Abstract Book

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KEYNOTE

Unraveling the Story of Aboriginal Australian Natural Pigments Using Nuclear and Spectroscopic Methods

Keynote Speaker: Rachel Popelka-Filcoff, PhD - University of Melbourne

Complex samples from natural and human-made processes offer a diversity of complicated and fascinating examples where characterization by multiple advanced analytical methods is the only way to understand their complete story. The use of radioanalytical and X-ray methods provide data which can be non-destructive to the sample and are often not influenced by matrix effects. These characteristics make these methods appealing for cultural heritage questions and objects. Radioanalytical methods such as neutron activation analysis (NAA), in combination with high-resolution spectroscopy methods such as X-ray fluorescence microscopy (XFM), provide insight to these questions. This presentation will provide an overview on recent research in our laboratory in the areas of cultural heritage, in particular Indigenous Australian natural mineral pigments, and the insights into cultural heritage questions by using national facilities and laboratory-based methods. Some key questions to be investigated include provenance and sourcing of materials, compositional analysis, and understanding applications of pigments on the micron scale. The combination of cultural context, and development of novel approaches leads to insightful answers for cultural research. The context for the samples can be diverse; however similar questions about composition (including multiple layers), intertwined material/chemical/physical properties and material provenance underlie the research objectives.

PLENARY ABSTRACTS

(PLEN1) Discovering the Subsurface of Materials by Intact Methods: The Contribution of Micro-SORS

Plenary Speaker: Claudia Conti, PhD - National Research Council Institute of Heritage Science

This presentation will focus on a recently developed method, Micro Spatially Offset Raman Spectroscopy (Micro-SORS). The technique combines conventional Spatially Offset Raman Spectroscopy [1] with microscopy concepts representing a new non-destructive analytical modality in Raman microscopy. The extension of SORS to the microscale paves the way for the non-destructive investigations of the molecular composition of subsurface, micrometer-scale-thick diffusely scattering layers at depths beyond the reach of conventional confocal Raman microscopy [2]. The applicability of micro-SORS has been demonstrated conceptually in several application areas including stratified polymers, glossy papers, layered materials in food analysis [3] and, in particularly, Cultural Heritage materials [4]; the use of micro-SORS provides a breakthrough in non-invasive and non-destructive analysis of the subsurface layers of artworks and is a key development in the understanding of conservation science for cultural heritage objects. The principles of micro-SORS, the existing instrumental modalities, its capabilities and limitations as well as applications areas will be presented and discussed. [1] P. Matousek, I. P. Clark, E. R. C. Draper, M. D. Morris, A. E. Goodship, N. Everall, M. Towrie, W. F. Finney and A. W. Parker, Appl. Spectrosc. 59, 2005, 393. [2] C. Conti, C. Colombo, M. Realini, G. Zerbi, P. Matousek, Appl. Spectrosc. 68, 2014, 686. [3] C. Conti, M. Realini, C. Colombo, K. Sowoidnich, N.K. Afseth, M. Bertasa, A. Botteon, P. Matousek, Anal. Chem. 87, 2015, 5810. [4] C. Conti, A. Botteon, C. Colombo, D. Pinna, M. Realini, P. Matousek, J. Cult. Herit. 43, 2020, 319.

(PLEN2) The role of reference methods and reference materials to support use of regulated nanomaterials in manufacturing industry

Plenary Speaker: Heidi Goenaga-Infante, PhD - LGC Limited

The EU definition of nanomaterials (2011/696/EU) as well as existing regulations (e.g. Cosmetics 1223/2009, Novel food EC 2015/2283 and Food Contact materials EC 1935/2004) have posed several analytical challenges as well as driven new measurement strategies and standards in the past ten years. A recent report from JRC (1) discussed key points to consider in the assessment of particulate materials according to the European Commission's Recommendation. Although analytical advances have been reported, there is still an increasing need for traceable methods and reference materials for quality assurance of nanoparticle characterisation in complex matrices. Such materials and methods will help industry with the implementation of upcoming regulation and enable quality control of existing products. This lecture will discuss key advances in measurement capabilities for the characterisation of nanomaterials and their impact on relevant standards. Focus will be on inorganic nanomaterials and on multi-modal platforms combining hyphenated ICP-MS with spectroscopy and microscopy techniques. The presentation will also focus on the determination of nanoparticle number concentration, size and size distribution for identification purposes and, examples of related analytical developments by our group will be provided. The author's viewpoint will be discussed on the remaining analytical challenges and drivers following the EU definition and regulations mentioned above. The lecture will finally touch

on the importance of developing reference materials (RMs) for nanomaterials, ranging from simple solutions (for method development and instrument calibration) to complex biological matrices (for method validation). The importance of reference methods that can help validate new and improved methods and/or can be directly implemented by industry will be highlighted. Finally, the relevance of international inter-laboratory comparisons for institutes to demonstrate competency and feasibility of existing methods will be discussed. (1) JRC SCIENCE FOR POLICY REPORT, Identification of nanomaterials through measurements-Points to consider in the assessment of particulate materials according to the European Commission's Recommendation, 2019, EUR 29942 EN, H. Rauscher, A. Mech, N. Gibson, D. Gilliland, A. Held, V. Kestens, R. Koeber, T. P. J. Linsinger, E. A. Stefaniak .

(PLEN3) 50 years of biomedical application of Raman spectroscopy

Plenary Speaker: Yukihiro Ozaki, PhD - Kwansei Gakuin University

50 Years of Biomedical Application of Raman Spectroscopy Nowadays, biological and medical application of Raman spectroscopy extends to a wide area of science covering biochemical application, biological application, medical application (experimental medicine) and clinical application. It uses a variety of methods such as Raman imaging, Raman microscopy, non-linear Raman, SERS, TERS, ROA and so on. Biological application of Raman spectroscopy started in 1970s when lasers prevailed widely. Raman and resonance Raman studies of proteins got a start on biological application of Raman spectroscopy. Note that even 1970s some notable investigations relevant to biomedical application were reported; virus research by Thomas et al. and an eye lens investigation by Yu et al. They succeeded in demonstrating that Raman is a powerful technique for non-destructive and non-invasive analysis of biomedical materials. In 1981 I reported a Raman work on cataractgenesis. This may be the first one that the mechanism of disease has ever been explored by Raman spectroscopy. However, 80s was a dark age for biomedical Raman spectroscopy because Raman spectra of disease materials suffered seriously from strong fluorescence. A new era came in 90s; the serious problems of fluorescence were mostly overcome by the advent of near-infrared semiconductor lasers. Our group used 1064-nm excitation (FT-Raman) to measure Raman spectra of human cancer tissues in 1994. SERS studies of biological molecules and biomedical martials including cells and tissues became popular in the latter half of 90s. Our group also reported several elegant examples of biomedical applications of SERS. TERS followed SERS from 2000s. In the last decade or so clinical application of Raman spectroscopy has made remarkable progress, and many medical doctors show keen interest in it. To develop further biomedical Raman spectroscopy the development not only of spectroscopic techniques and related techniques such as light fiber probes but also of spectral analysis including big data analysis are important. At last but not at least I would like to emphasize the importance of application of Raman spectroscopy to fundamental biology.

(PLEN4) Charge-Shifting Lock-In CCD Operation Combined with Shifted Excitation Raman Difference Spectroscopy

Plenary Speaker: Kay Sowoidnich, PhD - Ferdinand-Braun-Institut, Leibniz-Institut für Höchstfrequenztechnik

Non-Presenting Author: Mike Towrie - STFC Central Laser Facility

Non-Presenting Author: Martin Maiwald, Dr. - Ferdinand-Braun-Institut, Leibniz-Institut für Höchstfrequenztechnik

Non-Presenting Author: Bernd Sumpf, Dr. - Ferdinand-Braun-Institut, Leibniz-Institut für Höchstfrequenztechnik

Non-Presenting Author: Pavel Matousek, Professor - Science and Technology Facilities Council

Shifted excitation Raman difference spectroscopy (SERDS) is an established powerful tool allowing to extract chemically specific information from disturbing background interferences, e.g. fluorescence or ambient light. The effectiveness of SERDS can however be limited when fast changing backgrounds are present. This is due to the fact that rapid detection of Raman spectra, acquired at the two shifted excitation wavelengths required for SERDS, is usually restricted to sampling rates of about 10 Hz using conventional charge-coupled device (CCD) operation. To overcome this fundamental technical limitation, we present a charge-shifting optical lock-in detection approach permitting rapid alternating SERDS operation in the kilohertz range. As key components our system comprises a specialized dual-wavelength 830 nm diode laser and a custom-built CCD enabling retention and shifting of the charges back and forth directly on the CCD chip. In a first set of experiments, six heterogeneous and fluorescent mineral specimens were chosen and moved in an irregular motion during acquisition of the SERDS spectra. In comparison to conventional CCD read-out operation at the maximum rate of 5.4 Hz, the fast charge-shifting lock-in read-out was operated at 1,000 Hz demonstrating increased reproducibility between repeat spectra obtained from the same specimen. Using partial least squares-discriminant analysis a sensitivity of 99 % and a specificity of 94 % (using four latent variables) for the discrimination between various minerals was demonstrated in charge-shifting mode while conventional read-out obtained a sensitivity of 90 % and a specificity of 92 % (using six latent variables). A second set of experiments translated the charge-shifting approach towards sub-surface analysis by combining it with spatially offset Raman spectroscopy (SORS). A polytetrafluoroethylene (PTFE) layer, concealed behind an opaque and heterogeneous layer of 0.25 mm thickness, was used as test sample for the SERDS-SORS investigation. The spectra recorded in charge-shifting SERDS-SORS mode matched PTFE reference spectra much more closely and demonstrated a two-fold improvement in signal-to-background-noise-ratio compared to conventional CCD read-out SERDS-SORS spectra. In combination with its capability to suppress rapidly varying ambient light interference demonstrated by us earlier, the charge-shifting lock-in technique is expected to be particularly beneficial with heterogeneous fluorescent samples in field applications.

(PLEN5) Understanding Brain Neurochemistry: Imaging Neuromodulators with Near Infrared Fluorescent Nanocarbons

Plenary Speaker: Markita del Carpio Landry, PhD - University of California Berkeley

Neurotransmission – chemical communication of neurons in the brain – plays a critical role in brain function. Neuromodulators such as serotonin and dopamine are thought to signal across broader spatial regions than classical neurotransmitters, and aberrations in their signaling are implicated in psychiatric and neurodegenerative disorders including depression, addiction, and Parkinson's disease. Until recently, measuring the dynamics of dopamine and other neuromodulators could not be achieved at spatiotemporal resolutions necessary to understand how neuromodulators regulate the function of neural circuits, and how dysfunctions in this regulation lead to disease. We have developed several fluorescent nanocarbon-based tools that can image neuromodulators in the brain with high spatiotemporal resolution. We first describe the synthesis and implementation of a nanoscale dopamine probe constructed from single wall carbon nanotubes (SWNT) non-covalently functionalized with (GT)6 oligonucleotides [1]. We demonstrate this probe enables imaging of dopamine dynamics in striatal brain tissue and has uncovered endogenous variability in neurochemical responses to dopamine agonist or antagonist drugs [2]. We next describe an approach to evolve molecular recognition on SWNT surfaces

to generate a probe for serotonin [3], which presents a generic method to develop synthetic probes for other neuromodulators. We anticipate this probe and others of its class will enable synaptic-scale inquiries of the role of neuromodulators in brain health and disease. References: 1. Beyene, A. G. et al. Ultralarge Modulation of Fluorescence by Neuromodulators in Carbon Nanotubes with Self-Assembled Oligonucleotide Rings. Nano Letters, 2018 2. Beyene, A. G. et al. Imaging Striatal Dopamine Release Using a Non-Genetically Encoded Near-Infrared Fluorescent Catecholamine Nanosensor. Science Advances, 2019 3. Jeong, S. et al. High Throughput Evolution of Near Infrared Serotonin Nanosensors. Science Advances, 2019

(PLEN6) Interfacial Free Charge Density Gradients in Room Temperature Ionic Liquids

Plenary Speaker: Gary J. Blanchard - Michigan State University

Room Temperature Ionic Liquids (RTILs) hold much promise for use in areas ranging from energy storage and delivery to electronically-controlled optical devices and green synthesis. Despite the already wide use of these materials, a fundamental understanding of dynamics and properties such as the extent of dissociation in RTILs remains to be achieved. Our group has identified the existence of free charge density gradients in RTILs in contact with charged surfaces. These gradients can persist for ca. 100 □m in the RTIL and dilution data demonstrate persistent compositional heterogeneity in RTILs. We provide a summary of these unique RTIL properties and the current state of our understanding.

