widely used non-destructive imaging tool for the analysis of cells and tissues. Measuring the inelastic scattering of light by stimulating specific molecular vibrations, SRS provides vibrational information characteristic to the global changes of cellular DNA, lipids, and proteins with fast image acquisition speeds, high spatial and temporal resolution. Despite its unique advantages, one of the main challenges of SRS microscopy as a diagnostic imaging tool is its low detection specificity between similar biological species. Hence, the development of highly specific and multiplexed imaging technique suitable to detect specific biochemical variations in real-time would be beneficial for diagnostic applications. Here, a novel approach to immunofluorescence, called immuno-SRS microscopy, is designed to selectively detect a family of galectin proteins , which are considered critical in cancer progression, through the reaction of biotinylated antibodies with alkyne-modified streptavidin . Alkyne tags, which displays characteristic vibrational frequencies in the silent cellular region of the Raman spectrum (1800−2800 cm−1), are considered an attractive alternative to fluorophores due to their small size, exogenous character and synthetic accessibility. The successful synthesis of alkyne-modified streptavidin was confirmed by UV-Vis and Raman spectroscopy. This approach allows the specific detection of intercellular Gal-3 proteins in various permeabilised cell lines, such as MCF, HeLa and H1975, by demonstrating the breakthrough potential of alkyne tags for intracellular immune-labelling with high spatial and temporal resolution using rapid SRS imaging. These results displayed negligible background signal in off-resonance frequency. Additionally, increasing the number of triple bonds in the polyyne chain of the alkyne tag led to altering the detected Raman shifts, allowing the simultaneous visualisation of different molecular species, illustrating the multiplexed abilities of the technique.

(RAM-08.4) **Surface-enhanced Raman Scattering with Plasmonic Gold Nanostars for Biosensing and In Vivo Cancer Detection**
Yang Liu[1], David Kirsch[1], Tuan Vo-Dinh[1]; [1]*Duke University*

In fifteen words or less, explain the significance of this contribution (Novel Aspect).: We have developed a SERS nanoprobe for biosensing and in vivo cancer detection.

Abstract Text: Cancer is the second leading cause of death and it is of great significance to develop innovative methods for cancer biosensing and in vivo detection. We have developed a novel nanoplatform, plasmonic gold nanostars (GNS) with tip-enhanced plasmonics, for surface-enhanced Raman scattering (SERS) to perform in vivo biosensing and cancer detection. Experiment results demonstrated that our GNS nanoprobe can sensitively measure the change of pH, which is an important cancer biomarker because the tumor has a high rate of metabolic activity and poor perfusion. We have also identified a unique pH-sensing index, the shift of SERS peak position with pH change. Density functional theory calculation was performed to investigate the vibrational modes of observed SERS peaks related to pH sensing. The observed shift of SERS peak position was found to be due to the coupling between the benzene ring stretching and carboxylic group stretching, whose vibrational wavenumber decreases when the pH reporter, 4-mercaptobenzoic acid, changes from the protonated state to the deprotonated state. In addition, we have performed in vivo SERS study with a murine sarcoma animal model and demonstrated that the developed GNS nanoprobes can accumulate selectively in tumors due to the enhanced permeation and retention (EPR) effect and provide probes for in vivo cancer detection using non-invasive remote SERS sensing. As a result, our developed SERS nanoplatform could be applied for in vivo biosensing and cancer detection in future translation studies.

(RAM-08.5) **Raman spectroscopy as a neuropathological analysis tool: Rapid interoperative identification and genetic classification of diffuse brain tumors**

Sarah C. Shidler[1], Dale Boorman[2], James Livermore[3], Martin Isabell[2], Ian Bell[4], Natalie Voets[5], Connor Scott[3], John Walsby-Tickle[6], Joan Gannon[6], Puneet Plaha[5], Claire Vallance[6], Olaf Ansorge[3], Lucy Grainger[7], Tim Prusnick[8]; *[1]Renishaw Inc, [2]Renishaw Plc, [3]Nuffield Department of Clinical Neurosciences, [4]Renishaw, Plc, [5]Nuffield Department of Surgery, University of Oxford, John Radcliffe Hospital, Oxford, UK, [6]Department of Chemistry, University of Oxford, [7]Renishaw, Inc., [8]Renishaw Inc.*

In fifteen words or less, explain the significance of this contribution (Novel Aspect).: Genetic subtyping of diffuse brain tumors by Raman spectroscopy aids critical decision-making during surgical resection

Abstract Text: Raman spectroscopy is a valuable analytical technique which benefits from its ability to extract highly specific chemical information non-destructively, without requiring arduous and expensive sample preparation. With the development of compact, easy-to-use Raman spectrometers, the technique has become accessible to a wide range of users, and thus Raman is now being utilized not only within laboratory-based environments, but also within a range of clinical settings. Here, we demonstrate the application of Raman spectroscopy within the neurosurgery field. We show that Raman spectroscopy permits rapid (<15 min) analysis of fresh tissue samples, taken directly from the operating theatre, and the potential of the technique to distinguish tumor from normal tissue and classify the three most common and clinically relevant genetic subtypes of diffuse glioma. The results from this study demonstrate an extremely promising potential application of Raman spectroscopy for rapid and accurate tumor identification, where comparable techniques are limited. In addition, the genetic classification provided by Raman spectroscopy could allow surgeons to make more informed decisions as to whether the survival benefit of increasing surgical resection outweighs the risk of causing neurological deficit.

21SPECIAL01: Spectrochimica Acta Atomic Spectroscopy Award

Chair: Alessandro De Giacomo
On-site Chair: Jonathan Merten

(SPEC-01.1) **Atomic Spectrometry and SAB: Quo Vadis?**

Gary M. Hieftje[1]; [1]*Indiana University*

The foundations of atomic spectrophysics and atomic spectrochemistry can be traced back to such scientific giants as Newton, Bunsen, and Kirchhoff. However, it can be argued that many aspects of current practice in analytical atomic spectrometry were introduced during the period since 1939, the year when the journal Spectrochimica Acta was first published by the Vatican Press. Such developments include modern ways of producing and detecting atomic optical and mass spectra, the introduction of alternative sources for generating neutral atoms and atomic ions, advanced understanding of fundamental events that affect analytical figures of merit, an almost continuous improvement of such figures of merit, the melding of atomic and molecular spectrometry, and the application of atomic spectrometric methods to an almost unfathomable range of contemporary problems. However, even the most up-to-date methods, applications, and instrumentation for atomic spectrometry exhibit shortcomings. In this presentation, some of these limitations will be outlined and possible means to overcome them offered. These considerations will in turn suggest future directions that research in atomic spectrometry might profitably take.

(SPEC-01.2) **The Liquid Sampling-Atmospheric Pressure Glow Discharge: A Combined Atomic and Molecular (CAM) Ionization Source**

R. Kenneth Marcus[1], R. Kenneth Marcus[1], Edward Hoegg, David Koppenaal, Tyler Williams, Katja Hall, Jacob Bills; [1]*Clemson University*

The worlds of elemental/isotopic (inorganic) and molecular (organic) mass spectrometry have historically existed in different universes. The former predominately involves JCP-MS and TIMS, sources that involve decomposition of analyte species to atomic ion form; a total loss of chemical information. The latter includes El-, Cl-, ESI-, and MALDI-MS, whose principal mission is to provide molecular information; almost never considering metal elements and their isotopic composition. The ability to solve diverse problems requires more global approaches to obtaining information on a more holistic level. We present here, novel levels of information obtained by the coupling of a combined atomic and molecular (CAM) ionization source with a range of mass analyzers. Specifically, the liquid sampling-atmospheric pressure glow discharge (LS-APGD) microplasma has been employed for applications as diverse as uranium isotope ratio measurements, to nutrient monitoring in bioreactors, to LC-MS of drugs and proteins. By judicious selection of the sustaining mobile phase, spectra can be tuned between being atomic in nature (2% nitric acid) or much like atmospheric pressure chemical ionization (methanol:water). Likewise, operation in a single electrode mode yields molecular spectra while addition of a second electrode produces significant fragmentation. In this presentation, we will illustrate the diversity of information that can be generated using this simple, low power ionization source, with its versatility highlighted by its ready coupling to virtually any mass spectrometer capable of ambient atmosphere sampling.

(SPEC-01.3) **3rd SAB Award: Nanoparticle enhanced laser ablation inductively coupled plasma mass spectrometry**

Annarosa Mangone[1], Fabrizio Mastrorocco[1], Lorena C. Giannossa[1], Roberto Comparelli[2], Marcella Dell'Aglio[3], Alessandro De Giacomo[1]; [1]*University of Bari,* [2]*National Research Council (Italy),* [3]*CNR-NANOTEC*

The effect of metal nanoparticles (NPs) in enhancing the sensitivity of Laser Ablation Inductively Coupled Plasma Mass Spectrometry (LA-ICPMS) is discussed, namely Nanoparticle Enhanced LA-ICPMS (NELA-ICPMS). The enhancement is due to surface plasmon interaction from metal NPs on the laser field, effectively increasing the sensitivity of one order of magnitude with respect to the conventional technique at fixed physical

and chemical setup parameters. Tests were performed on different materials for both NPs and substrates, the latter being either metallic or dielectric, with various pairings.The enhancement extent is found to be dependent on features of both the NPs (typology, concentration and size) and the sample under analysis (investigated element and matrix), with best results from conductive matrices. Comparison of the laser-generated craters (depth and general morphology) and particles (size and composition) with and without NPs hints at the coupling of electromagnetic field from the laser and induced by the NPs as the main cause for the enhancement. The ablation in presence of NPs, in particular, locally appears more efficient below the power threshold for ablation in conventional LA-ICPMS, as demonstrated by the smaller size of laser-generated particles exhibiting better vaporization efficiency. Moreover, permanent contamination is prevented due to NPs being removed from sample surface after a limited number of laser shots. Correspondingly, NELA-ICPMS can result in no enhancement with respect to the standard technique with a sharp decrease in the number of laser pulses shone on the sample, making it an interesting tool for investigations requiring non-destructive approaches. Determination of surface-distribution patterns of very thin layers is also feasible with no underlying contaminationdue to the smaller crater size. Moreover, the technique can be particularly useful to cut down interference (i.e. Cr and Mn interfered by ArO and ArN) because it allows to increase the analyte signal without increasing the interferences, so increasing signal to noise ratio. In the end, the strength of this approach is undeniable due to its affordability and ease and rapidity of performance.

(SPEC-01.4) **2nd SAB Award: Hyperfine structures and isotopic shifts of uranium transitions using tunable laser spectroscopy of laser ablation plumes**

Sivanandan S. Harilal[1], Mark C. Phillips[2], Jeffrey Martin, Christopher Murzyn; [1]*Pacific Northwest National Laboratory,* [2]*Wyant College of Optical Sciences, University of Arizona, Tucson, AZ (USA)*

We report isotopic shifts and hyperfine structures of selected U transitions employing tunable spectroscopy viz: laser-induced fluorescence and laser absorption spectroscopy of laser ablation plumes. The plasmas were produced during ns laser ablation on a natural U metal target which contains 0.73% U-235. Our results show that isotopic shifts between U-238 and U-235 are entangled with hyperfine structures of U-235. Measurements obtained using laser-induced fluorescence are affected by the high absorbance of U-238. Time-resolved laser absorption spectroscopy is carried out for evaluating the optical absorption and estimating the hyperfine constants.