(PLEN7) Dielectric Spectroscopy in Flow Cytometry

Plenary Speaker: Philippe Renaud, PhD - EPFL

Dielectric spectroscopy in flow cytometry: Microfluidic technologies allow integration of microelectrodes inside microchannels in which biological cells can be flown in suspension. These electrodes can be used to measure the local electrical impedance when a cell or a particle is flowing in the channel. The local electric field gradients generated in the vicinity of the electrodes also induces dielectrophoretic forces on cells. Both electrical impedance and dielectrophoresis are dependent on the same complex dielectric properties of the cell and of the surrounding liquid. They can be treated in the same theoretical framework. We use the term dielectric spectroscopy as a generalization of electrical impedance spectroscopy and frequency analysis of dielectrophoretic effects on cells. Impedance flow cytometry is known since long time, since invention of the Coulter counter in the middle of 20th century. It is mostly used for enumeration and sizing cells. In the last two decades, microfluidic devices opened new perspectives for impedance measurement at high frequencies and for combination with other features such cell sorting or optical sensing. Electrical impedance spectroscopy allows measurement of more biophysical parameters, such as capacitance of the cell membrane or the osmolarity of the cytosol. Here we show several examples on extraction of relevant biological information like cell viability, developmental stage, parasite invasion or cell shape. Dielectrophoretic spectroscopy, classically done by electrorotation of single cells immobilized in electrode cages, extracts same biophysical parameters as in impedance spectroscopy. As a flow cytometry alternative, we have developed a multi-frequency dielectrophoretic device that sorts out the cells based on their dielectric spectra.

(PLEN8) Separations and Serial Crystallography in Microenvironments

Plenary Speaker: Alexandra Ros, PhD - Arizona State University

Unique migration phenomena have been unlocked with the development of ratchet migration mechanisms. While the importance of suitable microfluidic devices enabling ratchet migration for bioanalytes was underlined in the past, applications in the field of bioanalytical separations are still rare. We have recently developed a mechanism for ratchet migration induced through the interplay of sub-µm particles and tailored electric fields. This novel migration mechanism allows steering of sub-µm particles including constituents of cells, such as organelles, in opposing directions. To further unravel the power of this non-intuitive migration mechanism, high throughput microfluidic platforms were explored and the separation of mitochondria by size in a continuous separation approach was demonstrated. We expect this separation modality to be useful to unlock biomolecular differences in giant and normal organelles related to cellular dysfunction. Microfluidic devices created through high resolution 3D-printing can also be exploited in sample preparation and delivery for novel crystallography techniques. One such technique is serial crystallography with X-ray free electron lasers (XFELs), a powerful new approach to elucidate the structure of large proteins complexes such as membrane proteins. Microfluidic devices are essential for sample delivery, specifically of crystal slurries, but also allow to overcome its limitations resulting from the huge amount of sample wasted. Our solution constitutes a microfluidic droplet generator facilitating sample laden droplet generation and delivery in synchronization with a pulsed XFEL source for efficient serial crystallography of proteins.

TUESDAY FEATURED LIVE ORAL ABSTRACTS

(AAF-LP1) Art Chemistry and Forensics

(AAF-LP1.1) Integration of MA-XRF Scanning with Optical Spectroscopy for Technical Studies Research at Getty

Presenter: Karen Trentelman, PhD - Getty Conservation Institute

Much of the attention generated by early studies employing macro-XRF (MA-XRF) scanning focused on the dramatic – those studies in which hidden paintings were revealed with elemental specificity, allowing digital color reconstructions of the hidden images to be generated. While the discovery of previously unseen works by important artists such as Rembrandt and van Gogh is exciting and important, XRF scanning can do much more than uncover hidden paintings. It can reveal previously undetectable details about the materials and techniques employed by artists to create works of art, provide trace material information important for understanding the provenance or processing of materials, serve as a means of documenting past conservation interventions, and provide a common platform for communication between scientists, conservators and curators. Recently, efforts have begun to extend the capabilities of XRF scanning to allow the study of objects with increased surface topography, widening the range of objects that can be studied. The capabilities of this technique are even further enhanced when coupled with other non-invasive spectroscopic techniques that provide molecular information, such as reflectance imaging spectroscopy (RIS), Raman spectroscopy or fiber-optics reflectance spectroscopy (FORS). This talk will present an example of the type of research being carried out by the Technical Studies Research team at Getty Conservation Institute, showcasing how we are integrating MA-XRF scanning with other analytical techniques to address a wide range of questions relevant to conservation and art historical research.

(AAF-LP1.2) Treasures on Trial: The Role of Science in Decorative and Fine Arts Forgery

Presenter: Catherine R. Matsen, MS - Winterthur Museum

Scientific analysis – in conjunction with provenance and connoisseurship – is an important tool to aid in identifying whether art, antiques, and collectibles are genuine or fake. Though scientific testing alone cannot prove authenticity, it can often disprove it through the positive identification of materials that are inconsistent with a purposed date of manufacture, method of manufacture, or known working methods of an artist or craftsman. Through case studies in the decorative arts collection of Winterthur Museum and other objects, this presentation will highlight how materials analysis with XRF, FTIR, Raman and pyGC-MS provides a more informed understanding of a work of art.

(AAF-LP1.3) More Information in Less Time: New Approaches Toward the Treatment of Multidimensional Hyperspectral Data in Heritage Science

Presenter: Marc Vermeulen, PhD - Northwestern University

Non-Presenting Author: Marc Sebastian Walton, PhD - Northwestern University

Over that past decade, hyperspectral imaging has become a go-to technique for objects-based and objects-inspired scientific investigations in the field of Heritage Science. The technique generates large 3-dimentional data cubes (2 spatial and wavelength) that require post-processing in order to extract the molecular information it contains. The large datasets often contain hundreds of thousands of spectra which pose a challenge to scientists attempting to extract a maximum of information. With such large datasets it is clear that traditional methods for spectral analysis, that require examination of each spectrum, is not possible and there is a critical need for advanced machine learning and statistical methods that can supplant these common tasks. This presentation introduces the development of new and freely available software libraries released by the Center for Scientific Studies in the Arts to allow for quick and user-friendly treatment of hyperspectral imaging data cubes. As an improvement to principal component analysis (PCA), we describe the use of embedding techniques (either t-distributed Stochastic Neighbor Embedding, t-SNE, or, Uniform Manifold Approximation and Projection, UMAP) to reduce dimensions and cluster the data cube. The centroids of the identified clusters then serve as the initial inputs to a novel dictionary learning algorithm based on k-means clustering and singular value decomposition (K-SVD) with non-negativity constraints. This non-negative factorization algorithm quickly identifies endmembers and a per-pixel sparse representation that maps the spatial distribution of pigments found in the art object. The necessity for more robust and faster data treatment tools appeared as a requirement for the field and inspired the development of the libraries in the Julia programming language rather than Python. Julia is a high-level, high-performance, dynamic language that is built for speed. Using Julia, data treatment that would have otherwise taken several hours, originally written in Python, was reduced to less than 30 minutes. We will present the various steps required to use either Python or Julia for the non-programmer, will present the advantages and limitation of both platforms and will show how dictionary learning quickly leads to a robust identification of all representative pigments contained in large data cubes.

(AAF-LP1.4) A Consumer-Technology Approach to Near-IR Reflectography of Paintings and Murals

Presenter: McKenzie A. Floyd, Master of Science in Chemistry, Master of Science in Museum Studies - Freelance
Non-Presenting Author: Alexa Torres

Smartphone technology has the potential to make heritage science accessible to museums, individual collectors, and educators. Infrared reflectography (IRR) is a nondestructive analysis method used by museums to gain information about the provenance, history, and aging of artworks. We introduce a highly accessible, inexpensive apparatus for near-infrared (NIR) imaging of paintings: a long-pass filter with a 750 nm cut-on wavelength mounted on a Samsung HTC smartphone back-facing camera. This method proved effective in improving visibility of underdrawings, as well as in the detection of compositional changes by the artist, retouchings, and original composition elements obscured by damage or aging. Egg tempera test panels, historic oil paintings, and wall murals were all imaged with the NIR-smartphone apparatus. This research demonstrates the potential of adapted consumer technology for interdisciplinary scientific investigation in museums and at heritage sites, which has the potential to raise public understanding of heritage science and art forensics. This simple and affordable approach is also a promising tool for teaching the importance of and principles behind the scientific investigation of artworks.

(AAF-LP1.5) Application of Spectroscopic and Imaging Techniques in the Characterization of Organic Pigments

Presenter: Moupi Mukhopadhyay, MS, MA - Department of Conservation of Archaeological and Ethnographic Materials, University of California Los Angeles, A210 Fowler Building, 308 Charles E Young Dr N, Los Angeles, CA 90095-1510, USA

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Non-Presenting Author: Giovanni Verri - Art Institute of Chicago 111 S Michigan Avenue 60603 Chicago IL

Non-Presenting Author: Gianluca Accorsi - CNR-NANOTEC c/o Campus Ecotekne, University of Salento Via Monteroni - 73100 Lecce, Italy

Non-Presenting Author: Marco Pugliese - CNR-NANOTEC c/o Campus Ecotekne, University of Salento Via Monteroni - 73100 Lecce, Italy

Non-Presenting Author: Ioanna Kakoulli - Department of Materials Science and Engineering, HSSEAS School of Engineering and Applied Science, University of California Los Angeles, 420 Westwood Plaza, 3111 Engineering V, Los Angeles, CA 90095-1595, USA Molecular and Nano Archaeology Laboratory, HSSEAS School of Engineering and Applied Science, University of California Los Angeles, 420 Westwood Plaza, 3111 Engineering V, Los Angeles, CA 90095-1595, USA UCLA/Getty Conservation Program, University of California Los Angeles, A210 Fowler Building, 308 Charles E Young Dr N, Los Angeles, CA 90095-1510, USA

Non-Presenting Author: Tania Prontera - CNR-NANOTEC c/o Campus Ecotekne, University of Salento Via Monteroni - 73100 Lecce, Italy

This research applies spectroscopic and imaging techniques for non-invasive and minimally invasive analyses of organic pigments with focus on madder lake and indigo. These two colorants have been used since antiquity in painting, ceramics and textiles, and are important in the technical study of cultural heritage for the purpose of conservation. Madder lake was re-created based on ancient recipes provided in the Stockholm Papyrus, a 3rd century AD alchemical text, and characterized using a multi-analytical approach. Fiber optic reflectance spectroscopy (FORS) and surface enhanced Raman spectroscopy (SERS) have been used to explore the production technology and operational sequences (chaîne opératoire) for the synthesis of madder lake in antiquity. The characterization of the recreated madder lake helped understand how its photophysical and chemical properties are influenced by factors such as processing and chromophore ratios both in the source raw material, as well as the final product. The potential of infrared false-color imaging (IR-FCI) integrating reflectance data in the visible and infrared regions of the electromagnetic (EM) spectrum to characterize and map indigo blue directly on works of art in a non-invasive manner, has also been explored. Mockups with varying indigo blue pigment

concentrations, thicknesses of paint layer and substrates were prepared and analyzed using reflectance spectroscopy and imaging. Qualitative and quantitative assessments were further conducted to evaluate the effects of these variables and provide interpretation guidelines for the IR false-color images. This research has demonstrated the importance of combining spectroscopic and imaging techniques for the unbiased identification and mapping of colorants.

(MOLEC-LP1) Topics in Molecular Spectroscopy Part I

(MOLEC-LP1.1) Single Molecule Secondary Structure Determination of Protein through Infrared Absorption Nanospectroscopy

Presenter: Francesco Simone Ruggeri, PhD - Department of Chemistry, University of Cambridge

Biological processes rely on a wide class of biomolecular and macromolecular machines that have characteristic nanoscale physical dimensions and whose function emerges from a correlation between their chemical and structural properties. A fundamental objective of modern biophysics and biology is the comprehension of the biomolecular processes underlying life and disease by studying the biophysical, chemical properties and heterogeneity of single biomolecules. While innovative imaging methods have been developed to characterise biomolecular structures at high spatial resolution, acquiring vibrational absorption spectra, a benchmark for bulk sample characterization, on the single molecule level has remained elusive. Here, we introduce off-resonance, low power and short pulse infrared nanospectroscopy to acquire infrared absorption spectra and maps at the single molecule with a high signal-to-noise ratio (~10-20). The achievement of this high sensitivity 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, with similar accuracy than conventional bulk vibrational spectroscopy on samples with over a million fold larger mass. These results pave the way to probe directly the chemical and structural properties of individual biomolecules, as well as their interactions, in a broad range of chemical and biological systems.

(MOLEC-LP1.2) Advancing Liquid Phase Spectroscopy by Quantum Cascade Lasers & Balanced Detection

Presenter: Bernhard Lendl, Prof. Dr. - Technische Universität Wien

Non-Presenting Author: Andreas Schwaighofer - Technische Universität Wien

Non-Presenting Author: Georg Ramer, MSc PhD - Technische Universität Wien

Non-Presenting Author: Stephan Freitag, n/a - Technische Universität Wien

Non-Presenting Author: Christopher Karim Akhgar, n/a - Technische Universität Wien

Non-presenting author: Matthias Baer - Friedrich-Alexander-University Erlangen-Nuremberg

Non-presenting author: Bernhard Schmauss - Friedrich-Alexander-University Erlangen-Nuremberg

Two new experimental set-ups for liquid phase spectroscopy will be presented, both taking advantage of unique features of QCL sources (spectral power density and inherent polarization) and a recently introduced balanced TE-cooled MCT detector. Using this detector and an EC-QCL source a dual beam set-up for transmission spectroscopy is presented and used for measuring aqueous (H_2O) protein solutions. The S/N-ratio was significantly improved, allowing to record high quality protein spectra (amide I and amide II bands optical path 25 µm) enabling secondary structure analysis of the samples at concentrations of 0.1mg/mL. The second set-up introduces polarimetric ATR spectroscopy and exploits the fact that QCLs emit linear polarized light. The beam is sent into a 6 bounce trapezoidal ZnS ATR element so that the polarization plane of the beam is inclined at 45° with respect to the surface of the ATR element. After probing the sample, the exiting beam is divided in two beams by means of a ZnSe prism placed at the Brewster angle into the optical path. The exiting beam is thus divided into a beam

one only containing p-polarized light, whereas the other contains s- and p-polarized light. As the effective depth of penetration (de) is different for p- and s-polarized light, absorbance values can be calculated from the two beams. In doing so the long-term stability of the set-up is improved and pulse to pulse intensity fluctuations of the laser compensated. First data on EtOH in water are shown.

(MOLEC-LP1.3) Imaging isotopically labeled bacteria at the single cell level using high-resolution O-PTIR spectroscopy

Presenter: Roy Goodacre, PhD - University of Liverpool

Non-Presenting Author: Cassio Lima

Non-Presenting Author: Howbeer Muhamadali

Non-Presenting Author: Yun Xu

Non-Presenting Author: Mustafa Kansiz, PhD - Photothermal Spectroscopy Corp.

We will illustrate the identification of single bacterial cells that have been isotopically labelled using the far-field infrared imaging technique, optical photothermal infrared (O-PTIR) spectroscopy. This presents a significant advance as most studies using absorbance-based infrared spectroscopy have focused on evaluating communities of cells due to the poor spatial resolution achieved by classical infrared microspectrometers and, to date, there is no study evaluating the consumption of labelled compounds (13C-glucose and 15N-ammonium chloride) by bacteria at single cell levels. This complements studies where Raman spectroscopy has been used and shows an important step forward for measuring the metabolic activity of single bacteria, as this will allow for a better understanding of the interactions between microorganisms as well as the function of individual members and their interactions in different microbial communities.

(MOLEC-LP1.4) On the nature of interactions between aqueous ionic liquids and bio-molecules revealed by synchrotron-based UV Resonance Raman spectroscopy

Presenter: Barbara Rossi, PhD - Elettra Sincrotrone Trieste

Room temperature ionic liquids (IL) are a class of ionic compounds consisting of an organic cation and either an organic or inorganic anion, whose melting temperature is below 100 °C, making them liquid at or near room temperature. Recently, the studies on the toxicity of IL toward living organisms has stimulated several chemical–physical investigation on the interaction between IL and basic biological systems, such as saccharides, membranes, peptides/proteins and nucleic acids. For instance, it has been found that certain IL exhibit several positive effects on proteins, including an increased thermal stability, the suppression of aggregation and an enhanced refolding ability. Other examples concern the observed unusual stability of deoxyribonucleic DNA stored at room temperature in IL/water solutions that has stimulated growing interest in using IL as alternative solvents for DNA preservation and stabilization. Despite the increasing number of experimental and theoretical investigations on the chemical physics properties of systems made of IL and biomolecules, the deep knowledge on the basic principles able to organize and rationalize the vast variety of properties and phenomena displayed by these systems is still lacking. Synchrotron-based UV Resonance Raman spectroscopy (SR-based UVRR) is a powerful technique for revealing new insights on the nature of interactions between aqueous ionic liquids and bio-molecules on the most microscopic scales of their structure and dynamics. In this presentation, we will discuss selected case studies concerning the solvation properties of IL/water toward DNA with

particular emphasis on the role of hydration layer surrounding the biomolecule. Our results highlight the opportunity to use spectroscopic approaches for predicting properties of relatively extended families of IL/biomolecules combinations.

(MOLEC-LP1.5) Near-Infrared Discriminant Analysis Combined With Two-dimensional Correlation Analysis

Presenter: Hoeil Chung, PhD - Hanyang University

Non-Presenting Author: Woosuk Sohng

To improve accuracy for near-infrared (NIR) spectroscopic discrimination of adulterated olive oils, a strategy of combining temperature-induced NIR spectra and two-dimensional correlation (2D-COS) analysis has been attempted. Dynamic NIR spectral features induced by temperature change would be more informative for sample discrimination, and 2D-COS analysis was a reliable choice to characterize temperature-induced spectral variation. When 2D-COS analysis was performed using temperature-varied (20–41 °C) spectra and the resulting power spectra from 2D synchronous correlation spectra were used for PCA, identification of the two groups was noticeably enhanced and subsequent discrimination accuracy substantially improved to 86.4%. Two-trace two-dimensional (2T2D) correlation analysis was also proposed as a recognizer of dissimilar spectral feature able to improve accuracy of discriminant analysis. Since 2T2D correlation analysis is to capture asynchronous spectral behaviors in between two comparing spectra a sample, subsequent asynchronous correlation feature would become more sample-to-sample discriminant. For examination, 2T2D correlation analysis was performed using the sample spectra collected at 20 and 41 °C. When 2T2D slice spectra of each sample were used, the authentication accuracy improved further to 90.0%. Next, a simple strategy of utilizing an average spectrum of one sample group as a reference spectrum in 2T2D correlation analysis was proposed for two-group discrimination and evaluated for NIR identification of geographical origins of agricultural products (milk vetch root (MVR) and perilla seed samples). Since the average spectrum of one sample group was used for comparison, dissimilar constituent compositions of samples in the other group were better highlighted, thereby improving the accuracies in the discriminations of the geographical origins of both MVR and perilla seed samples.

(AAF-LP2) Archaeological Chemistry

(AAF-LP2.1) Applications of Scanning Electron Microscopy and Portable X-ray Fluorescence Spectrometry in the Identification of a 2000-year-old Tattoo Tool

Presenter: Andrew Gillreath-Brown, PhD Candidate - Department of Anthropology, Washington State University

Non-Presenting Author: Aaron Aaron Deter-Wolf, MA - Tennessee Division of Archaeology

Tattoo traditions of Native North America are integral aspects of Indigenous cultural expression, which have been long undervalued by Western scholars. Iconographic evidence suggests tattoo practices dated to as early as AD 1000 in the southwestern United States. However, the full temporal span of tattoo traditions in the region is unknown. We recently discovered a unique perishable tattoo tool from the Turkey Pen site, Utah, which dates to the Basketmaker II period (500 BC – AD 500). There are no set procedures for studying this type of unique, perishable tool, which is constructed from a skunkbush sumac stem (Rhus trilobata), prickly pear cactus (Opuntia spp.) spines, and yucca (Yucca spp.) split leaf wrapping. In analyzing the artifact, we sought to determine if there were defining characteristics for different taxa of cacti spines, which would aid in identification of cacti taxa used to make the artifact. It was not possible to distinguish between different cacti taxa using a standard stereomicroscope. However, a Scanning Electron Microscope (SEM) allowed identification of surface structure characteristics of cacti spines and plant crystals. We therefore used SEM to aid in the identification of the cactus spines from the artifact by comparing them to fifteen samples of modern cacti, agave, and yucca, representing at least 12 species. We discovered that the surface structures vary considerably between different cacti taxa, and were able to successfully identify the cactus spines of the 2,000 year old artifact as prickly pear cactus. We also used a portable X-ray fluorescence (pXRF; Model: Bruker Tracer IV GEO® energy-dispersive pXRF) spectrometer to determine the elemental composition of microcrystalline structures (i.e., pigment residue) discovered in the SEM images. pXRF and energy dispersive X-ray spectroscopy revealed that pigments were embedded in the cactus spine tips. This unusual tool is the oldest tattooing artifact identified in western North America and provides evidence extending the antiquity of Native American tattooing in the southwestern United States back to the first century AD.

(AAF-LP2.2) Non-Destructive Imaging Techniques for the Study of Archaeological Perishables

Presenter: Gina M. Watkinson, MA - University of Arizona

Perishable collections in museums remain understudied even though they have the considerable potential to offer rare insights into the archaeological understanding of prehistoric people. Not only do perishable collections from excavations await preliminary analysis but it is imperative that archaeologists restudy perishable material as there are innovative analytical techniques and tools that can address questions that were not within the capability of technology in the early twentieth century. The development of imaging techniques has advanced researchers ability to reveal how material has been constructed, created, and used. This presentation will discuss the non-destructive and relatively inexpensive and accessible imaging techniques that the Arizona State Museum conservators are utilizing to better understand perishable technology and can even be used to distinguish and reveal the chemical nature of an artifact. These techniques include microscopic reflectance transformation imaging,

multiband imaging, and computed radiography. Case studies using examples from the American Southwest will be presented.

(AAF-LP2.3) Investigating the Organization of Production for Inka Polychrome Pottery from Pachacamac, Peru Using Archaeometric Techniques

Presenter: James A. Davenport, M.S. - University of New Mexico

Inka Polychrome pottery played an integral role in strategies of control for the Inka empire, which rose to power in Andean South America from C.E. 1400 to 1532. It was used in state-sponsored ceremonies, feasts, and events that promoted imperial power and control in the provinces. This pottery was highly standardized and produced with a limited suite of forms and designs, making it a recognizable symbol of state power and reciprocity. Despite its uniformity, it was produced at multiple places across the empire and was produced by part- and full-time specialists who were both Inka and subject potters. This research asks the question: how did the Inka organize the production of this pottery for use in the provinces? To address this question, an assemblage of Inka Polychrome pottery from the Inka-built Temple of the Sun at the site of Pachacamac is examined. Pachacamac was the political capital of the Ychsma polity and the location of an important oracle that was subjugated by the Inka and transformed into a major provincial center. Using traditional archaeological techniques of ceramic analysis combined with interdisciplinary archaeometric techniques from analytical chemistry, geology, and radiography, this pottery is examined to reconstruct its production technology. Neutron Activation Analysis was applied to examine chemical composition of the pottery and investigate source of manufacture. Laser Ablation-Inductively Coupled Plasma-Mass Spectrometry is used to investigate surface pigment composition. Thin section petrography is used to examine mineral composition and source of manufacture, processing of raw materials, firing, and forming technology. X-radiography is used to evaluate forming and construction techniques and sequences. These data, combined with data from traditional ceramic analyses, are used to determine the technology and production sequences of this pottery. By understanding how the pottery was created, the identity of the potters and the location where the pottery was made can be learned, and the organization of production for this pottery reconstructed.

(AAF-LP2.4) Reconstructing hominin paleoenvironments through the analysis of n-alkanes and branched glycerol dialkyl glycerol tetraethers: vegetation and temperature regimes of Paleolithic sequences in the Qinling Mountains of central China

Presenter: Mathew L. Fox, PhD - University of Arizona
Non-Presenting Author: Jessica Tierney
Non-Presenting Author: Huayu Lu
Non-Presenting Author: Shejiang Wang

The Qinling Mountains of central China have been the focus of numerous, high-profile paleoanthropological research projects, as this region contains some of the oldest (~1.2 Ma) hominin occupations in China and is one of the only areas in eastern Eurasia yielding Acheulean-like handaxe technology. In order to reconstruct environmental conditions associated with hominin occupations, we utilize two geochemical approaches that have never been used in the region before. First, we analyze the carbon isotopic values of leaf wax lipids (n-alkanes) to reconstruct the ratio of C3 to C4 vegetation and, secondly, we analyze branched glycerol dialkyl glycerol tetraethers (brGDGTs) derived from soils to reconstruct temperatures. In the southern Qinling Mountains, isotopic and temperature variability is low.

Temperatures remain relatively consistent and vegetation regimes are dominated by C3 vegetation (85-90%) throughout large portions of the middle to late Pleistocene. Conversely, in the northern Qinling Mountains, isotopic and temperature variability is significantly higher. Temperature regimes have a range of approximately 15°C and, intermittently, C4 vegetation increases to 40%, which we interpret as the expansion of forest-grassland mosaics. Results from this research indicate that conditions associated with the southern Qinling Mountains are dominated by stable vegetation regimes. In contrast, the northern Qinling Mountains are characterized by much more variable environmental conditions. These results appear to be in good agreement with the archaeological record where Paleolithic industries in the south are dominated by Mode I industries for more than one million years. By contrast, the north is famous for the presence of Mode II (Acheulean-like) handaxes and higher levels of technological diversity. This suggests that environments associated with hominin occupations in the southern mountains were sufficiently stable to support simple and expedient technologies throughout most of the Pleistocene. This stands in contrast to the north, where higher levels of environmental variability may have triggered the need for more diverse toolkits and greater investment in the development of bifacial, handaxe technology. Our research demonstrates that there are key differences between the southern and northern Qinling Mountains, and that these basins are located within an inflection point along a steep monsoonal gradient.