(SPEC-01.5) **1st SAB Award: About detectability and limits of detection in single particle inductively coupled plasma mass spectrometry**

Francisco Laborda[1], Ana C. Gimenez-Ingalaturre[1], Eduardo Bolea[1], Juan R. Castillo[1]; [1]*University of Zaragoza*

The unique features of single particle inductively coupled plasma mass spectrometry (SP-ICP-MS) for the detection of particles, their quantification and size characterization, along with its availability in commercial instruments, have led to the success of this technique and its increasing application in different fields (environment, toxicology, foods…). However, as with any other analytical technique, SP-ICP-MS has limited detection capabilities. Moreover, because of the different types of information that SP-ICP-MS can provide, these capabilities are not only limited to the concentration domains (of particles and dissolved related species), but also to the mass of element per particle and the particle size domains (when additional informations about shape, composition and density of the particles are available). Discrimination and detection of particle events, based on the use of robust limits of decision (also known as critical values), and the estimation of the limits of detection in the different domains, require standardized metrological approaches that have not been clearly established yet. As a consequence, harmonized approaches and expressions to allow reliable comparisons between methods and instruments, as well as to process SP-ICP-MS data, are required. The objectives of this work are to highlight the peculiarities regarding detectability in SP-ICP-MS, as well as to propose a holistic approach with criteria and expressions for the estimation of the different critical values and limits of detection in terms of the different instrumental and experimental parameters involved, in an attempt to open a process of

harmonization in the SP-ICP-MS community about these topics. This work was supported by the Spanish Ministry of Science, Innovation and Universities and the European Regional Development Fund, project RTI2018-096111-B-I00 (MICINN/FEDER).

21SPECIAL04: Analytical Molecular Spectroscopy: Honoring the Contributions of Robert W. Hannah

Chair: DAVID Schiering
On-site Chair: DAVID Schiering

(SPEC-04.1) **Bob Hannah's Elegant Deconvolution Algorithm and How it Compares to Others**

James A. de Haseth[1]; [1]*University of North Carolina at Chapel Hill*

One of the earliest attempts to deconvolve spectra digitally was with the use of the Savitsky-Golay method of simplified least squares. The original algorithm was run in 1964 on a mainframe computer and the calculation was restricted to integer values. The Savitsky-Golay algorithm could calculate derivatives of spectra, hence peak width could be reduced. With the reduction in peak width it was possible to resolve some overlapped bands. In the early 1980s there was considerable interest in new computerized methods to extract more information from spectra. Although spectrometers had been interfaced to minicomputers for over a decade, it was not until that time that there was sufficient processing power and user access to the computer for programming that opportunity to introduce new software processing tools arose. Some of the earliest attempts to deconvolve spectra, or more appropriately to narrow peak width, were done by Fourier self-deconvolution and Fourier derivitization. Robert W. Hannah took a rather different approach to deconvolution and developed a method that used Savitsky-Golay smoothing and spectral subtraction. Subsequently, other algorithms have been developed. An examination of Hannah's deconvolution method will be undertaken in comparison to other methods, and the advantages and shortcomings of the different approaches will be explained. In recent studies, some authors have mistakenly assumed curve-fitting is deconvolution. The approaches are completely different, although deconvolution methods may be used as a starting point for some curve-fitting algotrithms.

(SPEC-04.2) **Strategies and Resources for Successful Infrared and Raman Spectral Interpretation**

Peter J. Larkin[1]; [1]*Solvay*

Infrared and Raman spectroscopies provide characteristic fundamental vibrations that are used extensively for the determination and identification of molecular structure. This is based upon well-established spectra-structure correlations for both organic and inorganic compounds. The successful application of both techniques has been limited by the lack of basic knowledge of spectral interpretation skills among potential users. In this work we introduce selected available resources as well as highlight some successful strategies typically utilized to analyze infrared and Raman spectra. The infrared and Raman spectral regions for characteristic group frequencies, software based approaches for spectral verification and identification are reviewed followed by two simple examples of an infrared and Raman based spectral identification. We highlight general types of chemical systems that Raman spectroscopy is particularly well suited as a structural identification technique. For more challenging and open ended problems, spectral interpretation by a knowledgeable analyst is required. We outline some of the general strategies used to solve structural problems using infrared spectroscopy and list commonly used resources. These resources include: digital spectral collections (both open access and commercially available), commercial software, reference books of collected infrared spectra as well as useful reference books to guide interested users to gain expertise in spectral interpretation.

(SPEC-04.3) **Application of Raman Spectroscopy to Achieve Process Understanding**

Brian Marquardt[1], Mel Koch; [1]*MarqMetrix Inc.*

Process analysis has provided immense value to the chemical related industries (pharmaceuticals, oil and gas, consumer products and foods) for years by optimizing productivity, quality and improving overall

process/product control. A few primary advances in this field have been the development and focus on process specific analytics and in the miniaturization of historical lab based analytical technology. These news tools when combined with advances in process sampling platforms are proving to be valuable approaches for implementing Process Analytical Technology (PAT) on a wider scale. This presentation will illustrate the path process analytical technology has taken to get here with emphasis on sampling technology and industrial process control applications. We will also honor the memory of Bob Hannah and his impact on PAT through our interactions with him at the Center for Process Analysis and Control.

(SPEC-04.4) **IR Spectral Search Systems - Automated Compound Identification and Functional Group Determinations**

DAVID W. Schiering[1], John P. Coates[2]; *[1]RedWave Technology, [2]John Coates Consulting LLC*

The need for automated methods for the identification of unknowns based on infrared (IR) spectral standards was recognized near the beginning of the use of IR spectroscopy in chemical analysis [1]. Bob Hannah was instrumental in developing and promoting computer aided chemistry in the time when scientific computers were in their infancy. One of his key contributions was in leading a team in the 1970s that developed the first automated, interpretative spectral search technology for Perkin-Elmer IR spectrometers using the Model 3600 data station [2], called simply, "SEARCH." The concept of SEARCH was consistent with Bob's strong opinion that analysts should possess spectral interpretation skills and SEARCH was a tool that aided the process and provided chemical candidates that might be the identity of the unknown. Spectral band position data and the functional groups, called "Possible Structural Units" (PSU), were used in scoring the potential chemical candidates. We will discuss the evolution of this IR spectral search system, describing its features, limitations, and performance. Modern search packages that implement automated mixture searching capabilities, filtered searches, and links to chemical and physical properties will be discussed in the context of in-field chemical threat identifications.

(SPEC-04.5) **Portable FT-IR Spectrometry and its Application in Field Forensics**

DAVID W. Schiering[1]; *[1]RedWave Technology*

Infrared (IR) spectroscopy is a mainstay analytical method for the identification and characterization of materials. According to the Scientific Working Group for the Analysis of Seized Drugs (SWGDRUG), IR spectroscopy is a "Category A" technique having "maximum potential discriminating power." Evolving threats to the soldier and public safety and security have served to significantly improve the capabilities of teams charged with responding to responding to chemical, biological, radiological, nuclear, explosives (CBRNE) and narcotics threats. FT-IR spectroscopy has become an indispensable method for the identification of threat materials in the field. These instruments are used in austere environments by persons with little formal scientific training. These smaller spectrometers have significantly contributed to a growth in adoption of FT-IR spectrometry and an expansion of its applications. This presentation shall be a review of the development of portable FT-IR spectroscopy instrumental methods for field forensics. We will review the requirements of portable and handheld FT-IR spectrometers for in-field analyses and the technological elements that have enabled the development and practical deployment of these systems. We will also address how the system design and integration of hardware and software benefits the operator in the environment and enables the threat mitigation. Finally, examples of analyses from real-world events will be presented.

21ART02: Archaeological, Geochemical, and Remote Sensing Applications in the Search for Pleistocene Landscapes of New England

Chair: David Leslie
On-site Chair: David Leslie

(ART02.1) **The Farmington River Valley during the Late Pleistocene: Paleoindian Occupations at Southern New England's Oldest Archaeological Site**

David Leslie[1], William Ouimet[2], Caroline Allen[2]; *[1]Archaeological and Historical Services, Inc.; University of Connecticut, Anthropology Department, [2]University of Connecticut, Department of Geosciences*

The Brian D. Jones Site (Site 4-10B) in Avon, Connecticut, is the oldest archaeological site in Southern New England (10,520 ± 30 14C yr. BP) with well-defined, stratified Paleoindian deposits from multiple occupations. The site is located approximately 1.5 meters below the existing ground surface in buried alluvial sediments, situated on a floodplain and levee geomorphological system of the Farmington River. In this study, we present geochemical analyses of terrestrial vibracores (pXRF, LOI, and FTIR), ground penetrating radar profiles, and updated radiocarbon dates associated with the core sediments, as well as a brief discussion of the archaeological site. These data are useful in understanding the sedimentation and paleoenvironment at the site, as well as the timing of human occupations during the Pleistocene along the Farmington River. The coring, radiocarbon, geochemical, ground penetrating radar, and archaeological datasets, paired with the geomorphological understanding of the Farmington river system, and compared to the Templeton and 4-15 sites, may also be useful in predicting the locations of other alluvially-buried Paleoindian sites in the Northeast that have yet to be discovered.

(ART02.2) **The Templeton Paleoindian Site: Investigating a Buried Terminal Pleistocene Age Artifact Bearing Deposit in Western Connecticut.**

Zachary Singer[1], Cosimo Sgarlata[2], David Leslie[3], William Ouimet[4], Dawn Beamer[5], John Wah[6]; *[1]Institute for American Indian Studies, [2]Western Connecticut State University, [3]Archaeological and Historical Services, Inc.; University of Connecticut, Anthropology Department, [4]University of Connecticut, Department of Geosciences, [5]University of Connecticut, [6]Matapeake Soil & Environmental Consultants*

The Templeton site is a stratified archeological site in western Connecticut, which contains a Paleoindian component buried approximately 1 meter below surface in silicate floodplain sediments. In this study, we present new data from 4 vibracores collected nearby the archaeological excavations. Sediment samples were analyzed via LOI, pXRF, and camsizer and new radiocarbon dates were obtained from the core material. Overall, the new dates and geochemical data provide an updated, high-resolution morphological model and relate floodplain and river phases to the timing of human presence on the landscape.

(ART02.3) **Pleistocene-Holocene Geomorphic Site Assessment for PaleoIndian Site 4-15, Avon, CT**

Samantha Dow[1], William Ouimet[1], David Leslie[2], Jonathan Leonard[3]; *[1]University of Connecticut, Department of Geosciences, [2]Archaeological and Historical Services, Inc.; University of Connecticut, Anthropology Department, [3]University of Connecticut, Department of Geography*

The Farmington River Valley in central Connecticut has recently yielded archaeological evidence of Early Paleoindian activity (site 4-10B) on a late Pleistocene terrace. Site 4-15, farther west along the Farmington River in Avon, CT is currently being investigated to determine if it is a potential second Paleoindian site. Here we present the results from a GPR survey and sediment core analysis at this new site in order to reconstruct the late Pleistocene through Holocene geomorphic history – in particular the landforms, stratigraphy, and incision history at the site. Regional geologic mapping suggests the area is characterized by a Holocene stream terrace. Closer examination of the topography at the site (including LIDAR DEM analysis) indicates multiple terrace levels and an abandoned paleo channel. Four sediment cores (ANR1- ANR4) were collected at the site using a vibracore apparatus – two on the lowest terrace levels and two in the abandoned paleo channel. Percent organic content of the sediment was determined using loss on ignition, and radiocarbon analyses were used to constrain stratigraphic ages. Core stratigraphy shows a change from fine grained glacial material or alluvial material underlying gravel indicative of an abandoned river channel, and finally the development of a wetland above the

fluvial material within the abandoned paleo channel. GPR data confirms a similar sequence of landscape change. Overall, this geomorphic information is useful in reconstructing the timing and environmental conditions of the region for Paleoindian activity.