(AAF-LP2.5) Tephrochronological Dating of Archaeological Deposits Using Non-Visible Glass Shards

Presenter: Jayde Hirniak, MA - Arizona State University

Non-Presenting Author: Eugene Smith

Non-Presenting Author: Racheal Johnsen

Non-Presenting Author: Minghua Ren

Non-Presenting Author: Jamie Hodgkins

Non-Presenting Author: Fabio Negrino

Non-Presenting Author: Curtis Marean

Chemical characterization of cryptotephra is critical for temporally linking archaeological sites across vast geographic areas. Cryptotephra, also known as microscopic volcanic ash, were recently identified at two Middle to Upper Paleolithic sites from northwest Italy, Arma Veirana and Riparo Bombrini. Results show cryptotephra are present as small (75 wt. %) and low FeO (<1 wt. %). Trace element analysis by LA-ICP-MS show depletions in Ba, Sr, and Eu and an enrichment in Th, U and Pb, indicating a geochemical signature rare for volcanoes in the Mediterranean region. Shards at Riparo Bombrini (P3) are from the same eruption as P1 shards at Arma Veirana, providing a distinct link between deposits at both sites. Geochemical characteristics suggest a potential source from Lipari Island (56-37.7 ka) in Italy, however, due to slight discrepancies in the chemistry, it is likely that P1 and P3 represent a highly fractionated explosive phase of Lipari Island eruption that has not yet been reported. These preliminary results need to be investigated further. Regardless, this study highlights how cryptotephra can benefit archaeology, even without a confirmed source eruption.

(MOLEC-LP2) Topics in Molecular Spectroscopy Part II

(MOLEC-LP2.1) Optical Mapping of Biological Water in Single Live Cells by Stimulated Raman Excited Fluorescence Microscopy

Presenter: Lixue Shi, PhD - Columbia University

Corresponding Author: Wei Min

Water is arguably the most common and yet least understood material on Earth. Understanding of the spatial and compositional heterogeneity of water inside cells remains elusive, largely due to lack of proper water mapping tools with high sensitivity and spatial resolution. Here we will present a hybrid technique of stimulated Raman excited fluorescence (SREF) which integrates ultimate single-molecule detection sensitivity and fine chemical specificity. Further, we extend and develop SREF into a live-cell water-sensing tool, by coupling with the vibrational solvatochromism of an environment-sensitive Raman mode. This technique allows us to directly visualize spatially-resolved distribution of water states inside living cells. Interesting observations of intracellular water heterogeneity will be discussed.

(MOLEC-LP2.2) Science Rick Van Duyne Inspired – Perspectives as a Student and Professor

Presenter: Amanda J. Haes, PhD - University of Iowa

In the presentation, I will reflect and provide examples as to how my Ph.D. advisor, Richard Van Duyne, inspired me as a scientist since first meeting him in 1999. Rick's creativity pushed me to think outside of the box, make new connections, and enjoy moments of success. As a student, hiis ability to help me think about what was scientifically possible rather than what was possible given the state of the lab in a given moment, was excellent preparation for me as a professor. I will share some stories that highlight how his mentorship continues to be payed forward.

(MOLEC-LP2.3) Photothermal induced enhancement of Raman scattering – Discuss

Presenter: Duncan Graham, BSc Hons, PhD, CChem, FRSC, FRSE, FSAS - University of Strathclyde

Non-Presenting Author: Karen Faulds, PhD - University of Strathclyde

Non-Presenting Author: Jennifer Gracie

Non-Presenting Author: Chunfang Wu

Non-Presenting Author: Tell Tuttle

SERS is a technique which has been around since the 70s and various proposals have been put forward to understand and explain the mechanism. There is still not one unified understanding of the mechanism however the majority of the explanations come from a physics or physical chemistry perspective which ignores the nature of the molecules adhering to the surface and how these molecules are bonded, where the electrons go and what happens to the atoms on the metal surface. This talk will discuss recent preliminary data which shows an exciting breakthrough in being able to understand and explain more at an atomic and molecular level, the nature of the interaction between the surface bound molecules and the metal surface providing the enhancement. There are still many unanswered questions and the presentation will aim to encourage and stimulate debate with the audience on this topic.

(MOLEC-LP2.4) Raman for biomedical safety und security.

Presenter: Jürgen Popp, PhD - 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 is currently advertised as a hot and ambitious analytical technology that has all of the features needed to rapidly characterize biomolecules and complex biological specimen without the need of complex sample preparations steps. Raman spectroscopy is rapid, easy to use, noninvasive, and it could complement established bioanalytical methods in the near future. Here, we will present various proof-of-concept studies demonstrating the great potential of Raman spectroscopy addressing important unsolved questions in biomedical safety and security. In this context the rapid on-site detection of microbial contamination in food, water and air is a permanent concern in food industry and health care. It will be shown that Raman spectroscopy in conjunction with extensive statistical analysis holds great promise as point-of-use approach for a rapid monitoring and identification of bacteria at different taxonomic levels in water, soil and food (e.g. meat, milk). The wealth of information derived from the spectral fingerprint Raman data of bacteria from various habits can be further improved by combining Raman spectroscopy with methods such as stable isotope probing to elucidate microbial interactions. One drawback of Raman spectroscopy is the intrinsically low scattering cross sections making the detection of low concentrated species very difficult. A variety of Raman techniques are now available to enhance the signals of the intrinsically weak Raman scattering effect. The most prominent approach is surface enhanced Raman scattering (SERS). The second part of this presentation demonstrates that SERS using metal colloids as SERS active substrate in combination with a microfluidic lab-on-a-chip (LOC) device enables high throughput and reproducible measurements with highest sensitivity and specificity. The application of such a LOC-SERS approach for the detection of e.g. antibiotics in waste and surface waters will be presented. Furthermore, we will introduce strategies to prepare SERS-active substrates as one-time use SERS substrates for specific food analysis (e.g. quantitative detection of toxic food colorants).

(MOLEC-LP2.5) SERS Imaging of Protein Receptors

Presenter: Zachary Schultz, PhD - The Ohio State University

The ability to monitor biochemical events in living cells is important in applications such as drug targeting and for understanding biological pathways. The enhancement of Raman signals by plasmonic nanoparticles, surface enhanced Raman scattering (SERS), or by a metalized scanning probe tip, tip enhanced Raman scattering (TERS), provide high sensitivity methods for exploring biological molecules in living cells. In this report we will examine how the Raman signals observed from functionalized nanoparticles can be used to study and differentiate protein receptor recognition based on the Raman signal observed. We will demonstrate the application of this technique in live cells. We will also examine the chemical origins of the observed signal to further elucidate the utility of these approaches.

WEDNESDAY FEATURED LIVE ORAL ABSTRACTS

RSC/IRDG Supplemental Programming: RSC and IRDG session on Vibrational Spectroscopy

(RSC/IRDG.3) Screening DNA-binding molecules with 2D-IR spectroscopy - structure, dynamics and function

Speaker: Neil Hunt, MA PhD - University of York

A spectroscopic platform enabling fast and efficient structure-based characterization of sequence-selective minor groove recognition by DNA-binding polyamides is described. The sequence selectivity profiles of a suite of pyrrole-imidazole polyamide analogues were interrogated using 2D-IR spectroscopy combined with analysis of variance principal component analysis (ANOVA-PCA). Polyamides incorporating larger N-terminal substituents induced greater perturbation of the duplex structure relative to smaller N-methyl substituents. More extensive duplex perturbation was associated with an altered conformation of the hairpin polyamide when bound to DNA sequences containing a GTAC binding sequence. The presence of this modified polyamide conformation not only correlated with large melting temperature stabilization of the dsDNA, but also led to modified vibrational energy redistribution pathways in the polyamide•DNA complex, thereby establishing a combined spectroscopic and dynamic profile of preferential polyamide binding to a target base sequence. Understanding the spectroscopic features that identify sequence preferences for polyamide•DNA binding provides the basis for the development of next-generation gene regulatory agents with enhanced selectivity.

(RSC/IRDG.4) Quantitative hyperspectral coherent Raman scattering microscopy for label-free bioimaging

Speaker: Paola Borri, PhD - Cardiff University

Coherent Raman scattering (CRS) microscopy is a powerful technique for imaging living cells and tissues label-free, with high chemical specificity and 3D spatial resolution. We have developed a broadband coherent anti-Stokes Raman scattering (CARS) instrument based on a single 5fs Ti:Sa laser source, capable of exciting a wide vibrational range from 1000cm-1 to 3500cm-1 which allows us to perform hyperspectral microscopy [1]. This in turn has led to the development of quantitative chemical imaging algorithms to represent the hyperspectral dataset as a superposition of Raman spectra and concentration maps of individual chemical components (e.g. proteins, lipids, DNA) [2,3]. With this technique, we have measured the 3D spatial distribution of lipid droplets in live mouse oocytes and early embryos [4] and elucidated the link between this distribution and the use of lipids in the egg metabolism (generation of ATP by mitochondrial oxidation of fatty-acids) [5]. We have also measured the lipid uptake in living human adipose-derived stem cells differentiating into pre-adipocytes over 9 days, observing a heterogeneous uptake which is droplet-size dependent, time dependent, and lipid dependent

[6]. Recently, we applied the technique to visualise lipid partitioning in single planar membranes bilayers exhibiting liquid ordered (Lo) and disordered (Ld) domains. We find that that domains are consistently resolved, both chemically and spatially, in a completely label-free manner. Quantitative Raman spectra of the domains are provided in relative units of susceptibility, alongside spatially resolved concentration components [7]. It should be stressed that imaging single membrane bilayers is pushing the detection sensitivity of CRS to its limit. It is therefore remarkable that, despite the low signal levels, when combined with our quantitative data analysis methodology, label-free hyperspectral CRS imaging does encode enough information to retrieve the spectra and concentration of the Lo and Ld domains. [1] Optics Express (2013); 21, 7096. [2] Anal. Chem. (2013); 85, 10820. [3] Anal. Chem. (2019); 91, 2813. [4] Development (2016) 143, 2238. [5] J. of Cell Science (2019); 132, jcs228999. [6] Anal. Chem. (2016); 88, 3677-3685. [7] Anal. Chem. (2020) submitted.

(SPECIAL 2) Spectrochimica Acta B Best Paper Award Session

(SPECIAL 2.1) Listening to Laser Sparks: A Link Between Laser-Induced Breakdown Spectroscopy, Acoustic Measurements and Crater Morphology

Presenter: Baptiste Chide - Institut Supérieur de l'Aéronautique et de l'Espace (ISAE-SUPAERO), Toulouse, France

Non-Presenting Author: Sylvestre Maurice - Institut de Recherche en Astrophysique et Planétologie (IRAP - CNRS), Toulouse, France

Non-Presenting Author: Naomi Murdoch - Institut Supérieur de l'Aéronautique et de l'Espace (ISAE-SUPAERO), Toulouse, France

Non-Presenting Author: Jérémie Lasue - Institut de Recherche en Astrophysique et Planétologie (IRAP - CNRS), Toulouse, France

Non-Presenting Author: Bruno Bousquet - Université de Bordeaux

Non-Presenting Author: Xavier Jacob - Université Paul Sabatier (Toulouse III), Toulouse, France

Non-Presenting Author: Agnès Cousin - Institut de Recherche en Astrophysique et Planétologie (IRAP - CNRS), Toulouse, France

Non-Presenting Author: Olivier Forni - Institut de Recherche en Astrophysique et Planétologie (IRAP - CNRS), Toulouse, France

Non-Presenting Author: Olivier Gasnault - Institut de Recherche en Astrophysique et Planétologie (IRAP - CNRS), Toulouse, France

Non-Presenting Author: Pierre-Yves Meslin - Institut de Recherche en Astrophysique et Planétologie (IRAP - CNRS), Toulouse, France

Non-Presenting Author: David Mimoun - Institut Supérieur de l'Aéronautique et de l'Espace (ISAE-SUPAERO), Toulouse, France

Non-Presenting Author: Roger C. Wiens, PhD - Los Alamos National Laboratory

Scheduled for landing on Mars in February 2021, the Mars 2020 Perseverance rover will carry the SuperCam remote sensing suite, a multifunctional spectroscopy instrument to analyze Martian rocks and soils with Laser-Induced Breakdown Spectroscopy (LIBS), time resolved Raman and luminescence, Visible and Infrared Reflectance Spectroscopy, and color context imaging. It also includes a microphone that will record acoustic pressure fluctuations in the 100 Hz to 10 kHz frequency bandwidth. It will support LIBS investigation by listening to laser-induced sparks. As part of the preparation of surface operations, the acoustic signal associated with the plasma formation during LIBS is studied with respect to the shot-to-shot evolution of the laser induced crater morphology and plasma emission lines. A set of geological targets are sampled in depth with up to 300 shots, using a specifically designed LIBS setup coupled with an acoustic test bench under ambient terrestrial atmosphere. Results show that the decrease of the acoustic energy as a function of the number of shots is correlated with the target hardness/density. It also demonstrates that the acoustic energy can be used as a proxy for the ablated volume of the target. Listening to LIBS sparks thus provides new information relative to the ablation process that is independent from the LIBS spectrum itself. A further set of experiments have been performed under a controlled Martian atmosphere, which will allow us to compare the results obtained with different sets of atmospheric parameters with the ones related to ambient terrestrial atmosphere, published in the awarded paper.

(SPECIAL 2.2) Application of gold nanoparticles embedded in the amyloids fibrils as enhancers in the laser induced breakdown spectroscopy for the metal quantification in microdroplets

Presenter: Zita Salajkova, MEng - Central European Institute of Technology

Non-Presenting Author: Antonia Mallardi

Non-Presenting Author: Raffaele Mezzenga

Non-Presenting Author: Leonie van 't Hag

Non-Presenting Author: Nicola Cioffi

Non-Presenting Author: Gerardo Palazzo

Non-Presenting Author: Alessandro De Giacomo, PhD - University of Bari

Corresponding Author: Marcella Dell'Aglio, PhD - CNR-NANOTEC

In this work Nanoparticle Enhanced Laser Induced Breakdown Spectroscopy (NELIBS) has been employed for quantitative metal detection using amyloid fibrils coated with gold nanoparticles as enhancers. Amyloid fibrils represent, from one hand, an extremely interesting system for novel technologies, ranging from water purification to medical applications, and on the other hand, an ideal system for investigating the performance of laser-matter interaction in biological systems. The results obtained in this work show the potentiality of NELIBS for the quantification at sub-ppm (mg/kg) level of metallic elements (Cr, Pb, Tl, and Cd) even in the protein and/or biological environment, employing amyloid fibrils with gold nanoparticles. Moreover, the single-shot measurements reveal the promising use of this technique in applications where high sensitivity and/or limitation in the sample amount are demanded.

(SPECIAL 2.3) Imaging rare-earth elements in minerals by laser induced plasma spectroscopy: Molecular emission and plasma induced luminescence

Presenter: Michael Gaft, Prof - Ariel University

Non-Presenting Author: Vincent Motto-Ros, PhD - Institut Lumiere Matiere

LIBS, PIL and Luminescence μ-mapping for optically active centres of minerals interpretation M. Gaft1, V. Motto-Ros2 1Ariel University, Department of Physics, Israel 2University Claude Bernard, Lyon, France One aim of physics of minerals is the identification of luminescence and colour centres. It is often not a trivial. Time-resolved luminescence and excitation spectra, optical absorption spectrum, decay time and their behaviour with temperature give information on energy of emitting and absorbing levels. Based on this information, potential optically active centres may be proposed. Nevertheless, in certain cases it is not enough. Particularly important example is transition metals, which play important role in physics of minerals as common luminescence and colour centres. For example, Cr^{3+}, Mn^{4+} and V^{2+} have the same 3d3 configurations and the similar optical behaviour and their interpretation is often ambiguous. More than this, their optical properties depend strongly on the local crystal field strength, and Cr^{3+} emission may be similar to Ti^{3+} with 3d1 configuration. The correlation of the observation of the specific luminescence and colour with particular impurity concentration may indicate the source but it is not proof of the origin. One of the reasons is that luminescence is an extremely sensitive technique with lower detection limit than most "controlling" methods. LIBS may overcome this problem because its sensitivity for Cr, Mn, Ti, Fe, V, is comparable with luminescence technique. Additional advantage is

the possibility of µ-mapping with 5 µm spatial resolution correlating emissions and colour zoning with relevant elements distribution. Single laser source, such as 266, 355 and 532 nm form Nd-YAG, allows reliable matching between laser-induced time resolved luminescence, atomic and ionic narrow emission lines of specific elements, such as Cr, Mn, Ti, Fe, V, molecular emission, which is especially relevant for TiO, MnO, and FeO and plasma-induced luminescence which is the mostly effective for luminescence centres with long decay time, such as $Mn2+$, $Fe3+$ and $Cr3+$. The specific example is mineral kyanite $Al2SiO5$ where multiple luminescence and colour centres present, which may be interpreted as belonging to different elements, such as Cr, Mn, V, Ti, Fe, located in four structurally different positions of aluminum.

(SPECIAL 2.4) Laser Ablation Based Techniques

Presenter: Rick Russo, PhD - Applied Spectra, Inc

Thirty eight years seems like a long time, and it is considering that it is more than half the number of years since the invention of the laser (mid 1960's) and its application to ablation. Analytical atomic spectroscopy was recognized early in this time as a viable use of laser ablation (LA) – for sampling and for direct elemental analysis. It was obvious at the beginning that LA was a unique science with a deep rooted fundamental basis in physic and chemistry – the beginning of an entire field of study. Fundamentals are imperative and they still are addressed. However, empirical studies seem to now dominate as the goal is to achieve reproducible behavior – the backbone for applications. Early instrumentation was developed but could not meet the needs of industrial requirements; not because of the technology itself, but because lasers, spectrometers (optical and mass), and computers also were just being developed, and were unreliable. Times have changed! Laser, spectrometers and computers have been perfected (always more to come) to the point that sensitive, accurate and precise laser ablation chemical analysis is becoming mainstay. Companies now commercializing LA based instruments for LIBS and ICP are seeing increased demand for products as the world awakens to the use of this 21st century technology to replace the antiquated chemical digestion approach to analysis.

(ATOM-LP1) Topics in Atomic Spectroscopy Part 1

(ATOM-LP1.1) Comparing the Dynamics of Molecular and Atomic Emission of Excited Species in Laser Induced Plasmas: Potential Benefits for the Determination of Halogens.

Presenter: Nerea Bordel, PhD - University of Oviedo

Non-Presenting Author: Luis Javier Fernández-Menéndez - University of Oviedo

Non-Presenting Author: Cristina Méndez-López - University of Oviedo

Non-Presenting Author: Cristina Gonzalez-Gago - University of Oviedo

Non-Presenting Author: Jorge Pisonero, PhD - University of Oviedo

Detection of molecular emission (e.g. from CaF) has become a resource in LIBS for the indirect determination of halogens, such as fluorine, in different kind of samples. Limits of detection achieved using the molecular emission improve those obtained from atomic emission; maintaining the simplicity of LIBS technique since no complementary procedures or devices are required (such as He atmosphere or the need of an ablation chamber). The optimization of the experimental conditions for molecular emission detection has shown the need of using long delay times to guarantee that the atomic emission does not interfere or hide the molecular signals, and wide acquisition gates due to the low plasma emission at such long delay times. In this work, a detailed study of the temporal and spatial evolution of CaF emission has been carried out and compared with the results obtained for the atomic emission in LIBS. The results show that not only time (delay and gate time) is a parameter of interest to optimize molecular signals but also the region of the plasma plume, whose emission is detected, can play an important role. In particular, the molecular emission prevails in a small stripe of the plasma, close to the sample, while the atomic signals are emitted mainly from higher distance to the surface.

(ATOM-LP1.2) Automated elemental analysis of dietary supplements using laser ablation ICP-MS

Presenter: Todor I. Todorov, PhD - US Food and Drug Administration, Center for Food Safety and Applied Nutrition

One of the missions of the U.S. Food and Drug Administration is to ensure the safety of the American food supply, which includes monitoring essential, non-essential, and potentially toxic elements in a wide variety of foods and dietary supplements. Traditionally, this has been done by acid decomposition followed by ICP-MS and/or ICP-OES analysis. LA-ICP-MS is an alternative to conventional ICP-MS analysis, as it not only eliminates time-consuming microwave assisted digestion techniques, but also provides a path for the analysis of matrices that may be resistant to acid digestion, such as multivitamins and dietary supplements. However, due to sample cell size and geometries, the use of LA-ICP-MS historically lacked high throughput. In this study, we will present results from a laser ablation system equipped with a self-seal microchamber and a carousel autosampler. The advantages of the system are that (1) it allows a large number of samples to be analyzed unattended, (2) has an easy to clean micro-chamber and (3) has a small laser window (cost-effective compared to replacing a large format laser chamber window). Multivitamins and dietary supplements were combined with a cellulose matrix fortified with internal standard elements, pressed as pellets, and analyzed by LA-ICP-MS. Comparison of quantification approaches with spiked cellulose, matrix matched standards, and standard addition calibrations will be discussed.

(ATOM-LP1.3) Long-range Isotope Detection with Laser Plasma-Based Spectroscopy

Presenter: Vassilia Zorba, PhD - Lawrence Berkeley National Laboratory & UC Berkeley

Non-Presenting Author: Jose Chirinos - Lawrence Berkeley National Laboratory

Non-Presenting Author: Xianglei Mao - Lawrence Berkeley National Laboratory

Non-Presenting Author: George Chan - Lawrence Berkeley National Laboratory

Non-Presenting Author: Richard Russo - Lawrence Berkeley National Laboratory

The detection of isotopes in solid samples at a distance represents a significant challenge. In this work we present our latest results on the combination of Laser Ablation Molecular Spectrometry (LAMIS) with femtosecond laser filamentation for the detection of isotopes from solid samples at extended distances. Specifically we focus on strategies for improving sensitivity, and for controlling the filament length and propagation characteristics by tailoring the femtosecond laser beam. This work provides new insights into the use of laser plasma-based techniques for elemental and isotopic analysis at long distances.

(ATOM-LP1.4) Identification and Quantification of Arsenic Species in Pacific Geoduck Clam (Panopea generosa)

Presenter: Lee Yu, PhD - National Institute of Standards and Technology

Non-Presenting Author: Caleb Luvonga - National Institute of Standards and Technology

Non-Presenting Author: Tomohiro Narukawa - National Metrology Institute of Japan

Produced exclusively in the northwestern US and Canada, geoduck clam (Penopea generosa) is a delicacy in East Asian cuisine. Trading of the commodity requires assessment of toxic elemental contents, including arsenic. The National Institute of Standards and Technology (NIST) is developing a Standard Reference Material (SRM) to meet the needs in food safety and quality assessment of the commodity. Fresh geoduck clams were frozen, cryomilled, and packaged in glass jars. For the assessment of the arsenic contents, the candidate SRM was extracted with dilute nitric acid and water for evaluation of the regulated toxic inorganic arsenic and the nonregulated organic arsenic species. The total arsenic in the geoduck was determined by ICP-MS after a robust nitric acid digestion in the microwave. Arsenic acid (AsV) was the only inorganic arsenic species found at 0.2 mg/kg, which accounts for about 5% of the total arsenic in the geoduck. The arsenic species in the candidate SRM was identified based on the retention time using cation and anion exchange chromatography. Arsenobetaine (AB), arsenosugar 328 (As328) and arsenosugar 482 (As(482) were identified as the major arsenic species in the geoduck while dimethylarsinic acid (DMA) and arsenosugar 392 (As(392) were present in minor quantities. The level of monomethylarsonic acid (MMA) and trimethylarsine oxide (TMAO) was undetectable in the sample, which allowed the arsenicals to be used as internal standards in speciation measurements. The optimization of the extraction and measurement leading to the results of the arsenic species will be presented and discussed.

(ATOM-LP1.5) Advances in matrix-matched standards for biomedical analysis

Presenter: Matthieu Baudelet, PhD - University of Central Florida
Non-Presenting Author: Mauro Martinez

Laser-induced breakdown spectroscopy (LIBS) is now a widely accepted technique for direct sampling of hard biological materials for elemental analysis. The calibration strategy is crucial for the analytical robustness of the method and encompass the sample treatment, the calibration material, and the instrumental conditions to obtain the signal. This presentation will focus on the calibration material. These reference materials need to be similar to the sample, reproduce the laser ablation as well as the plasma conditions. In this presentation, we will present the development of matrix-matched reference hard biological materials to analyse teeth, fingernails and hair. We will show the synthesis protocols, the comparison with laser ablation behaviour and plasma conditions for these samples and show their performance with real samples in comparison with ICP-MS analysis. The discussion will also focus on the new directions that can be taken to improve the number of elements in these reference materials, their analytical performance as well as their characterization.