(ART02.4) **Rediscovering Buried Pleistocene Landscapes using Ground-Penetrating Radar**

Peter Leach[1], David Leslie[2], Zachary Singer[3]; *[1]Geophysical Survey Systems Inc., [2]Archaeological and Historical Services, Inc.; University of Connecticut, Anthropology Department, [3]Institute for American Indian Studies*

Ground-penetrating radar [GPR] is an efficient, non-invasive, and readily available technology utilized in many field disciplines. As an archaeological method it facilitates a landscape-scale assessment of site layout, the detailed geophysical imaging of potential cultural features, and the vertical contextual evaluation of site components. Despite its increasing importance to archaeology, the geoarchaeological potential of GPR is often overlooked in the context of archaeological investigations. This is of particular concern for deeply buried Pleistocene-aged sites where individual features may have minimal dielectric contrast with surrounding media, occupation evidence may exhibit low density and cover a large area, or buried occupation surfaces are beyond the reach of traditional mid-frequency GPR antennas. Additionally, in active depositional areas (like floodplains) the paleotopography of Pleistocene landscapes may be markedly different than that of the modern surficial topography and could present difficulties for standard archaeological evaluation. This paper will discuss the geoarchaeological applications of GPR by presenting our research at two Paleoindian sites in Connecticut, USA. At the Templeton site, a relatively flat modern alluvial setting disguises a variable subsurface paleolandscape that was occupied in the middle to late Paleoindian period. At the Brian D. Jones site, a thick alluvial sequence has buried an expansive paleolandscape with a robust Paleoindian component. We deployed a newly developed GSSI 200MHz HyperStacking GPR antenna to perform “digital deep testing” to map and characterize these buried landscapes. Ground-penetrating radar data, interpretation, and results will be discussed, as will recommendations for performing similar research at other Pleistocene-aged sites.

(ART02.5) **Investigating post-glacial landscape recovery, river incision and landform stability in river valleys of southern New England**

William Ouimet[1], David Leslie[2], Amber Lee Nicoulin, Tom Schenck; *[1]University of Connecticut, Department of Geosciences, [2]Archaeological and Historical Services, Inc.; University of Connecticut, Anthropology Department*

Rivers terraces are ubiquitous features in the river and stream valleys of southern New England that attest to post-glacial landscape recovery, river incision and landform stability throughout the region. Here, we present a summary of numerous studies aimed at better understanding these landforms, their stratigraphy, and incision history. First and foremost, widely available high-resolution LiDAR Digital Elevation Models (DEMs) provide a vast improvement on previous datasets dealing the identification and mapping of terraces and paleo channel features throughout the region. Along the Farmington and Housatonic Rivers in Massachusetts and Connecticut, for example, LiDAR was used to extract longitudinal river profiles and compile information for 300 stream terraces ranging 2-20 meters above current water levels. More terrace levels and greater amounts of incision are consistently found downstream of bedrock knickpoints, highlighting the high degree of spatial variability that exists and the role that local bedrock and base-level controls have on river incision and the formation of terraces. Second, terrestrial vibracoring allows for the detailed investigating of the stratigraphy of terraces and paleo channel features, and can yield new insight on the timing of incision in the region. In one study, we used sediment cores within river adjacent kettles and sediment traps along rivers in Connecticut to document the drop in sedimentation associated with incision by the local river at the site. Analysis of four cores around the state revealed that incised consistently occurred between 12,000 to 9,000 yr BP. In other vibracoring studies, we have used multiple sediment cores collected in the vicinity of active archaeological sites on terraces in the region to

better characterize, date and correlate units across the site, and study the history of sedimentation and paleo-flood deposit in relation to human occupation and preservation of the discovered artifacts. Overall, this research is fundamental to understanding post-glacial landscape evolution in southern New England, and the methods used here can be easily transferred for use in other deglaciated regions around the world.

21AWD01: RSC Sir George Stokes Award Symposium Honoring Tuan Vo-Dinh

Chair: Tuan Vo-Dinh
On-site Chair: Laura Fabris

(AWD-01.2) **Applications of SERS in Biology and Medicine: from the Bench to the Clinic**

Laura Fabris[1], Manjari Bhamidipati[2], Hao Wang[3], Kholud Dardir[2], Sasanka Ulapane[2], Kevin Christian[2];
[1]Rutgers, the State University of New Jersey, [2]Rutgers University, [3]Duke University

The integration of biosensors into various industries can transform the ability to monitor personal and public health. Nanostructured biosensors, in particular, have pushed detection limits down to femtomolar and even attomolar concentrations by utilizing diverse sensing modalities. In particular, high sensitivity and specificity have been realized using surface enhanced Raman spectroscopy (SERS), which has been proven very useful in biomarker analysis. Our research has been focusing on designing and implementing sensing, and importantly, diagnostic platforms based on SERS with reduced costs and increased applicability, that however retain sensitivity and selectivity, and can be implemented in multiplex. In my talk, I will report on some recent results of our group, focused in particular on the detection of communicable and non-communicable diseases, on the low-cost forensic analysis of opioids carried out with portable Raman equipment, and on the development of streamlined approaches for substrate characterization and data analysis that can increase SERS quantification power without internal standards. I will discuss how gold nanostars can be leveraged to design switchable SERS/fluorescent probes for the detection of influenza A viral particles in buffer and individual intact cells with high selectivity, how SERS-based biosensors can be used for phenotype characterization in individual cancerous cells, and how these devices allow to efficiently stratify prostate cancer patients. Finally, I will discuss how we leveraged a low-cost portable Raman module to identify fentanyl in urine with LODs of 5 ng/mL and to detect it when laced in other drugs of abuse.

(AWD-01.3) **New Methods and Paradigms in Optical Sensing**

Brian Cullum[1]; *[1]University of Maryland Baltimore County (UMBC)*

This talk will describe the evolution of optical sensing in the Vo-Dinh laboratory over 40+ years, and his efforts and drive to continuously improve technology to answer pressing questions of the day, from energy and environmental monitoring to biomedical sensing. In addition, it will describe several of the latest advances in optical sensing technologies and paradigms from his laboratory as well as the Cullum lab and former students/postdocs and the bright future in optical sensing that lies ahead due to his expertise and inspiration. This talk will give an overview of the advent of new technologies/spectroscopic techniques developed and pioneered in Tuan's laboratory and the labs of his former students, postdocs such as synchronous luminescence, optical nanoprobes/nanosensors, optical coherence tomography, handheld Raman instrumentation, nanostars, SERS nanoimaging and the recently discovered THORS (Thermally-induced Optical Reflection of Sound).

(AWD-01.4) **Physical Ultrasonics – From Microspheres to Manipulation**

Joel Mobley[1]; *[1]The University of Mississippi*

Physical ultrasonics encompasses a broad range of topics from the physics of wave propagation to the physical effects of high power sound. This talk will describe multiple lines of research in ultrasonics beginning with my time as a postdoc in the Vo-Dinh group at ORNL, as well as Tuan's influence on my career. The topics

discussed will include the work with Tuan on transcranial ultrasound and photoacoustics, to subsequent research on complex media, inertial cavitation and near-field acoustic tweezers.

(AWD-01.5) **Bioengineering for COVID-19: Rapid Acceleration of Diagnostics (RADx) at Unprecedented Speed and Scale**

Bruce J. Tromberg[1]; [1]*National Institute of Biomedical Imaging and Bioengineering/NIH*

Abstract The NIH Rapid Acceleration of Diagnostics (RADx) initiative was launched on April 29, 2020, just 5 days after a Congressional directive to expand the number and type of SARS-CoV2 diagnostic technologies. This talk introduces principles of the RADx Tech "innovation funnel" designed to evaluate, validate, and scale up promising technologies for laboratory, point of care, and home settings. More than 700 applications were submitted to the innovation funnel and reviewed on a rolling basis over a ~3-month period. Early stage funding was provided to more than 100 projects, many of which involved cutting edge nanotechnologies and materials. Approximately 4% of applicants successfully competed for larger phase 2 contracts to support manufacturing expansion and clinical studies. Phase 2 companies received 23 FDA EUAs and increased US capacity by >390 million new tests and test products between September 2020 - May 2021. RADx has accelerated innovation by compressing the typical multi-year tech commercialization process into ~6 months. This technologic transformation is driving a paradigm shift from lab-based testing of symptomatic individuals to more accessible home-based screening and surveillance for personalized medicine.

21CHEM02: Chemometric Theory in Practice

Chair: Karl Booksh
On-site Chair: Karl Booksh

(CHEM-02.1) **Handling Noise in Portable Instrumentation**

Karl S. Booksh[1], James A. Jordan[2], Barry K. Lavine[3], Caelin Celani[1], Michael E. Ketterer[4]; [1]*University of Delaware,* [2]*National Geospatial Intelligence Agency,* [3]*Oklahoma State University,* [4]*Northern Arizona University*

Discretization that is intelligently converting a continuous distribution of instrumental responses to a smaller set of digital outputs, is a powerful technique to declutter and denoise data collected from hand-held instrumentation. The increasing availability of hand-held portable spectrometers is ushering a paradigm shift in how classification applications are approached – we are shifting from a smaller number of high resolution and high signal to noise measurements to rapidly analyzing larger numbers of samples in the field. The characteristics and properties of these samples are consequently determined by classification methods such as PLS-Discriminant Analyses or Support Vector Machines. However, the greater levels of noise and clutter in hand-held instrumental applications requires new approaches to maximizing the performance of hand-held analyses strategies. This talk will look at discretization as a superior method to External Parameter Orthogonalization (EPO) for classification problems employing hand-held Laser Induced Breakdown Spectroscopy (LIBS) and hand-held X-Ray Fluorescence (XRF) analyses.

(CHEM-02.2) **Variable Selection applied to Portable Instrumentation**

Barry K. Lavine[1], Collin White, Karl S. Booksh[2], Michael E. Ketterer[3], James A. Jordan[4]; [1]*Oklahoma State University,* [2]*University of Delaware,* [3]*Northern Arizona University,* [4]*National Geospatial Intelligence Agency*

Hand-held instrumentation is playing an increasingly prominent role in scientific investigations. With hand-held instruments, analysts can more deftly sample and analyze large numbers of samples in the field than can be accomplished using laboratory based methods. The hand-held sensing strategy offers the advantage of increasing the sample throughput, thereby providing results in real-time and enabling a dynamic experimental design that adapts to areas of circumstances of particular interest. The key to realizing successful applications of

hand-held instruments can be found in the development of multivariate methods – that is chemometrics and machine learning – to maintain a favorable trade-off between the increase in mobility and ease of use while minimizing losses of signal to noise and chemical resolution that are an inherent part of instrumentation miniaturization. In this presentation, a genetic algorithm for variable selection that enhances the discrimination of collections of instrumental data by improving the confidence level for the classification of samples that lie in multiple discrete locations in the pattern space is discussed. Although problems in overfitting and validation of classification models have been the subject of numerous studies, confidence estimates for model inference have been largely ignored. Three data sets will serve as testbeds to demonstrate the value of this new methodology for variable selection: (1) classification of Dalbergia sub-species using hand-held laser induced breakdown spectroscopy (LIBS), (2) differentiation of commercial colored glasses based on their metal oxide or metal sulfide content by LIBS, and (3) discrimination of pine trees to identify their region of origin using LIBS and X-ray fluorescence (XRF) spectroscopy. Successful data analysis methods developed in the test experiments such as those described here will become part of routine analytical practice in the hands of experimenters using LIBS and XRF spectroscopy.