(CHEM-LP) Topics in Chemometrics

(CHEM-LP.1) Classical Least Squares Calibration using Partial Analyte's Knowledge

Presenter: Hamid Abdollahi, PhD - Institute for Advanced Studies in Basic Sciences

Classical Least Squares Calibration using Partial Analyte's Knowledge H. Abdollahi*, S. Khalili Ali Abad, N. Omidikia, Department of Chemistry, Institute for Advanced Studies in Basic Sciences, Zanjan, 45195-1159, Iran. ABSTRACT : Calibration is finding a relationship between instrument response and physical or chemical property of analyte(s) [1]. Multivariate calibrations are divided into two directions, Classical Least-Squares (CLS) and Inverse Least-Squares (ILS) [2]. ILS methods such as Principle Component Regression (PCR), Partial Least Squares (PLS) are more popular than CLS because inverse methods allow one to study multicomponent samples where only one or few analytes are of interest, but the concentration, spectra, and chemical identities of other components in the calibration samples are unknown while the complete knowledge of chemical and physical components such as concentration, baseline, offset and etc. is necessary for CLS [3]. On the other hand, CLS method allows one to do qualitative and quantitative analysis, simultaneously due to estimated regression coefficients since they are pure spectra of components. The need for full information about the all constituents involved in responses of calibration set has been stated as the disadvantage of CLS. In this contribution a new procedure is proposed for CLS which it can work with the same input information of ILS methods. To this goal, a series of random concentration vectors were augmented to compensate unknown components contributions. Finally, an algorithm was developed to optimize the required number of random augmented vectors and was tested on simulated and several real data sets. Keywords: "Multivariate Calibration, Modified-CLS, Duality Concept" References: [1] H. Martens, T. Næs, "Multivariate calibration" John Wiley and Sons, New York, 1989, pp. 168–213. [2] D. K. Melgaard, D. M. Haaland, C. M. Wehlburg, "Concentration residual augmented classical least squares (CRACLS): a multivariate calibration method with advantages over partial least squares, Appl. Spec. 56 (2002) 615- 624. [3] Olivieri, Alejandro C. Introduction to Multivariate Calibration: A Practical Approach. Springer, 2018

(CHEM-LP.2) On-board Chemometric Models with Portable Raman Spectroscopy for Identifying Plasticizers in Food Contact Materials

Presenter: Betsy Jean Yakes, PhD - U.S. Food and Drug Administration
Non-Presenting Author: Katherine Carlos - U.S. Food and Drug Administration
Non-Presenting Author: Eric Crump - Oak Ridge Institute for Science and Education
Non-Presenting Author: Timothy Begley - U.S. Food and Drug Administration

Having a sufficient supply of nutritious and safe food is at the heart of food security. Rapid screening methods and chemometric modeling can support food security through ensuring the safety of food and reducing contamination events that could lead to food waste. In recent years, plasticizers used to increase flexibility of food contact materials have come under scrutiny for potential transfer to foods and potential toxicity to humans. In the dairy industry, one such set of compounds that is being evaluated for frequency of use, potential routes of exposure, and risk to consumers is ortho-phthalates which can be used in flexible tubing, liners, and gaskets throughout milk extraction and processing equipment. In order to understand the use of phthalate versus non-phthalate plasticized food contact materials, a robust, rapid, and portable analytical method must be developed for on-site screening and use determination.

This presentation will overview our research using Raman spectroscopy to create a chemometric model that can identify phthalate versus non-phthalate plasticized dairy tubing, liners, and gaskets. This classification model can rapidly and accurately identify ortho-phthalate containing polyvinyl chloride (PVC) and has the potential to be employed as a future field screening technique for regulators and the dairy industry.

(CHEM-LP.3) Interpretable Deep Neural Networks for Infrared Microscopic Images in Clinical Studies

Presenter: Axel Mosig, PhD - Ruhr-University Bochum
Non-Presenting Author: David Schuhmacher

In many applications of infrared microscopy in biomedical applications, it is straightforward to train deep neural networks to classify specimen into diagnostically relevant classes. However, the resulting neural networks are difficult to interpret -- it is generally difficult to determine whether the classifier has learned relevant features of disease, or whether it has rather learnined confounders that do not gerneralize beyond a given study. It is thus of predominant importance to train classifiers that can segment disease-associated regions in medical images. While numerous deep learning approaches, most notably U-Nets, exist to learn segmentations, these approaches typically require precise reference segmentations as training data. As a consequence, obtaining precise annotations of histopathological samples has become a major bottleneck to establish segmentation learning approaches. Beside state-of-the-art approaches of neural network interpretation, this talk introduces a neural network approach to avoid the annotation bottleneck in the first place: the introduced approach requires two-class labels such as cancer vs. healthy at the sample level only. Using these sample-labels, a meta-network is trained that infers a segmenting neural network which will segment the disease-associated region (e.g. tumor) that is present in the cancer samples, but not in the healthy samples. This process results in a network, e.g. a U-Net, that can segment tumor regions in arbitrary further samples of the same type. We showcase some recent applications of this approach in both technical validation studies as well as ongoing clinical studies.

(CHEM-LP.4) A Quantitative Reliability Metric for Querying Large Databases

Presenter: Peter B. Harrington, PhD - Ohio University
Non-Presenting Author: Zewei Chen, MS - Clippinger Laboratories, Department of Chemistry and Biochemistry, Ohio University
Non-Presenting Author: Vivekananda Shetty, PhD - Houston Forensic Science Center
Non-Presenting Author: Preshious Rearden, PhD - Houston Forensic Science Center
Non-Presenting Author: Angelica Noyola, MBA - Houston Forensic Science Center

A modern tool for identifying data objects is by comparison to libraries of reference objects. Similarity metrics, such as correlation, are used to search for a list of the closest matching objects. In some instances, the correct identification is not the closest match, therefore having a quantitative measure of the search reliability can help the analyst determine whether the queried data object is contained in the reference collection or finding the best match that may not be the closest match. The quantitative reliability metric (QRM)1 can be used with any library search. Instead of relying on a one-to-one comparison between the query and reference objects, the QRM will use a number of K comparisons.

The QRM was largely ignored with only 11 citations since its publication. It recently was refined to assist in the identification of novel synthetic opioids (NSOs) by gas chromatography/mass spectrometry (GC/MS). A customized library of more than 223,000 mass spectra was queried with several thousand NSO samples. The QRM provided a good measure of the search quality but also proved invaluable for library optimization. The QRM was first developed in 1987 but was modified to eliminate the selectivity factor that allowed for a comparison of different libraries and modified so that it ranges from 100 to 0. It computes a weighted sum of squares of the differences between the intralibrary similarity measures and the similarities with the query spectrum. A QRM may be calculated for each object in the list of closest matching objects in the search results. The QRM takes advantage of the match list from the search of the library spectrum against the library to form a constellation of the closest matching spectra that can be used for evaluation. The key advantage is that it can be used with any kind of library or database and any kind of similarity metric. 1. Harrington, PB; Isenhour, TL; A Quantitative Measure of the Reliability of Searches of Spectral Libraries. Analytica Chimica Acta, 1987, 197, 105-119.

(CHEM-LP.5) On the use of deep learning for small biomedical data sets

Presenter: Thomas W. Bocklitz, PD Dr rer nat habil - 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

Non-Presenting Author: Rola Houhou - 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

Non-Presenting Author: Parijat Barman

Non-Presenting Author: Michael Schmitt, PhD - Friedrich-Schiller University Jena, Germany

Non-Presenting Author: Tobias Meyer

Non-Presenting Author: Pranita Pradhan

Non-Presenting Author: Nairveen Ali

Non-Presenting Author: Jürgen Popp, PhD - 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

Deep learning (DL) techniques have a large impact on the way predictive models are generated for molecular sensitive spectral and image data. That results from the fact that DL outperformed traditional chemometric and machine learning (ML) approaches because for DL the feature extraction does not need to be manually investigated and found for every data set on an individual basis. Rather the feature extraction is learned implicitly based on the structure of the model determined by its hyperparameters [1]. This works quite well in cases large data sets with standardized data (either images or spectra) are existent. Nevertheless, these requirements aren't met in studies where biomedical data is generated outside of the clinical standard workflow. For such biomedical data we investigate deep learning to solve the inverse problem linked to the measurement process [2], which generates standardized data leading to a lower sample size requirement. Beside this we research the influence of transfer learning on the generalization performance and also how the estimated generalization performance is changing when

the hyperparameters of the models are fixed but the selection of training / validation / testing data are shuffled. These studies are a starting point to investigate how the generalization performance of DL can be predicted in small sample size scenarios and how these DL performances can be compared with ML performances typically estimated by cross validation.

(ATOM-LP2) Topics in Atomic Spectroscopy Part 2

(ATOM-LP2.1) Advances in Elemental Analysis of Non-metals by Plasma Assisted Reaction Chemical Ionization Mass Spectrometry

Presenter: Kaveh Jorabchi, PhD - Georgetown University

Non-Presenting Author: Joseph E. Lesniewski - Georgetown University

Non-Presenting Author: Kunyu Zheng - Georgetown University

Non-Presenting Author: Michael Dolan Jr. - Georgetown University

Non-Presenting Author: Samuel White - Georgetown University

Non-Presenting Author: Wanqing Li - Georgetown University

High-sensitivity elemental mass spectrometry (MS) as a chromatographic detector provides attractive capabilities such as facile screening of compounds containing an element of interest, and quantitation without compound-specific standards. These capabilities complement the identification of compounds by molecular MS, together offering a universal non-targeted approach for rapid detection, identification, and quantitation of compounds. However, application of this approach to organic compounds has been limited. This is because high-sensitivity elemental detection of common heteroatoms such as halogens, sulfur, and phosphorus has been challenging using conventional plasma-based MS. These challenges stem from low efficiency for heteroatom ion generation in the plasma as well as plethora of polyatomic isobaric interferences originating from the plasma, solvents, and sample compositions.

To address these challenges, we have developed a plasma-based elemental ionization technique termed plasma-assisted reaction chemical ionization. In this technique, a plasma is used to convert analytes into element-specific polyatomic neutrals which are then ionized in the afterglow using variety of chemical ionization methods. Accordingly, plasma and ionization characteristics can be tuned independently to maximize the ion formation efficiency. Moreover, the ionization occurs at a relatively low temperature, facilitating use of a variety of commercially available MS platforms to resolve isobaric interferences. In this presentation, the progression of the technique will be discussed, culminating in the recent advances. In particular, we will focus on formation of novel element-specific ions enabled by sodiation in the gas phase, e.g. Na_2F^+ for F detection. Implementation using a variety of plasmas including dielectric barrier discharge and inductively coupled plasma will be discussed, demonstrating versatile nature of this ionization technique. Finally, an example for non-targeted analysis will be presented, highlighting facile combination of elemental and molecular MS on a single platform to conduct non-targeted detection of fluorinated compounds.

(ATOM-LP2.2) LIBS for Planetary Exploration: The Sky is NOT the Limit

Presenter: Roger C. Wiens, PhD - Los Alamos National Laboratory

Non-Presenting Author: Xiong Wan - Shanghai Institute of Technical Physics

Non-Presenting Author: Jérémie Lasue - Institut de Recherche en Astrophysique et Planétologie (IRAP - CNRS), Toulouse, France

Non-Presenting Author: Sylvestre Maurice - Institut de Recherche en Astrophysique et Planétologie (IRAP - CNRS), Toulouse, France

In 2012, ChemCam became the first LIBS instrument to explore another planet. Over the last decade, we have tracked its progress as it returned > 700,000 spectra from targets within 7 meters of the Curiosity rover on Mars, leading to exciting discoveries. In addition to providing quantitative abundances of major rock-forming elements, results included the first Mars analyses of H, Li, B, and F. Each of these elements has provided new insights into the geochemistry, groundwater, and climate history of the red planet. ChemCam continues its exploits as the Curiosity rover climbs Mt. Sharp, traveling into ever-younger terrain over > 350 m of vertical ascent. ChemCam's successor, SuperCam on NASA's Perseverance rover, is scheduled to land on Mars in February 2021. SuperCam retains the LIBS capability while adding remote time-resolved green Raman and luminescence spectroscopy by frequency doubling the Nd:YAG laser used for LIBS. SuperCam also includes an infrared spectrometer, a color high-resolution imager, and a microphone. The latter will be used to listen to atmospheric phenomena and LIBS plasma shock waves. The latter provide information on the physical properties of targets, including rock hardness. Other space-faring nations are also using LIBS for planetary science. The Indian Chandrayaan-2 lunar rover included a small LIBS instrument with fixed focus designed to observe rocks and soils directly beneath the rover. The Vikram lander's descent in September 2019 deviated within ~2 km of the lunar surface and it crashed. The Chinese Space Agency has developed a LIBS instrument called MarsCode for its Tianwen-1 rover to land on Mars in spring 2021. It has many similar design features to ChemCam, including the capability to target rocks and soils several meters from the rover. MarsCode is located in the body of the rover and uses a periscope mirror to observe its targets. An infrared spectrometer is also part of the MarsCode package. If both SuperCam and MarsCode are successful, the Earth should benefit from a flood of new LIBS data from the red planet. Various organizations are actively seeking to extend the reach of LIBS to other planets in the near future.

(ATOM-LP2.3) Background Subtraction In Near-Surface Analysis With GD-OES - Adding A New Dimension With The High- Resolution CCD Spectrometer

Presenter: Arne Bengtson, PhD - SWERIM AB

Non-Presenting Author: David Malmstroem

Glow Discharge Optical Emission Spectroscopy (GD-OES) for near-surface compositional depth profiling (CDP) is a powerful tool, but the accuracy is limited by background radiation in the very early stage of the plasma. There are two known sources of this background: 1) emission from diatomic molecules formed by light elements; 2) a broad continuum from dissociation of H_2 molecules. These signals are mainly caused by release of adsorbed molecular compounds from the interior lamp walls, with some added contribution of organic surface contamination of the sample. The extent of these start-up effects from molecular species depends on the condition of the lamp and the quality of the lamp argon. With the introduction of high-resolution CCD spectrometers with fast readout rate of complete spectra, the capability to identify and subtract background signals has been dramatically improved. It is possible to measure emission signals in real time at any spectral position. With high resolution (< 30 pm), spectral positions adjacent to the analytical lines can be utilised for accurate background correction. In addition, the full spectral coverage allows identification of the least affected analytical lines for each element of interest, reducing the risk of analytical errors due to background emission even further. Two methods have been devised to separate "true" from "false" elemental surface profiles. The first method uses at least two well separated spectral lines from one element, provided several lines from the element are available. If surface peaks with the same shape of the intensity-time profile are observed for all lines, the observed elemental surface enrichment is most likely true; if absent for at least one line it is

definitively false. The second method uses an adjacent background spectral position, free from elemental lines. If the elemental line and background position both give very similar profiles both in shape and intensity, the apparent elemental surface enrichment is definitively false. In this work, examples of near-surface quantified depth profiles will be presented using background correction as outlined above. Also, spectra from the plasma start-up will be shown to illustrate how true elemental emission can be distinguished from molecular and H2 continuum backgrounds

(ATOM-LP2.4) Challenges in the Laser Ablation of Soft Tissues

Presenter: Pavel Porizka, PhD - Brno University of Technology

Non-Presenting Author: Anna Šindelářová

Non-Presenting Author: Jakub Buday

Non-Presenting Author: Pavlína Modlitbová

Non-Presenting Author: Katerina Kubickova, Mrs.

Non-Presenting Author: Milan Kaska

Non-Presenting Author: Marcela Buchtova

Non-Presenting Author: Jozef Kaiser, Prof - Brno University of Technology

The main benefit of laser-induced breakdown spectroscopy (LIBS) in elemental imaging of biological samples is namely the capability to provide large scale analysis (i.e. whole slide imaging). Thus, LIBS may serve as a complementary technique providing a fast scan of the sample and guiding the researcher in further analysis across applications, incl. cancer research. However, elemental imaging of biological samples still remains a challenging task, mainly due to the soft nature of biological tissues and its heterogeneous composition. Laser-tissue interaction is a complex phenomenon that is simultaneously influenced by several parameters (laser energy, spot size, ambient atmosphere, etc.). Thus, in our work, we have suggested a way how to prepare and optimize soft-tissue samples in order to get the best possible analytical signal response. Concurrently, we aim to stick to the histological preparation of the sample while it is a golden standard used in clinical research. Therefore, we only used paraffin embedding and avoided homogenization. We analyzed samples in the form of paraffin blocks and 10 μm slices on a glass slide. Moreover, we outlined two techniques of optimization of elemental imaging. Together with the signal response, we have also investigated the plasma properties and its temporal and spatial evolution through shadowgraphy. We contributed to the development of LIBS in clinical research while analyzing human skin melanoma. We have selected several analytes (Ca, Mg, K, etc.) and imaged their distribution in the tissue. Finally, the relevance of the obtained results in the analysis of cancerous tissues is discussed.

(ATOM-LP2.5) Tracing element processes and pathways with reliable isotopic composition data

Presenter: Michael Wieser, PhD - Physics and Astronomy, University of Calgary

Chemical elements including (but by no means limited to) calcium, copper, zinc, and sulfur play critical roles in biochemical and geochemical processes. Disruptions in the delicate balance among these elements in living systems are implicated in diseases such as Alzheimer's, Parkinson's, and cancer and are of significant concern in human-environmental interactions. The challenge is to gain insights into the physical and chemical mechanisms affecting these elements in living systems and thus understand how these metals are processed in the geosphere and biosphere. Many analytical methods rely on

quantification of amount of element, but these data can be ambiguous and do not provide the detail needed to follow an atom through a system. Knowledge of isotopic composition of the element adds a dimension to the data to elucidate source(s) of the element to the system and physical or chemical processes responsible for incorporation and translocation of the element. This is because isotopes of an element contain the same number of protons and different numbers of neutrons. The same number of protons means that neutral atoms have the same number of electrons and participate in chemical reactions in a similar manner. However, different numbers of neutrons result in different masses and this impacts kinetic reaction rates and thermodynamic equilibria of different isotopes. Careful quantification of the redistribution of an element's isotopes provides valuable insights into processes on an atomic scale. The thoughtful application of isotope abundance data can help to understand why changes in the isotopic composition of Ca, Cu, Zn, and S occur and we can exploit this knowledge to understand the role of these elements in biogeochemical interactions. Success with the isotope approach requires that contamination of the samples is controlled, and analytical biases are monitored and addressed. In this presentation, how critical aspects of the analytical method are brought into focus such that meaningful results are obtained from small amounts of sample.

(BIM-LP) Topics in Biomedical & Bioanalytical

(BIM-LP.1) Next-Generation Synthetic Receptors for Advancing Selective Virus Detection: Augmenting Analytical Techniques in Sensitivity and Selectivity

Presenter: Boris Mizaikoff, Prof. Dr. - Ulm University / Institute of Analytical and Bioanalytical Chemistry

Biomimetic recognition utilizing molecularly imprinted polymers (MIPs) has proven its potential by providing synthetic receptors for numerous analytical applications including liquid chromatography, solid phase extraction, biomimetic assays, and sensor systems. The inherent advantages of synthetic receptors and functionalized membranes in contrast to biochemical/biological recognition and immobilization schemes include their robustness, synthetic versatility, and potentially lower costs. In principle, molecularly imprinted/templated materials are an ideal molecular capturing matrix tailorable for selective recognition or immobilization of a wide range of molecules, and even of entire organisms. Of particular interest is the development of biomimetic recognition schemes for selectively binding proteins and large biomolecules, e.g., at the surface of biomedical devices for promoting or preventing adhesion of selected biomolecules, as well as for controlled molecular release. While materials with recognition capabilities even for larger biomolecules have achieved substantial advancements, the synthesis of molecularly imprinted materials with virus recognition properties remains challenging. We will discuss innovative strategies for developing biomimetic virus capture materials yielding excellent selectivity and binding capacity, which could be achieved by a novel and generic strategy for suppressing non-specific binding via coating with deliberately selected blocking agents, a.k.a., 'enhanced selectivity by passivation'. Next to competitive studies with non-template viruses, applications in real-world biotechnological scenarios corroborate the practical potential of this approach. Finally, superior strategies for visualizing virus binding to the particle surface will be shown. Selected References: [1] Gast, M., et al., Analytical Chemistry, 90, 5576−5585 (2018) [2] Gast, M., et al., Analyst, 143 (11), 2616-2622 (2018) [3] Gast, M., et al., Material Science and Engineering: C, 99, 1099-1104 (2019) [4] Gast, M., et al., Trends in Analytical Chemistry, 114, 218-232 (2019) [5] Gast, M., et al., Human Gene Therapy Methods, 30, 235-244 (2019) [6] Gast, M., et al., Analytical Chemistry, 92, 3050-3057 (2020)

(BIM-LP.2) Higher Harmonic Generation for Instant Pathology: Results from the Clinic

Presenter: Marloes Groot, Prof. dr - Vrije Universiteit Amsterdam

Non-Presenting Author: Laura Van Huizen

Non-Presenting Author: Theodora Radonic

Non-Presenting Author: Frank Van Mourik

Non-Presenting Author: Danielle Seinstra

Non-Presenting Author: Chris Dickhoff

Non-Presenting Author: Johannes Daniels

Non-Presenting Author: Idris Bahce

Non-Presenting Author: Jouke Annema

During lung cancer operations a rapid and reliable assessment of tumor tissue can reduce operation time and potentially improve patient outcomes. We show that third harmonic generation (THG), second harmonic generation (SHG), and two-photon excited autofluorescence (2PEF) microscopy reveals relevant, histopathological information within seconds in fresh unprocessed human lung samples. We used a compact, mobile microscope and recorded images within 1 – 3 seconds using a power of 5 mW. The generated THG/SHG/2PEF images of tumorous and non-tumorous tissues are compared with the corresponding standard histology images, to identify alveolar structures and histopathological hallmarks. Cellular structures (tumor cells, macrophages, lymphocytes) (THG), collagen (SHG), and elastin (2PEF) are differentiated and allowed for rapid identification of carcinoid with solid growth pattern, minimally enlarged monomorphic cell nuclei with salt-and-pepper chromatin pattern, and adenocarcinoma with lipidic and micropapillary growth patterns. THG/SHG/2PEF imaging is thus a promising tool for clinical intra-operative assessment of lung tumor tissue.

(BIM-LP.3) Deep Learning-enabled Computational Microscopy and Sensing

Presenter: Kevin De Haan, MASc - UCLA

Non-presenting author: Aydogan Ozcan, PhD - University of California, Los Angeles

Deep learning is a class of machine learning techniques that uses multi-layered artificial neural networks for automated analysis of signals or data. The name comes from the general structure of deep neural networks, which consist of several layers of artificial neurons, each performing a nonlinear operation, stacked over each other. Beyond its main stream applications such as the recognition and labeling of specific features in images, deep learning holds numerous opportunities for revolutionizing image formation, reconstruction and sensing fields. In fact, deep learning is mysteriously powerful and has been surprising optics researchers in what it can achieve for advancing optical microscopy, and introducing new image reconstruction and transformation methods. From physics-inspired optical designs and devices, we are moving toward data-driven designs that will holistically change both optical hardware and software of next generation microscopy and sensing, blending the two in new ways. Today, we sample an image and then act on it using a computer. Powered by deep learning, next generation optical microscopes and sensors will understand a scene or an object and accordingly decide on how and what to sample based on a given task – this will require a perfect marriage of deep learning with new optical microscopy hardware that is designed based on data. For such a thinking microscope, unsupervised learning would be the key to scale up its impact on various areas of science and engineering, where access to labeled image data might not be immediately available or very costly, difficult to acquire. In this presentation, I will provide an overview of some of our recent work on the use of deep neural networks in advancing computational microscopy and sensing systems, also covering their biomedical applications.

(BIM-LP.4) A Bioinspired Plasmonic Sensing Platform to Fingerprint Bacterial Metabolism

Presenter: Regina Ragan, PhD - University of California, Irvine

Non-Presenting Author: Allon I. Hochbaum

Even in the 21st century, bacterial infections are still treated empirically. Antibiotics are prescribed preemptively, because a proper diagnosis relies on culture growth which takes a day or more and can delay patient treatment, increasing morbidity. Yet the unnecessary administration of powerful, broad-spectrum antibiotics leads to the proliferation of antibiotic resistance. A bioinspired sensing platform, composed of 2-dimensional physically activated chemically (2PAC) assembled surface enhanced Raman

scattering (SERS) sensors coupled with machine learning (ML) algorithms, has been developed for rapid phenotypic antibiotic susceptibility tests (AST). This platform provides new diagnostic tools for antibiotic stewardship, complementing existing genomic tests that only provide feedback on known antimicrobial resistance mechanisms. Just as one can smell the difference between coffee and chocolate amongst multiple odors, SERS+ML rapidly measures and classifies spectral features of bacterial metabolite signatures in response to antibiotics, which are correlated with antibiotic lethality mechanisms. As individual odor receptors are incapable of identifying odorant molecules, so are individual SERS spectra unable to differentiate in a complex background. Data will be presented on how 2-PAC SERS surfaces having molecular control of nano-architecture and surface chemistry provide high quality and reproducible data needed for robust ML analysis. When benchmarked against lithographically fabricated commercial sensors, 2PAC sensors exhibit quantification down to femtomolar concentrations using ML analysis of single molecule sensing events whereas commercial sensors cannot. Our results show differentiation of bacterial populations of ESKAPE pathogens based on antibiotic susceptibility in 30 min when using SERS + ML. Using the generative nature of the variational autoencoder (VAE), the VAE latent space enables a data informed transfer learning approach, in which spectral features associated with antibiotic efficacy are identified. An easy to acquire combinatorial metabolite database is used for further training and dramatically improves differentiation to identify effective antibiotic treatment. Greater than 99% accuracy is achieved with unsupervised Bayesian Gaussian Mixture analysis when using data informed transfer learning with only a few training examples. This enormously reduces the amount of time needed to validate phenotypic AST with conventional growth assays and outlines a promising approach towards practical SERS AST.

(BIM-LP.5) Exploring the Clinical Potential of Raman Imaging with SERS-based Nanoparticles for Improved Cancer Detection: Regulatory Concerns and Alternative Solutions

Presenter: Cristina Zavaleta, PhD - University of Southern California

Corresponding Author: Cristina Zavaleta, PhD - University of Southern California

Raman imaging with surface enhanced Raman scattering (SERS) nanoparticles has gained interest in the molecular imaging community due to its ultra-high sensitivity properties as well as its unique multiplexing capabilities. Nanoparticles have great potential as diagnostic contrast agents for cancer detection. Compared to their small molecule counterparts they can offer increased sensitivity due to their loading capacity and increased tumor binding efficiency due to the multiple targeting ligands displayed on their surface. Several groups have demonstrated the potential of this optical imaging technique with active tumor targeting SERS nanoparticles. Ongoing attempts towards developing new nano-based contrast agents have faced major problems in gaining regulatory approval due to their potential systemic toxicity and prolonged accumulation in vital organs. My lab has investigated the biodistribution of gold silica Raman nanoparticles after oral ingestion in living mice to mimic a topical administration to the gastrointestinal tract as a potentially less toxic alternative to intravenous (IV) injection. Some researchers choose to administer these nanoparticles topically to epithelial targets in order to mitigate the potential systemic administration toxicity issues. This approach is highly advantageous when coupled with endoscopic or intra-operative techniques as it resolves the low depth of penetration constraint often associated with optical imaging strategies and can provide important molecular information to clinicians during clinical procedures like endoscopic examination or surgical tumor resection. The focus of this talk will predominantly cover the preclinical evaluation of SERS nanoparticles as we assess their tumor targeting potential, biodistribution, and systemic toxicity post administration. I will also briefly discuss

my own experience in filing an application to the FDA as we attempt to translate our Raman imaging approach to the clinic along with these SERS-based contrast agents.