(CHEM-02.3) **Application of Hand-Held Technologies and Chemometrics to Forensic Investigations in Illicit Narcotics**

James A. Jordan[1]; [1]*National Geospatial Intelligence Agency*

Hand-held instruments such as XRF and LIBS, are playing an increasingly prominent role in scientific investigations. These relatively small and low power devices have fueled much interest in the forensics community, by providing timely analyses while minimizing issues with sample collection, transport, and storage. The growing emphasis on field testing using analyzers in non-laboratory settings has become essential for interdiction of illicit materials such as heroin and fentanyl seized at ports of entry. The key to successful applications of hand-held and portable instrumentation lays in the development of matrix matched reference materials and multivariate analyses methods – that is chemometrics – to maintain a favorable trade-off between the increase in mobility and ease of use while minimizing losses in signal-to-noise (S/N) and chemical resolution. S/N, chemical resolution, and calibration are often sacrificed when miniaturizing an instrument for placement in a mobile platform. However, chemometric methods, e.g., quantitative multivariate calibration and factor analyses have the potential to mitigate these detrimental effects. The increasing role of hand-held devices depends upon extending methods and data processing task to devices not explicitly designed for the task. This project addresses critical knowledge that is lacking for the development and implementation of classification models to low resolution data of the type of data that the Drug Enforcement Administration (DEA) or Customs and Border Protection (CBP) would expect to obtain from applying hand-held chemical analyzers in a challenging field environment. A primary goal of this project is the examination of illicit narcotics by hand-held XRF and LIBS. Additionally, this project investigates chemometric methods for classification which incorporate variable selection and data fusion to maximize statistical confidence model inferences aimed at identification of the illicit material and its relationship to known reference materials.

(CHEM-02.4) **Characterization of Chinese Celadon Ceramics by LIBS and XRF**

Marcie Wiggins[1], Marcie Wiggins[1], Richard R. Hark[2], Chandra S. Throckmorton[3], Katherine Peters, Amreet Kular, Aniko Bezur[2]; [1]*Yale Institute for the Preservation of Cultural Heritage,* [2]*Yale University, Institute for the Preservation of Cultural Heritage,* [3]*Signal Analysis Solutions*

Yale's Institute for the Preservation of Cultural Heritage (IPCH) has carried out an investigation into the chemical makeup of over 100 Chinese celadon and celadon-style ceramic objects dating from the 9th to 14th centuries from the Yale University Art Gallery (YUAG) collection. The goal of this study was to characterize these ceramics according to the chemical elements present in the clay bodies and glazes to inform our investigation into the geographic provenance of these objects. Objects with similar elemental signatures may

have been created in the same regions/provinces. Laser-induced breakdown spectroscopy (LIBS) and X-ray fluorescence spectroscopy (XRF) were both used due their differing elemental sensitivity. Datasets from each technique, as well as a fused dataset, were used for classification using several chemometric algorithms to group objects based on their elemental signatures. The materiality of these celadon and celadon-style artifacts combined with an art historical perspective have added to our understanding of how the style and craftsmanship of these ceramics spread throughout China. Additionally, this project allowed us to evaluate the utility of field-ready, portable instrumentation (handheld LIBS and pXRF) for the large-scale survey of cultural heritage objects.

(CHEM-02.5) **Analysis of Geological Sample Suites with Laser-induced Breakdown Spectroscopy**

Richard R. Hark[1], Russell S. Harmon[2], Chandra S. Throckmorton[3], Michael A. Wise[4], Peter A. Defnet[5], Keith Hilferding[6]; *[1]Yale University, Institute for the Preservation of Cultural Heritage, [2]Department of Marine, Earth and Atmospheric Sciences, North Carolina State University, [3]Signal Analysis Solutions, [4]Department of Mineral Sciences, National Museum of Natural History, Smithsonian Institution, [5]Department of Chemistry, University of Washington, [6]Department of Chemistry, Juniata College*

Laser-induced breakdown spectroscopy (LIBS) combined with chemometric analysis provides a powerful and straightforward technique for the examination of geological materials. Since all elements emit in the 200–900 nm spectral range of the LIBS optical emission geochemical fingerprinting can be achieved using the full broadband spectrum. The advent of commercial handheld LIBS instruments allows for rapid, high throughput, in situ analysis of a wide variety of geomaterials. This presentation will describe how the application of machine learning tools, such as principal component analysis (PCA), partial least squares discriminant analysis (PLS-DA), and linear support vector machine classification (SVM), to LIBS data obtained from large spectral reference libraries allows one to address important questions related to mineral identification, elemental distribution, stratigraphic correlation, provenance determination, and natural resource exploration. Examples will include investigation of carbonate minerals and rocks, so-called 'conflict minerals' (e.g., columbite-tantalite, cassiterite), native gold, and obsidian. In addition, recent work on the validation of labels assigned to garnets specimens in museum collections and discrimination of LCT (lithium-cesium-tantalum) and NYF (niobium-yttrium-fluorine) pegmatites based on garnet geochemical fingerprinting will be addressed. In that example, electron microprobe analysis accompanied the LIBS data collection for a suite of 208 garnets from 24 countries. The success rate for many of these classification tasks is often >90%, further demonstrating the value of chemometric analysis of LIBS data for addressing questions of importance in the earth science field.

21FORENS04: Analytical Chemistry of Nuclear Materials

Chair: Robert Lascola
On-site Chair: Robert Lascola

(FORENS-04.1) **Applications of laser-induced breakdown spectroscopy for chemical analysis of nuclear materials**

Ashwin P. Rao[1], Ryan E. Pinson[1], Phillip R. Jenkins[1], Anil K. Patnaik[1]; *[1]Air Force Institute of Technology*

We present recent advances using laser-induced breakdown spectroscopy (LIBS) coupled with chemometric and machine learning techniques for rapid chemical analysis of materials of interest to the nuclear community, to include lithium compounds, nuclear debris, and plutonium alloys. Recent work demonstrates the capability of LIBS to perform rapid quantification of oxidation ingrowth compounds in different Li-based materials to study the effects of environmental aging. Additionally, a portable LIBS device coupled with tree-based ensemble regression methods is shown to perform in-situ analysis of trace elements, such as Ga, in Pu alloys with detection limits in the low tenths of a percent. Finally, a comprehensive machine learning design experiment was conducted using spectra of cerium-gallium samples taken with a high resolution Echelle spectrograph.

These tuned models yielded robust predictive regressions, with techniques such as extra trees able to generate models with detection limits as low as 60 ppm Ga. The integration of novel machine learning methods to these complex analytical spectroscopy problems sheds light on the potential of several promising new techniques for rapid, robust elemental analysis of nuclear material spectra.

(FORENS-04.2) **In situ Raman/Infrared Surface Spectroscopy to Monitor Environmental Changes of Solid Materials**

Tanya L. Myers[1], Danielle L. Saunders[1], Russell G. Tonkyn[1], Catherine A. Banach[1], Kai-For Mo[1], Ashley M. Bradley[1], Carlos G. Fraga[1], Timothy J. Johnson[1]; [1]*Pacific Northwest National Laboratory*

In fifteen words or less, explain the significance of this contribution (Novel Aspect).: Sampling methodology for monitoring environmental (T, RH) change in solids via Raman and hemispherical infrared.

Abstract Text: We have recently been investigating the chemical transformation of multiple solid materials in outdoor environmental settings. As many compounds weather and age, e.g. due to hydrolysis, the parent chemicals transform to other species. We have developed simple and effective microchambers to provide fixed atmospheres, namely bale jars outfitted with temperature and humidity sensor/loggers. To monitor the chemical changes, we have developed simultaneous IR and Raman sampling apparati and measurement protocols to directly measure both the IR and Raman spectra from an extended sample surface without any sample preparation. Both the FT-Raman and FT-IR have proven very effective at probing chemical change without disturbance for solid materials. The method is demonstrated for solids such as methyl phosphonic anhydride, ammonium nitrate, etc.

(FORENS-04.3) **Laser-Induced Annealing of Luminescence in Aged PuO2**

Eliel Villa-Aleman[1], Don Dick[1], Jonathan Christian[1], bryan Foley[1]; [1]*Savannah River National Laboratory*

In fifteen words or less, explain the significance of this contribution (Novel Aspect).: Luminescence and Raman spectroscopy can provide information on the age of PuO2 since last calcination.

Abstract Text: Savannah River National Laboratory (SRNL) conducted spectroscopic investigations of Plutonium dioxide (PuO2) calcined at different temperatures using vibrational (Raman and infrared) and luminescence spectroscopy. In these studies, alpha particle-induced damage of the PuO2 crystal lattice was tracked by observing changes in the full-width half maximum (FWHM), band intensity, band position, and growth of defect bands in the Raman spectrum. Time dependent broad luminescence was also observed and was found to grow as the material ages. An automated laser-induced annealing technique was developed to reverse damage to the crystal lattice while allowing in situ observation of the PuO2 Raman and luminescence spectrum. High resolution laser-induced annealing of lattice damage in PuO2 was demonstrated by following the defect band intensity and the bands' FWHM as the temperature was raised to 1400□C. Thus, we show, for the first time, the spectrum of aged PuO2 and its laser-induced annealing curve. The annealing curve and its correlation with the laser-induced annealing of the Raman bands PuO2 will be discussed.

(FORENS-04.4) **Spectroscopic Analysis of Aged Kapton®**

Greg Klunder[1], Christian Grant[1], Amitesh Maiti[1], Christy Fox[1], Mihail Bora[1], Ari Reider[1], Jeremy Armas[1], Richard gee[1]; [1]*Lawrence Livermore National Laboratory*

In fifteen words or less, explain the significance of this contribution (Novel Aspect).: Determining materials aging and compatibility is important for predicting performance lifetimes.

Abstract Text: Kapton® is a robust inert polyimide which has many applications due to its chemical inertness, and excellent electrical, mechanical, physical properties over a large range of environmental conditions. Understanding how Kapton® material changes with aging is critical for long term applications. The chemical name of Kapton® is pyromellitic dianhydride-co-4, 4′-oxy-dianiline (PMDA-ODA) and can be prepared with calcium biphosphate an anti-slip additive (Kapton® HN). Depending on the applications, Kapton® can be applied by spin-coating as a solution onto a substrate and thermally curing or applied as a precast film using an adhesive and thermally curing with pressure. In this study, we are focusing on Kapton® films and layered parts which have been artificially aged under various environmental conditions. Determining performance changes due to aging is a destructive process and there is great benefit to identify the state of the material prior to failure. Spectroscopic methods including visible-near infrared, FTIR and Raman have been employed to assess changes due to accelerated aging. For Kapton® films, subtle changes in the visible spectra provided a linear correlation with aging, however, no correlation was observed with ATR-FTIR spectroscopy. In the layered parts, the spectroscopic changes that were observed can be attributed to the adhesive and not necessarily the Kapton®. This presentation will address the experimental aging conditions, spectroscopic measurements and data analysis. This work was performed under the auspices of the United States Department of Energy by Lawrence Livermore National Laboratory under Contract DE-AC52-07NA27344.

(FORENS-04.5) **Online Monitoring of Offgases Associated with Nuclear Materials Processing**

Robert Lascola[1], Patrick O'Rourke[1], Ron Jeffcoat[1], David Immel[1]; [1]*Savannah River National Laboratory*

In fifteen words or less, explain the significance of this contribution (Novel Aspect).: High precision spectroscopic measurements of radiological gas streams in nuclear materials processing

Abstract Text: We have developed and installed a Raman spectrometer for online monitoring of offgases produced during the dissolution of nuclear materials at the Savannah River Site's H Canyon processing facility. The instrument produces real time measurements of percent concentrations of NOx, H2, N2, O2, and other gases. The effects of facility operations and events within the dissolver are observed in real time. Measurement precision of NO2, the primary component of the gas stream when it reaches the analyzer, is approximately 0.01%. This is sufficient to permit detection and real time prediction of the dissolution endpoint against background levels associated with the evolution of dissolved gas. This capability is critical for facility operations, as various components of the nuclear fuel bundles dissolve at different rates. Incomplete dissolution of charged material delays the introduction of subsequent charges to the dissolver and slows overall processing rates. This application is a relatively rare example of the use of Raman spectroscopy for the monitoring of gas streams in radiological applications. Lessons learned and opportunities for improvement will be discussed.

21IR07: Advances in Determination of Molecular Orientation and Interactions by Infrared Spectroscopy

Chair: Benedikt Schwarz
On-site Chair: Rohith K Reddy

(IR-07.1) **Imaging 3D molecular orientation by concurrent-polarization infrared microscopy**

Shuyu Xu[1], Chad Snyder[1], Young Jong Lee[1]; [1]*National Institute of Standards and Technology*

In fifteen words or less, explain the significance of this contribution (Novel Aspect).: This analytical method extracts 3D orientation angles from 2D polarization-controlled IR imaging.

Abstract Text: Microscopic molecular orientation affects the macroscopic physical and chemical properties of materials and is critical to performances and long-term reliability of materials. For molecular orientation measurement, conventional optical imaging methods are based on two-dimensional (2D)-projected,

polarization-controlled signals, which cannot measure the 3D orientation angles, particularly the out-of-plane angle, of molecules. Due to the inaccessibility to out-of-plane directions, 2D-projected orientation measurement can characterize the orientational distribution properly only for samples which symmetry axis is pre-defined onto the polarization plane. In this work, we propose and demonstrate a numerical method of polarization-controlled infrared (IR) microscopy that can determine the full 3D angles and the order parameter of the molecular orientation at each image pixel by concurrently analyzing the 2D polarization-dependent absorption of two orthogonal transition dipoles. We applied this concurrent analysis to map the 3D orientations of polymer chains in a semicrystalline poly(e-caprolactone) film from hyperspectral data measured from hyperspectral data measured by a wide-field quantum cascade laser (QCL) IR microscope. By comparing the images of the 3D orientations of mechanical deformation and orientational relaxation. We further show the ability to distinguish the orientational differences in both the crystalline and amorphous phases, which unravel the orientational structures of chains and lamellae in the spatially heterogeneous semicrystalline polymer.

(IR-07.2) **Crystallography without X-Rays: New Insights into Metal-Organic Framework Film Structures Using Polarization-Dependent IR Spectroscopy**

Bettina Baumgartner[1], Ken Ikigaki[1], Kenji Okada[1], Masahide Takahashi[1]; [1]*Osaka Prefecture University*

In fifteen words or less, explain the significance of this contribution (Novel Aspect).: Polarization-dependent infrared spectroscopy of metal-organic framework films fills the information gap left by diffraction methods.

Abstract Text: Material development and utilization constantly demands for new experimental tools to contribute to the understanding of structure-function relationships. Metal-organic framework (MOF) films consist of organic linkers and metal-containing units that form porous materials with great variety and multiplicity regarding constituents' geometry, pore size and functionality. Their fields of applications are equally manifold and include photonics, energy-related, catalysis, gas and fuel storage, or (bio-)sensing. The structure-function relationship of MOFs largely relies on pore alignment and linker orientation. We report a simple and fast infrared spectroscopic method allowing to assess this information so far not accessible with standard methods employed until now e.g. diffraction techniques or infrared reflection absorption spectroscopy (IRRAS). Cu-based MOF films were studied with polarization-dependent FTIR spectroscopy in transmission and attenuated total reflection(ATR) configuration. The polarization-dependent ratio of the band areas of the carboxylate vibration in the IR spectra recorded in transmission allowed for determine the degree of in-plane orientation. The obtained values were in great agreement with conventional XRD data. The high sensitivity of multibounce ATR crystals bears comparison with IRRAS but at the same time provides structural information in all three axes. This enabled us to clearly distinguish MOF films in different crystallographic orientations. Besides confirming the film orientation and comparing favorably with results from XRD measurements just obtained at shorter time scales, additional structural information was retrieved: The orientation of the aromatic linker in the 3D MOF Cu2(BDC)2DABCO (BDC: 1,4-benzenedicarboxylate, DABCO: 1,4-Diazabicyclo[2.2.2]octane), to date inaccessible with conventional techniques, and highly essential for the accessibility of the pores, was determined to be parallel to the 2D MOF sheets and perpendicular to the bridging carboxylate plane. Furthermore, the initial orientation of MOF films, otherwise only feasible with synchrotron techniques due to the low amount of material, could be investigated. Experimental IR spectra correlate with theoretical explanations, paving the way to expand the principle of orientation studies with polarization-dependent IR spectroscopy to oriented, organic-inorganic hybrid materials beyond MOFs.

(IR-07.3) **Transport and Interactions of Hazardous Chemical Agents with Metal-Organic Frameworks**

Isabella Goodenough[1], Binh-An Nguyen[1], Mattheus De Souza[2], Prasenjit Das[2], Nathaniel Rosi[2], Eric Borguet[1]; *[1]Temple University, [2]University of Pittsburgh*

In fifteen words or less, explain the significance of this contribution (Novel Aspect).: We developed a novel, label-free vibrational spectroscopic method to monitor molecular transport in Metal-Organic Frameworks.

Abstract Text: Porous Metal-Organic Frameworks (MOFs) have potential as superior sorbent materials capable of capturing, transporting and neutralizing hazardous chemical agents, such as chemical warfare agents (CWAs). The UiO family of MOFs, in particular, offer a high degree of chemical, structural and thermal stabilities making them amenable for a wide-range of protective applications. A combination of in situ FTIR spectroscopy and Temperature-Programmed Desorption Mass Spectrometry (TPD-MS) are applied to understand the uptake, transport and desorption interactions of the nerve agent simulant, dimethyl methylphosphonate (DMMP), acetone and isopropanol with UiO-series MOFs. Acetone and isopropanol have been suggested as a simple and benign alternative to some traditional CWA simulants, providing detailed information on the structure-activity relationship of key structural functionality of live agents and MOFs, while minimizing experimental exposure risk. Using in situ FTIR, we monitor analyte transport as molecules diffuse isothermally from the external MOF surface into the interior MOF pore environment. Through this label-free vibrational spectroscopy, we find that the local environment surrounding the μ3-OH groups on the MOF zirconium node is sensitive to polar analytes, where the hydrogen bond strength is impacted by the polarity of the analyte. Ultimately, this multi-technique approach enables a fundamental understanding of CWA simulant interactions and informs the rational design of MOF materials with diverse functionality capable of selective analyte uptake and transport mechanisms necessary for superior filtration devices.

(IR-07.4) **Optical and Chemical Characterization and Identification of Crystalline Structures in Cannabis Solvent Extracts**

Otyllia R. Abraham[1], Ruth Waddell Smith[2]; *[1]Microtrace LLC, [2]Michigan State University*

In fifteen words or less, explain the significance of this contribution (Novel Aspect).: Characterization and identification of cannabis solvent extracts to differentiate marijuana and hemp products.

Abstract Text: Marijuana and hemp represent two broad classes of Cannabis sativa plants that are distinguished based on the concentration of the psychoactive cannabinoid delta-9-tetrahydrocannabinol (Δ9-THC). Cannabinoids are commonly extracted from marijuana and hemp using organic solvents or supercritical CO2 to generate cannabis solvent extracts. These solvent extracts typically contain total concentrations of Δ9-THC or cannabidiol (CBD) (depending on extraction from marijuana or hemp) that are six to eight times more potent than the plant material alone. Macroscopically, cannabis solvent extracts appear orange/brown in color and can range in texture from glass-like shards to sticky, amorphous materials. Microscopically, at low magnification, the amorphous material is composed of two distinct components: well-formed crystalline material and an amorphous, waxy matrix. Cannabis solvent extracts are typically analyzed by gas chromatography-mass spectrometry (GC-MS) to identify the cannabinoids present. However, using this method, the bulk extract is analyzed rather than the crystalline and wax components separately. As such, characterization and identification of the major cannabinoids present in either the wax matrix or the crystalline component have not yet been accomplished. In this work, the crystalline components of marijuana-derived and hemp-derived solvent extracts were optically and chemically characterized and identified. For optical characterization, representative crystals were analyzed via polarized light microscopy (PLM). Morphological and crystallographic differences, including optic sign, crystal system, and principle refractive indices, were used to differentiate marijuana-derived extracts from hemp-derived extracts. Analysis of the crystals via infrared (IR) spectroscopy indicated the presence of tetrahydrocannabinolic acid (THCA) in the marijuana-derived crystals

and cannabidiol (CBD) in the hemp-derived products. Definitive identification was achieved using single-crystal X-ray diffraction, which also reflected and confirmed the crystal systems determined by PLM. Results from each technique will be presented and comparison of characterization methods and chemical compositions will be discussed.

(IR-07.5) **Integration of Mid-Infrared Spectroscopy and Luminescence-based Optical Sensing for *In Situ* Studies on Biofilm Formation**

Diellza Bajrami[1], Stephan Fischer[2], Holger Barth[2], María Fernández García[3], Boris Mizaikoff[4]; *[1]Institute of Analytical and Bioanalytical Chemistry, Ulm University, Germany, [2]Institute of Pharmacology and Toxicology, Ulm University Medical Center, Germany, [3]Institute of Dairy Products of Austurias, IPLA-CSIC, Villaviciosa, Spain, [4]Institute of Analytical and Bioanalytical Chemistry, Ulm University and Hahn-Schickard, Institute for Microanalysis Systems, Ulm, Germany*

In fifteen words or less, explain the significance of this contribution (Novel Aspect).: Development of a new generation of combined chemical sensing technologies integrating complementary optical sensing principles

Abstract Text: Bacterial contaminations are critical in the food industries as sources of contaminating pathogens initiating foodborne illnesses and other bacteria-related diseases associated with biofilm formation. Biogenic amine producers (BAPs) formed in food via the activity of lactic acid bacteria invoke toxicological effects on the digestive and respiratory systems. Among them, biofilms of *Lactobacillus parabuchneri* produce histamines rendering understanding of the involved molecular mechanisms of biofilm formation and metabolic pathways essential. In the present study, we demonstrated a bi-functional integrated chemical sensing technology for fundamentally understanding biofilm formation at a molecular level by combining mid-infrared (MIR) spectroscopy and fluorescence sensing schemes. MIR absorption via attenuated total reflectance (IR-ATR) is an established analytical method providing insight into chemical changes during the early stages of biofilm formation. Complementarily, dye-based luminescence sensing enables the simultaneous determination of, e.g., oxygen, pH and CO_2. Hence, the integration of IR with orthogonal sensing technologies based on a novel IR-ATR flow cell concept with fluorescence dye sensing spots immobilized in between the discrete evanescent field sensing spot along an ATR crystal surface is an innovative approach. Thereby, contactless fiberoptic oxygen sensors, retractable microsensors, and flow-through cells with integrated oxygen sensors enable monitoring oxygen (O_2) concentration gradients within biofilms as a relevant metabolic parameter. Simultaneous IR and oxygen measurements during biofilm formation of gram-positive *L. parabuchneri* confirm the utility of this method. The characteristic IR spectra show significant changes of the amide bands, lactate, and extracellular polymeric substances, which are the major components of biofilm maturation involved in the initial adhesion processes. The microaerophilic environment promotes oxygen depletion after the early stages of biofilm formation via oxygen concentrations dropping from 9 mg/L essentially to zero, thereby confirming the metabolic consumption by facultative *L. parabuchneri* within the biofilm. In summary, the biofilm formation process has been dynamically monitored, molecularly analyzed, and structurally understood facilitating the subsequent development of suitable prevention strategies. **Keywords:** biofilm formation, mid-infrared, IR-ATR spectroscopy, orthogonal sensing, luminescence optical microsensors, oxygen monitoring, *L. parabuchneri* biofilms

21PMA01: Manufacturing of the Future: Innovative PAT Tools and Advanced Process Control

Chair: Claudia Corredor
On-site Chair: Claudia Corredor

(PMA-01.1) **How healthy is your culture? Cell death detection using dielectric spectroscopy**

Suyang Wu[1], Stephanie Ketcham, Claudia Corredor[2], James K. Drennen[3], Carl A. Anderson[1]; [1]*Duquesne University,* [2]*BMS,* [3]*Duquesne University Graduate School of Pharmaceutical Sciences, Duquesne Center for Pharmaceutical Technology*

In the last decade, biologics, especially the monoclonal antibody (mAb), have experienced rapid development. However, the price of biologics is often prohibitively high because of the low process efficiency. Delay of the inevitable death of production cells during the cell culture process improves productivity. A successful delay relies on the monitoring of the onset of cell death, which indicates the timing of preventive action and/or sampling for information-rich off-line analysis, e.g. proteomics. Apoptosis, the primary regulated cell death pathway, incurs various physical properties change in cells, e.g. ion exchange and premature membrane permeabilization. These lead to changes in the dielectric property, which can be monitored by the dielectric spectrometers, such as ABER Futura Biomass Probe and Hamilton Incyte Biomass Sensor. This project proposes to monitor early cell death events in fed-batch production bioreactors using dielectric spectroscopy. Early cell death was measured by flow cytometery using stains marking caspase activation, loss of plasma membrane asymmetry, and premature plasma membrane permeabilization. The multivariate analysis was applied to quantitatively correlate the dielectric spectra and early cell death. The observed early cell death occurred hours before the viability drop described by the trypan blue, providing a time window for subsequent control actions. In addition, a flow-through microscopic camera system was implemented in the production bioreactor run. The artificial intelligence classification system recognizes the cell aggregates and cell debris. The size of aggregates, along with the number of aggregates and debris, correlated with cell death progress. Therefore, they were used as secondary cell death indicators for cell death.

(PMA-01.2) **Ensuring good analytical sampling by fiber optic probes inserted into moving powder beds**

Stephen V. Hammond[1], Philip Doherty; [1]*Expo Pharma*

The foundation of any analytical method is good sampling. If sampling is not representative of the material to be analyzed the whole measurement system is compromised. This basic rule holds true for PAT measurements, but is sometimes neglected, especially when considering moving powder beds. Engineering solutions have been developed that allow the insertion of diffuse reflection probes into powder streams. Each probe interface is designed to be compatible with the characteristics of the sample material. The capability to rapidly sample the powder bed of different processing steps, measure performance and understand the how the process is operating. In the same way, the use of a NIR probe inserted into the feedframe of a tablet press, as a means to support the fast release of the product has emerged as a viable option for a continuous process, that can easily be applied to common batch processes. By use of an off-line device that simulates the feedframe of a tablet press, the sampling characteristics and capability of diffuse reflectance probes inserted into a tablet feedframe can be assessed using a minimum amount of material outside of a GMP manufacturing environment. Key drivers considered for development of the measurement systems were speed of sampling, relevant (unit dose) contributing mass of material, and the ability to detect and react to disturbances in the system. In this area information on sampling characteristics have been captured and used to integrate a probe into at multiple points is SOD manufacturing. An important facet of the practical implementation of these engineering devices 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. A software platform has been developed that can in realtime filter out such disturbances allowing only “clean” spectra to be used for prediction of powder characteristics. This presentation will describe the spectroscopy, engineering development and supporting software that has contributed to a highly capable measurement and control system deployed in SOD manufacturing.

(PMA-01.3) **Smart PAT for development and manufacturing; inform, understand, monitor and optimise**

Aparajith Bhaskar[1], Darren Whitaker, John Mack; [1]*Perceptive Engineering*

Process Analytical Technology (PAT) is used widely to facilitate continuous pharmaceutical manufacturing. It provides greater insight and depth into processes and products in both the process development and routine manufacturing environments. Technologies such as vibrational or electronic spectroscopy are rich data sources that when coupled with multivariate data analysis have the power to provide vital information about the critical quality attributes (CQAs) of any process. This plays a key role in applications that provide advisory "open loop" functions such as predicting product Quality Attributes and in-process monitoring. The ability to provide accurate and fast Quality Attribute predictions also offers the opportunity to implement Real Time Release of product. Most "traditional" applications of PAT involve taking pre-determined measurements from the process to generate spectroscopic data and passing this through modelling techniques such as Partial Least Square (PLS) Regression and Principal Component Analysis (PCA). The models generated are then used to predict the CQAs of the process and develop applications as described above. While this scheme reflects the common operating mode, there are many other use cases of PAT where a model is not needed, and the data can be acted on immediately. Furthermore, PAT can be employed in the implementation of Advanced Process Control (APC) and, Machine Leaning based self-optimisation or "smart data" generation for process development. PAT powered APC implementations deliver improved control of inferred CQAs via feedback control of the process. These approaches generally require an already developed and reasonably well understood process. On the other hand, the use of PAT during the development process enables the use of ML to drive inferred CQAs to desired optimal values even when the operating space is not that well understood, this can reduce both time and material usage. In this talk, we will present a series of case studies in Pharmaceutical Manufacturing that highlight the use of PAT in these areas.

(PMA-01.4) **Raman Feed-Back Control During Fed-Batch Platform Processes: Beyond Glucose Control**

thaddaeus A. Webster[1], Brian Hadley[1], carrie Mason[1]; [1]*Lonza*

In fifteen words or less, explain the significance of this contribution (Novel Aspect).: Inline Raman monitoring of amino acids for feed-back control of complex feeds

Abstract Text: The adjustment of nutrient feed rates requires careful monitoring of critical process parameters (CPPs) to ensure that mammalian cell cultures are provided with the correct level of nutrients during fed-batch production. Historically, nutrient feed-rate adjustment has relied on daily offline measurements of CPPs that applies a static feed-rate to a dynamic environment. For platform processes, this can lead to cell lines with high utilization rates being under fed while cell lines with lower utilization rates are being overfed. Both scenarios are undersireable as this can lead to premature cell death and lower culture productivity. Ideally, dynamic feed rate adjustment in response to a change in a CPP should ensure that as the culture progresses it is being provided the nutrients it requires. In order to achieve this an inline monitoring method that can provide near real time information about CPP's is required. One potential solution is the use of inline Raman spectroscopy to continuously monitor the process for novel parameters that can be used to adjust the resulting nutrient feed-rate during culture. Inline Raman spectroscopy has been shown to enable monitoring and control of glucose containing feeds during fed-batch mammalian cell culture on platform processes, leading to improvements in product titers. This presentation expands the use of Inline Raman spectroscopy for feed-back control beyond previous work done with glucose. Raman models for two amino acids were developed to continuously monitor changes in their concentration during fed-batch culture of CHOK1SV GS-KO® cell lines cultured on a platform process. Utilizing inline Raman models for these two amino acids enabled real time adjustments to the feed-rates of two different nutrient feeds in response to the dynamic metabolic activity of the culture. Moreover, maintaining the concentration of these two amino acids at specified targets enabled more consistent process performance across different cell lines with different metabolic behaviors cultured on a platform process. Coupled with a previous Raman model for glucose, the data presented highlights how all nutrient feeds within a platform process can be automated via Raman spectroscopy, greatly reducing operator intervention and errors while delivering more robust process performance between runs.

21RAM03: IRDG Raman Spectroscopy Session

Chair: Karen Faulds
On-site Chair: Jason Dwyer

(RAM-03.1) **Towards improving SERS-based Sensing using Automated Nanoparticle Synthesis**

Samuel Mabbott[1], Suhash Chavva[2], John Dean[2]; [1]*Department of Biomedical Engineering, Texas A&M University; Center for Remote Health and Technologies & Systems, Texas A&M Engineering Experiment Station,* [2]*Texas A&M, Biomedical Engineering*

Since the accidental synthesis of a gold sol by Michael Faraday in the basement of the Royal Institution in 1857, researchers have employed noble metal nanoparticles in many applications, including biosensing. Over 100 years after Faraday's sol synthesis, British researchers, Faulk and Taylor, demonstrated one of the first biological applications of gold nanoparticles (AuNPs). The researchers described a method for antibody conjugation to colloidal gold enabling the visualization of salmonellae surface antigens using electron microscopy. Dubbed the "revolution in immunochemistry," this led to functionalized AuNPs being used in a wide range of biomolecule recognition assays. As the number of applications for noble metal nanoparticles grew, so did the research dedicated to understanding their synthesis and controlling their physical, catalytic, and optical properties. Researchers have carried out detailed studies of solution-based nanoparticle fabrication methods for controlling the dimensions of noble metal particles and many of them emphasize the careful selection of chemical reducing agents and the regulation of variables such as temperature, pH, and mixing rate. However, batch-to-batch irreproducibility of nanoparticles synthesized in research laboratories still remains an issue. In our case, it has often caused a bottleneck in the optimization of SERS biosensors. To overcome these issues, we have conceptualized and prototyped an instrument named the NanoSynth, which is capable of automatically synthesizing noble metal nanoparticles according to published methods. The apparatus controls reagent delivery, heating/cooling cycles, and mixing speeds to produce nanoparticles of a selected size without the need for user manipulation. In the talk, I will describe how the instrument works, the improvement it has made to developing our SERS sensors, and outline our future plans.

(RAM-03.2) **Identification of pathology specific signatures of tauopathy in mouse brain by FTIR spectroscopic imaging**

Michelle Bailey[1], Ryan S. Edginton[1], Charlie Jeynes[1], Mark D. Frogley[2], Gianfelice Cinque[2], Francesco Tamagnini[3], Francesca Palombo[1]; [1]*University of Exeter,* [2]*Diamond Light Source,* [3]*University of Reading*

In fifteen words or less, explain the significance of this contribution (Novel Aspect).: FTIR spectroscopic imaging was used to discriminate between transgenic and wild-type mouse models of tauopathy.

Abstract Text: Dementia is a progressive neurodegenerative disease and affects around 50 million people worldwide [1]. Alzheimer's disease (AD) is the most common form of dementia and is associated with the progressive accumulation of extracellular amyloid-B (AB) plaques and intracellular neurofibrillary tangles (NFTs). Previous work has demonstrated the capability of FTIR spectroscopic imaging and Raman microscopy to detect AB-plaque associated astrogliosis in AD mouse brain [2]. Mechanical mapping based on Brillouin microscopy enabled us to distinguish various regions within AB plaques characterised by different viscoelasticity [3,4]. In this work, we investigated tauopathy using infrared hyperspectral imaging with both benchtop (globar) and high brightness synchrotron sources, applied to a mouse model of tau disease. Principal component analysis (PCA) was used to highlight differences between transgenic (TG) and wild type (WT) mouse brain samples; classification of these data showed the ability to discriminate between TG and WT samples with high specificity and sensitivity. Synchrotron-FTIR spectroscopic imaging (Diamond Light Source, UK) was used to measure these samples with higher (cellular scale) spatial resolution. Significant differences were observed at the level of Amide I & II and lipid ester bands, plausibly associated with the presence of NFTs-bearing neurons. [1] The Global Dementia Observatory Reference Guide. Geneva, Switzerland: World

Health Organization; 2018. [2] Palombo, F., Tamagnini, F., Jeynes, J. C. G., Mattana, S., Swift, I., Nallala, J., ... & Stone, N. (2018). Detection of Aβ plaque-associated astrogliosis in Alzheimer's disease brain by spectroscopic imaging and immunohistochemistry. Analyst, 143(4), 850-857. [3] Mattana, S., Caponi, S., Tamagnini, F., Fioretto, D., & Palombo, F. (2017) Viscoelasticity of amyloid plaques in transgenic mouse brain studied by Brillouin microspectroscopy and correlative Raman analysis. Journal of Innovative Optical Health Sciences, 10(6), 1742001. [4] Palombo, F., Masia, F., Mattana, S., Tamagnini, F., Borri, P., Langbein, W., & Fioretto, D. (2018). Hyperspectral analysis applied to micro-Brillouin maps of amyloid-beta plaques in Alzheimer's disease brains. Analyst, 143(24), 6095-6102.

(RAM-03.3) **Bimolecular radiation response monitoring using Raman spectroscopy and data analytics**

Andrew Jirasek[1], Kirsty Milligan[1], Alison Deng[2], Ramie Ali-Adeeb[1], Phil Shreeves[2], Alexandre Brolo[3], Julian Lum[4], Jeffrey Andrews[1]; *[1]University of British Columbia, [2]University of British Columbia - Okanagan, [3]University of Victoria, [4]BC Cancer*

The recent technical advances in radiation therapy for cancer treatment have enabled exquisite dose sculpting to tumour volumes. Arc therapies, image guided treatment, and high dose fractions have enabled the ability to maximize radiation dose to a tumour volume while concomitantly minimizing dose to surrounding healthy tissue. However, there currently remains a lack of capability for accurate, dedicated technologies for the prediction and monitoring of patient response to radiation therapy, resulting in doses being prescribed based on prior population statistics rather than inherent patient radiobiological considerations. Raman spectroscopy (RS) has been shown to be a capable technique in monitoring biological response to ionizing radiation. The multiplex advantage of RS in being able to identify multiple classes of biochemicals allows for a unique capability for biomolecular dynamics of cellular and tissue environments to be probed within their host environment, thereby providing an overall picture of radiation response dynamics. However, this multiplexed advantage can come at a cost of complexity in spectral interpretability. Variable reduction techniques such as Principal Component Analysis (PCA) do not segregate loading vectors along physically meaningful vectors (in our case individual bio components and radiation), hence identifying subtle biochemical variations in response to radiation becomes difficult. We here employ alternate variable reduction strategies to identify biomolecular dynamics in cellular and tissue radiation response. We modify a standard non-negative matrix factorization (NMF) algorithm to restrict group and basis factors (GBR-NMF) to known biochemicals. Random Forrest (RF) classification allows for top response variables to be identified. As a result, we are able to identify the top, rank ordered, biochemical variations within a biological environment exposed to radiation. We show that using GBR-NMF, RF, and standard regression techniques, we are able to concomitantly identify known dynamics within cellular environments (e.g. glycogen glucose cycle). When applied to irradiated patient biopsy samples we show that the RS-GBR-NMF-RF technique is capable of segregating tissue samples along clinical parameters (e.g. Gleason score) and to also predict PSA vectors post treatment. In sum, RS with advanced data analytic strategies shows excellent potential for future progress in understanding and monitoring radiation response in cancer therapies.

(RAM-03.4) **Development of SESORS for Optical Medical Imaging Applications**

Matthew Berry[1], Evita Ning[1], Gareth Turnbull[1], Samantha McCabe[1], Hayleigh Kearns[1], Wenmiao Shu[1], Duncan Graham[1], Karen Faulds[1]; *[1]University of Strathclyde*

In fifteen words or less, explain the significance of this contribution (Novel Aspect).: The first report of SESORS for the through tissue imaging of bacterial pathogens.

Abstract Text: 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, nanotags were developed for the through tissue detection and multiplexed imaging of multiple bacterial pathogens including Escherichia coli (E. coli) and methicillin resistant Staphylococcus aureus (MRSA). The nanotags, which comprise of silica coated gold nanoparticles functionalised with Raman reporters and strain specific antibodies, were designed to bind selectively with target pathogens and display a unique optical response from deep within tissue barriers. The bacteria were cultured within 3D printed biofilm models and after incubation with the nanotags, multiplexed imaging was achieved using a handheld spectrometer within porcine tissue up to depths of 24 mm. This is the first time that the through tissue imaging of bacterial pathogens has been reported, and, to the best of our knowledge, the highest depth at which SESORS imaging has been reported using a handheld device with a backscattering SORS configuration. Future work will involve the design of more clinically relevant assays by incorporating bone and prosthetics into tissue to better mimic deep tissue infections. Furthermore, a method was developed for predicting the depth of nanotags embedded within porcine tissue samples up to 40 mm. By increasing the tissue barrier between the nanotags and the laser in small increments and tracking the intensity ratio of nanotag specific SERS bands to a tissue Raman band, a linear model was developed that allowed for the relative nanotag contribution in SESORS spectra to be correlated with nanotag depth.

(RAM-03.5) **Tuning, Torturing, and Touching Up a SERS Substrate**

Jason R. Dwyer[1]; [1]*University of Rhode Island*

We used a hand-held flameless (Tesla coil) cigarette lighter to easily create a micro- and nanostructured surface on a silicon wafer that is suitable for SERS after metallization. The tortured surface showed promising resistance to biofouling when challenged with a model marine organism. An alternative, flow-based SERS substrate fabrication approach combines the benefits of nanoparticle substrates, including favorable mass transport properties, with the convenience of monolithic substrates. We are using chemical synthesis to further tune the SERS substrate surface for improved sensing, but without using thiols for the surface attachment. We are focused especially on improving the robustness of the bond between the molecular overlayer and the coinage metal surface. Finally, we use a commercially available monolithic nanopillar array SERS substrate to explore the importance of oxygen plasma cleaning of SERS substrates while highlighting potential perils.

21SPSJ02: VUV/FUV Spectroscopy

Chair: Yusuke Morisawa
On-site Chair: Igor Lednev

(SPSJ-02.1) **Multielectron-ion coincidence spectroscopy of atoms in intense EUV-FELfields**

Mizuho Fushitani[1]; [1]*Nagoya University*

Strong free-electron lasers (FEL) in the EUV and X-ray region have opened various applications based on unprecedented nonlinear processes where inner-shell electrons play a key role. Two-photon formation of double-core-hole (DCH) is one such novel process that is expected to be used for a sensitive probe in atomic element within molecule even though they are situated in different chemical bond states. However, these electron signals are usually weak so that they are often smeared out by dominant other electron signals generated in one-photon processes. To circumvent the problem, we incorporate electron spectroscopy with ion spectroscopy where electrons as well as ions generated in photoionization processes are simultaneously detected. By taking an advantage of ion charged states which carry information on how many photons are absorbed, one can single out weak two-photon signals. We applied this idea to multi-photon ionization of Xe by EUV-FEL irradiation as a benchmark system. By relating electrons signals to Xe^{4+} ions generated by two-

photon process, we successfully extracted electron signals that are associated with two-photon DCH formation of Xe 4d inner-shell states. The present work can be extended to molecular systems and is expected to be used as a new method for local chemical analysis within molecules.

(SPSJ-02.2) **Cavity Enhanced XUV Generation at 60 MHz for Photoemission Spectroscopy**

Arthur K. Mills[1], MengXing Na, Alexandra Tully, Rysa Greenwood, Sarah Burke, David Jones; [1]*Quantum Matter Institute, University of British Columbia*

Time- and angle- resolved photoemission spectroscopy (TR-ARPES) experiments performed with laser-based extreme ultraviolet (XUV) radiation have historically been limited, almost entirely, by the XUV light sources themselves. The nonlinear optical processes required to generate such XUV light –four-wave-mixing in noble gases for <12 eV photons or high harmonic generation (HHG) >10 eV photon– necessitate lasers with repetition rates below 100 kHz and ultrashort pulses (below 100 fs) to achieve the highest driving pulse intensities and thus the best conversion nonlinear efficiencies. These two conditions lead to a host of experimental complications: (i) low and often unviable data acquisition rates; (ii) poor energy resolution resulting from the broadband XUV pulses and the distorting space charge caused by their high intensity (too many electrons emitted per pulse); and (iii) the high pump fluence needed for adequate signal to noise, which is often well above any perturbative regime. While advances in high average power laser systems (enabled notably by Yb-doped fiber technologies) have succeed in increasing the repetition rate to a few MHz an accompanying decrease in the achievable energy resolution has persisted. In an alternate approach we employ a passive femtosecond enhancement cavity (fsEC), wherein the pulses of a mode-locked laser are coherently 'stacked'. Such fsECs provide ideal conditions for driving low-efficiency optical conversion processes that require high intensities at repetition rates >10 MHz. The source has a useable photon energy range of 8-40 eV, a temporal resolution of 190 fs, an energy resolution of 22 meV, and operates at a repetition rate of 60 MHz. It has been in service as a dedicated TR-ARPES source with several months of continuous operation. In this talk, we discuss the design and construction of the source and present an experimental characterization of its properties, including its long-term stability, time/energy resolution, Finally, we will summarize the results of two measurement campaigns using this source: (i) electron-photon coupling in graphite; (ii) a study of few-layer C60 films grown on single crystal Au(111).

(SPSJ-02.3) **Far- and deep-ultraviolet spectroscopy applied for organic semiconductor/ionic liquids interfaces**

Ichiro Tanabe[1]; [1]*Osaka University*

The interface of organic semiconductor films is of particular importance with respect to various electrochemical devices such as transistors and solar cells. In this study, we developed a new spectroscopic system, namely electrochemical attenuated total reflectance ultraviolet (EC-ATR-UV) spectroscopy, which can access the interfacial area. Ionic liquid-gated organic field-effect transistors (IL-gated OFETs) were successfully fabricated on the ATR prism. Spectral changes of the organic semiconductor were then investigated in relation to the gate voltage application and IL species, and the magnitude of spectral changes was found to correlate positively with the drain current. Additionally, the Stark shifts of not only the organic semiconductor, but also of the IL on the organic semiconductor films were detected.

(SPSJ-02.4) **Study for electronic states of water in high-concentrated aqueous solutions of lithium salts**

Yusuke Morisawa[1], Nami Ueno[2]; [1]*Kindai University,* [2]*Innsbruck University*

Ultraviolet (UV) spectroscopy in the 145-200 nm region has recently been a matter of intense interest because many kinds of materials in the condensed phase. Rapid progress of the studies has been introduced by the development of attenuated total reflection spectroscopy in the FUV region (ATR-FUV), which has enabled us to measure the spectra in the complete Far-UV region for liquid and solid samples without facing problems

such as peak saturation.1 Moreover, significant progresses of quantum chemical calculations for electronic excitation states of molecules improve our interpretations of the FUV spectra. There are series of studies investigated the FUV region of various molecules by ATR-FUV spectroscopy. In the aqueous solution of alkali halides, charge-transfer-to-solvent bands of halide anions, I-, Br- and Cl- were observed in the region of 185-250, 175- 220, and 170-190 nm, respectively. Moreover, the effect of alkali cation (Li+, Na+, K+, Rb+ Cs+) on the first electronic transition of liquid water which is seen around 150 nm were studied by ATR-FUV spectroscopy. We also have studied the changes in electronic states of polyethylene glycol by coordination with Li+ in the highly concentrated solution. These studies show the electronic states of solvents were changed by ions in the solution and the changes in electronic states were reflected in the electronic transition of the solvents. In this presentation we will show results of ATR-FUV spectra of super-concentrated aqueous electrolytes (SAE) (>21 mol/kg), called hydrate melt (HM). These very concentrated aqueous solution have strong attention because Li-ion batteries used SAE as electrolyte have the high performance over 3.5V, although water is used as solvent. As a reason for a large potential window, We will have presented results of ATR-FUV spectra of SAE. We concluded that the band gap of water in SAE is very much enlarged by coordination with Li ion.

(SPSJ-02.5) **Investigation of electronic structure and transitions of biological molecules by using ATR-FUV spectroscopy**

Kosuke Hashimoto[1], Yusuke Morisawa[2], Hidetoshi Sato[1], Mariagrazia Tortora[3], Barbara Rossi[4], Yukihiro Ozaki[1]; *[1]Kwansei Gakuin University, [2]Kindai University, [3]AREA SCIENCE PARK, Elettra-Sincrotrone Trieste, [4]Elettra-Sincrotrone Trieste*

The purpose of this study is to explore the potential of ATR-far-ultraviolet (FUV) spectroscopy in investigating electronic structure and transitions of various kinds of biological molecules. ATR-FUV spectra were measured for several kinds of proteins with the different secondary structures, several kinds of carbohydrates. Band assignments have been made for all kinds of biological molecules investigated based on our previous ATR-FUV studies on n-alkanes, alcohols, esters, and amides. All the carbohydrates studied yielded a band near 170 nm due to n-Rydberg transition of ether. In addition, acetylcarbohydrates give an additional band near 190 nm originating from π-π* transition of amide at 2' carbon. The spectra of proteins show a characteristic band near 190 nm due to π-π* transition of amide groups. The position of this band varies a little with the secondary structure of proteins. Its intensity of some protein solution changes significantly depending on not concentration but composition of buffer, which may reflect protein adsorption on the internal reflection element (IRE). The present study has demonstrated that ATR-FUV spectroscopy is a new powerful technique in exploring electronic structure and transitions of biological molecules, in general. It is also possible to use ATR-FUV spectroscopy for quantitative and qualitative analysis of biological molecules. Moreover, it is of note that information regarding electronic transitions collected by ATR-FUV spectroscopy is useful for UV resonance Raman (UVRR) spectroscopy studies of biological molecules. A combined ATR-FUV spectroscopy and UVRR spectroscopy method may provide a novel analytical tool for molecular and electronic structure of biological molecules.

21AWD09: 2021 FACSS Innovation Award Finalists

Chair: Robert Lascola
On-site Chair: Robert Lascola

(AWD-09.1) **EASI: A New Paradigm for Mass Spectral Identifications**

Glen P. Jackson[1], Samantha Mehnert, J. Tyler Davidson; *[1]West Virginia University*

This presentation describes a novel algorithm for the identification of compounds from their mass spectra. The algorithm is intuitive, based on fundamental principles, uses common statistical tools and, most importantly, is able to provide reliable measures of uncertainty in drug identifications. As a proof of concept, the algorithm is demonstrated through the ability to distinguish spectra of cocaine from spectra of its diastereomers allococaine,

pseudococaine and pseudoallococaine. The algorithm takes advantage of the fact that the variance in ion abundances of replicate spectra are not independently variable, as has long been assumed. Instead, idiosyncratic and instantaneous instrument conditions influence the observed unimolecular fragmentation rates and spectral distortion, both of which cause correlations and anticorrelations in ion abundances in each compound's replicate spectra. The algorithm uses a general linear regression model (GLM) to predict the ion abundances of each of the n most abundant ions in a mass spectrum of a questioned sample (e.g. n=20 for cocaine). A binary classifier then uses simple measures of similarity between predicted ion abundances and measured ion abundances for each questioned sample to decide whether or not to identity the questioned spectrum as cocaine or not. Using external validation spectra of hundreds of replicate spectra, the algorithm predicts abundances with a precision that is typically 3-4 times better than models that assume a fixed exemplar, which is the current state of the art. For the hundreds of tested spectra, the algorithm enabled zero false negatives and zero false positives, even when identifying cocaine and its diastereomers from spectra collected on instruments that differed from the one on which the training set was built. The approach is extendable to any substance and any fragmentation technique in mass spectrometry through which replicate spectra of standards can be acquired.

(AWD-09.2) **Ultra-high-throughput LIBS analysis of PGE-bearing drill cores**

Marie-Chloé Michaud Paradis[1], François Doucet[2], Kheireddine Rifai[3], Lütfü Özcan[3], Nawfel Azami[4], François Vidal[5]; *[1]Université de Montréal, [2]ELEMISSION inc., [3]ELEMISSION INC., [4]INPT-UM6P, [5]INRS-EMT*

Mineral mapping and pattern recognition in the mining industry pose huge challenges for developing selective chemical sensors, as dissemination and alteration are natural phenomena hard to reproduce in the lab. Laser-induced breakdown spectroscopy (LIBS) sensors are known for rendering all-state elemental contents. This technology is a laser ablation technique and the spectrum collected at each spot is consequently the atomic emission fingerprint of the chemical composition of the ablated surface. This fingerprint being unique for each mineralogy, pattern recognition has been studied more and more in the last decade to assign LIBS spectra to crystal phases. This atomic emission spectroscopic technique has a lot of potential in the mining industry, even more so as a LIBS analyzer can serve as its own validation tool for critical minerals, such as platinum group elements (PGE)-bearing massive sulfides. Such a content analysis is not possible using infrared reflectance hyperspectral imaging (IR-HSI) or X-ray fluorescence (XRF) technologies used at the moment in the industry. Another advantage of LIBS analyzers is that single laser-induced plasma integration times are lower than those of XRF and IR-HSI instruments. Therefore, the potential of ultra-fast analysis is a huge advantage of LIBS sensors in the mining industry. In this paper, we will introduce an overview of the analytical capabilities of the ECORE drill core scanner. A mineralogical library was first built by using scanning electron microscope – energy dispersive spectroscopy (SEM-EDS) mineral maps for the supervised learning of mineral phases contained in three core samples drilled from a drill core tray containing PGE-bearing rocks. All teaching classes were further validated by comparing characteristic emission peaks to each classes' empirical formula for each selected spectrum. After the teaching process, the whole core tray was scanned in air in the ECORE ablation chamber and a mineralogical map was rendered by processing the teaching classes into a machine learning pattern recognition algorithm. The 20 mm x 3.81 m map reaching 7.62 megapixels is the biggest hyperspectral single image produced to our knowledge using a LIBS scanner and first makes LIBS real-time analysis possible at big data scales.

(AWD-09.3) **On-the-fly Raman image microscopy by reinforcement machine learning**

Tamiki Komatsuzaki[1], Koji Tabata, Hiroyuki Kawagoe, James Nicholas Taylor, Kentaro Mochizuki, Jean-Emmanuel Clement, Yasuaki Kumamoto, Atsuyoshi Nakamura, Yoshinori Harada, Katsumasa Fujita[2]; *[1]Hokkaido University, [2]Osaka University*

To bridge information science and measuremental science provides some different approaches in measurements; compressed sensing has made us "measure" samples at super-resolution by assuming sparsity in

real space or Fourier domains. However, most approaches are regarded as “one-way bridge” so that data acquired by a measurement are fed into information science protocol or analysis (post-analyzed) after measurements are accomplished. In this talk, we present our recent study combined information science so-called Bandit algorithm with spontaneous Raman microscope, which aims at accelerating the measurements by designing and generating optimal illumination pattern “on the fly” during the measurements. We first define the information we aim at knowing, such as the question of whether the sample to be measured includes cancer cells or not. We construct a descriptor to quantify the likelihood as a function of Raman signal. Second, we start to measure a given sample with random point-illuminations as an initial guess. Third, by referring the set of Raman spectra randomly distributed over pixels, we feed the optimal condition (i.e., illumination point distribution) back to the Raman measurement system. We repeat this procedure until we can identify whether the sample includes at least one cancer cell or no cancer cells in the sample at least with a probability of 1-δ ($0 \leq \delta \ll 1$: allowed error rate). We present our simulation studies using Raman image in the diagnosis of follicular thyroid carcinoma, whose diagnosis is known to be difficult by relying on morphological information of cells and tissues, and show that this protocol can accelerate 80-100 times in speedy and accurate diagnoses faster than the standard line-scanned Raman microscope that requires the full detailed scanning over all pixels. We also examine nonuniformly distributed polystyrene beads/polymethyl methacrylate (PMMA) beads mixture systems by our Raman image microscope combined with a spatial light modulator, and found that we can diagnose whether PMMA beads exist in the sample and where they are densely distributed much faster than conventional microscope. Our results demonstrate that optimal design of the experimental conditions on the fly can accelerate the measurement.

October 1, 2021

21SCIFRI01: SciFri

Chair: Jean-François Masson

(SCI-01.1) **The Trowel and Error Experiences of a Spectroscopist Doing Field Archaeology**

Mary Kate Donais[1]; [1]*Saint Anselm College*

The challenges and highlights of collaborative archaeometry research will be discussed with particular emphasis on the role portable spectroscopy played in our work. Applications involving different instrumental techniques and artifact types will be used to show how the research team's approach to answering questions about our archaeology sites has broadened and evolved with the addition of in situ chemical measurements into the research design.

(SCI-01.2) **Title TBD**

Karl S. Booksh[1]; [1]*University of Delaware*

(SCI-01.3) **SHERLOC: Looking for clues in Jezero Crater**

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On February 18th 2021, the Perseverance rover landed in Jezero crater, Mars. This site was chosen because orbiter data analysis provides evidence that the crater hosted a stream-fed lake at a time in the Martian Noachian period. The Octavia Butler landing site is located ~1.9 km east of the remnants of a river delta. Deltaic and lacustrine sediments can preserve biosignatures, making Jezero crater a prime target for Mars sample return science. One of the seven instruments of Perseverance's science payload is SHERLOC –Scanning Habitable Environments with Raman and Luminescence for Organics and Chemicals. . SHERLOC combines fluorescence and Raman spectroscopy with microscopic imaging to analyze surface material to better understand the history of the aqueous environments recorded in the rocks of Jezero crater and to search for potential biosignatures. SHERLOC imaging consists of two microscopic cameras, the Autofocus and Context Imager (ACI), and the Wide-Angle Topographic Sensor for Operations and eNgineering, (WATSON). These subsystems obtain high spatial resolution images of geological targets to characterize grain-scale structure and texture. SHERLOC spectroscopy enables high-sensitivity detection, characterization, and spatially-resolved correlation of trace organic materials with the mineral matrix. SHERLOC's 248.6 nm deep UV laser generates a 100 µm-diameter spot and is mapped across a natural or abraded surface. Raman scattering and fluorescence emission are collected and spectra are downlinked to Earth for analysis. The spectral maps are combined with the ACI and WATSON image using to generate mineral and compositional maps. An overarching theme of past and future Martian exploration focuses on characterizing its aqueous history, determining its habitability potential, and searching for evidence of life. The mineral and organic maps generated by SHERLOC will be combined with measurements from Perseverance's instrument suite to understand the geological history and context of rocks and regolith. This will enable the coring and caching of astrobiologically-relevant samples for eventual return to Earth as part of the Mars Sample Return (MSR) campaign.

(SCI-01.4) **Round Table on Concepts of Field-Deployable Spectroscopy (10 minutes)**