(SPSJ) Supplemental Programming: Perspective for Biomedical Raman Spectroscopy

(SPSJ.2) Raman and SRS for Cellular Analysis

Speaker: Duncan Graham, BSc Hons, PhD, CChem, FRSC, FRSE, FSAS - University of Strathclyde

Non-Presenting Author: Will Tipping

Non-Presenting Author: Karen Faulds, PhD - University of Strathclyde

Raman and stimulated Raman scattering (SRS) are techniques that can be used to provide a rich amount of information on a number of different biological systems. This presentation will cover the use of Raman and SRS spectroscopy to understand more about the biological nature of cancer and in particular lipid changes in response to drug treatments. Data will be presented on the use of Raman spectroscopy to understand more about the effect of small molecule drugs on cancer cells relative to non-cancer cells and in particular on the lipid synthesis. To achieve this, we have used 2D mapping in the high wavenumber region and ratiometric analysis used to understand the effect of small molecule drugs on the increase or decrease on lipid synthesis on prostate cancer cells. The results have shown some interesting selectivity for a particular small molecule relative to a normal cell in that this small molecule has a preference for a cancer cell and appears to up regulate the lipids. This was a very simple approach to understanding the effect of small molecule drugs and the data presented will show how Raman spectroscopy and ratiometric analysis can be used to provide an accurate analysis on the effects of small molecule drugs on cancer cells and whether further in depth testing of the efficacy of these drugs as anti-cancer agents should be pursued. A second area of investigation involves using tumour sections and analysis of the lipid changes to understand more about the mechanisms of resistance to treatment regimes. These two examples show how Raman spectroscopy can be used to probe biology and provide useful data to inform future experiments and thinking.

(SPSJ.3) Validation of Detection Limits of Drugs of Abuse Using a Portable SERS Instrument

Speaker: Peter B. Harrington, PhD - Ohio University

Non-Presenting Author: Ling Wang, PhD - Florida International University

Non-Presenting Author: Mario Vendrell-Dones, BS - Florida International University

Non-Presenting Author: Chiara Deriu, MS - Florida International University

Non-Presenting Author: Sevde Doğrue, PhD - Florida International University

Non-Presenting Author: Bruce McCord, PhD - Florida International University

Validation of Detection Limits of Drugs of Abuse using a Portable SERS Instrument Peter de B. Harrington1, Ling Wang2, Mario Vendrell-Dones2, Chiara Deriu2, Sevde Dogruer2, Bruce Mccord2 1Department of Chemistry & Biochemistry Ohio University Athens, OH 45701-2979 USA 2Department of Chemistry and Biochemistry Florida International University 11200 SW 8th Street Miami, FL 33199 USA Recently there is an upsurge in reports that illicit seizures of cocaine and heroin have been adulterated with fentanyl. Surface enhanced Raman spectroscopy (SERS) provides a useful alternative to current screening procedures that permits the detection of trace levels of fentanyl in mixtures. Samples are solubilized and allowed to interact with aggregated colloidal nanostars to produce a rapid and sensitive assay. In this study we present the quantitative determination of fentanyl in heroin and

cocaine by SERS, using a point-and-shoot handheld Raman system. Our protocol is optimized to detect pure fentanyl down to 0.20 ± 0.06 ng/mL and can also distinguish pure cocaine and heroin at ng/mL levels. Multiplex analysis of mixtures is enabled by combining SERS detection with super partial least squares discriminant analysis (sPLS-DA), which allows for the determination (w/w) of fentanyl as low as 0.05% in simulated seized heroin and 0.10% in simulated seized cocaine samples. In this project, a method for computing confidence intervals for detection and decision limits will be advocated using the uncertainty in the estimate of analytical sensitivity. Improved methods for calculating uncertainty would assist researchers in making statistical comparisons and avoid overestimating the precision of the LoD. Multivariate procedures for validation using principal component analysis using Hotelling T2 confidence ellipses, soft independent modeling for class analogies (SIMCA) using Q statistics, and sPLS-DA will be compared.

(SPSJ.5) Assessment of Oocyte Quality and its Maturation using Raman Spectroscopy

Speaker: Mika Ishigaki, PhD - Shimane University

Oocyte quality is related to early embryonic survival, and establishment and maintenance of pregnancy. Therefore, the assessment of embryonic survival competence has been paid attentions in the area of infertility treatment for human. In our recent study, it has been aimed to establish the assessment method of oocyte quality in non-destructive, non-staining, and non-invasive manner. Developmental competence is gradually acquired during the prolonged process of oogenesis. To evaluate the maturation of oocyte, we investigated the cytoplasmic changes in mouse oocytes over time after ovulation by Raman microscopy and explored the biomarkers characteristic of oocytes having high developmental competence. I would like to introduce our recent results showing that the cytoplasmic variation with the oocyte maturation, while discussing the relationship between the respiratory activity and developmental competence.

(SPSJ.6) Label-free Raman analysis of live cell and tissue

Speaker: Hidetoshi Sato, DSci - School of Science and Technology, Kwansei Gakuin University

Raman spectroscopy is one of the strongest tools for life analysis. It is able to obtain molecular information of inherent cells and tissues without any labelling and fixing. The biomedical application of Raman spectroscopy depends totally on the development of its instruments and spectral analytical technologies. Developments of varied stable solid-state lasers for excitation light source expand the Raman applications into biological samples that emits strong autofluorescence. Resonance Raman spectroscopy is powerful to extract information of a specific pigment in the cell and tissue without any sample processing. The resonance Raman spectra of whole blood showed varied features to the excitation wavelengths from visible to near-infrared (NIR) energies, which gave information of carotenoid, hemoglobin and protein independently. If it is necessary, we can employ a fiber optic probe to reach even inside of a body. The Raman analysis of growing colorectal tumor successfully detected reduction of collagen in a live mouse with a miniaturized Raman probe and endoscope. A Raman microscope enables us to observe organelle of live cells. When a human infectious virus was added in the cultured human cell, the Raman analysis detected its molecular changes within only 3 hours. In contrast, the immunostaining method could detect the early protein that was generated at first in the virus propagation in the cells at 12 h after the infection. The Raman analysis is highly sensitive to the molecular compositional changes of cells and tissues. Besides, the molecular changing is often detected

by Raman spectroscopy much earlier than the usual biological techniques, according to my experiences. Although Raman spectroscopy has not been used widely in biology yet, it may open a new era in analysis and application of life science.

THURSDAY FEATURED LIVE ORAL ABSTRACTS

(CLIRSPEC) Supplemental Programming: Advancing the Understanding and Diagnostics of Medical Conditions with Novel Vibrational Spectroscopic Approaches

(CLIRSPEC 1.2) Towards Raman subcellular imaging of endothelial dysfunction

Speaker: Malgorzata Baranska

Non-Presenting Author: Ewelina Matuszyk

Non-Presenting Author: Katarzyna Majzner

Non-Presenting Author: Ewelina Bik

Non-Presenting Author: Adriana Adamczyk

Non-Presenting Author: Basseem Radwan

Non-Presenting Author: Stefan Chlopicki

Endothelial cells lining the lumen of all the vessels in the body, from the heart to the capillaries can be regarded as the unique organ of the body which maintains cardiovascular homeostasis and accomplish multiple roles by its endocrine, paracrine and autocrine function. Healthy endothelium is essential for undisturbed functioning of the cardiovascular system, while endothelial dysfunction is recognized as a hallmark of various cardiovascular diseases. As far, Raman imaging has proved to be a promising tool in endothelium-oriented investigations combining chemical specificity with a microscopic resolution. Broad Raman studies on animal models and tissue samples have revealed a set of universal spectroscopic markers to be related with an endothelial dysfunction. Endothelial dysfunction is closely interconnected with oxidative stress, that has been recognized by specific changes in Raman spectral signature of dysfunctional endothelial cells, connected to changes in protein and phospholipids content of endoplasmic reticulum. However more detailed studies on specific biochemical changes taking place in cellular organelles, although desirable, are limited by the non-labelled Raman imaging method. Moreover, the speed of conventional Raman microscopy suffers from low measurements speed. As an response to this drawback the stimulated Raman scattering (SRS) microscopy due to high sensitivity and possibility of ultrafast high-resolution imaging, as well as linearly dependent response to molecule concentration becomes an advantageous technique in studies on biological processes at a subcellular level. So far number of molecules has been proposed to target specific subcellular sites like mitochondria, nucleus or lysosomes by so-called Raman reporters, allowing for their selective imaging due to characteristic Raman signature. Therefore, the combination of high-speed offered by SRS with organelle-specific Raman reporters presents a new approach towards subcellular imaging of endothelial dysfunction.

(CLIRSPEC 1.4) Raman spectroscopic analysis of spontaneous mineralization in breast cancer cells

Speaker: Pascaline Bouzy, PhD - University of Exeter

Non-Presenting Author: Shane O'Grady - University College Dublin

Non-Presenting Author: Honey Madupalli - Central Michigan University

Non-Presenting Author: Mary Tecklenburg - Central Michigan University

Non-Presenting Author: Keith Rogers - Cranfield University

Non-Presenting Author: Francesca Palombo, PhD - University of Exeter

Non-Presenting Author: Maria Morgan - Royal College of Surgeons in Ireland

Corresponding Author: Nick Stone, PGDip, MSc(Dist), MSc(Dist), MBA, PhD, CSci, FSAS, FIPEM, FRSC - University of Exeter

Breast cancer is the second most common cause of death from cancer in women, accounting for more than 1 million deaths globally per year. Microcalcifications, resulting from abnormal deposition of calcium in the mammary gland can provide an early marker for breast cancer detection. Two types of microcalcifications could be found: type I composed of calcium oxalate and type II composed Hydroxyapatite (Hap). However, the occurrence of microcalcifications in the breast and the underlying mineralization process are still not fully understood. The aim of this study was to use Raman micro-spectroscopy, which is non-destructive, non-invasive, label-free, and chemically specific to assess the deposition of these microcalcifications over time from an in vitro model of mineralization. Here, the mineralization of the MDA-MB-231 breast cell line was induced by two osteogenic agents: the inorganic phosphate (Pi) and β-glycerophosphate (βG) for 14 days. This work evidenced that the uptake of the Pi induced an early mineralization after 3 days while the cells cultured with βG initiated the process after 11 days. A curve fit analysis of the Raman spectra demonstrated a heterogeneity of the mineral deposits. In fact, the maturation process involved different phosphate species i.e. octacalcium phosphate (OCP), β-tricalcium phosphate (β-TCP) during Hap crystal formation. In parallel, it was also observed variations of protein and DNA peak intensities and an increasing of the mineral-to-matrix ratio (MMR) during the cell mineralization. This cellular model could give us a better understanding of the microcalcification maturation process and could be useful to link with the pathological process for discriminating changes between benign and malignant lesions in breast biopsy samples.

(CLIRSPEC 1.5) GBM vs. Lymphoma: Are all serum molecular weight regions vital for stratification?

Speaker: Ashton Theakstone, BFSc (Hons), PhD - University of Strathclyde

Non-Presenting Author: James Cameron

Non-Presenting Author: Matthew Baker

Non-Presenting Author: Paul Brennan

Non-Presenting Author: Michael Jenkinson

Non-Presenting Author: Benjamin Smith

Non-Presenting Author: Katherine Ashton

Non-Presenting Author: Khaja Syed

Discrimination of brain cancer vs. non-cancer patients using serum-based ATR-FTIR diagnostics was first developed by Hands et al. achieving sensitivity and specificity values of 92.8% and 91.5% respectively. Cameron et al. then went on to stratifying between specific brain tumours: glioblastoma multiforme (GBM) vs. primary cerebral lymphoma and was successful in providing a sensitivity of 90.1% and a specificity of 86.3%. Expanding on this study, 30 GBM and 30 lymphoma patients were selected in order to improve the sensitivity and specificity values between the two cancer types. Centrifugal filters that are commercially available have been used to separate fractions of the serum with different molecular weights (MW) and results in a greater spectral quality. Membrane filters with molecular weight cut offs of 100k, 50k, 30k, 10k and 3k were purchased in order to remove the most abundant high MW proteins. This will reveal the lower MW components that are usually hidden within the spectra and has potential to improve the sensitivity of detections for each disease type. As serum is a complex matrix with over 20,000 different proteins, as well as sugars, lipids, peptides and metabolites, it is important to sample fractionate in order to detect and monitor the features relating to the lower MW proteins. These low MW fractions contain potential cancer-specific diagnostic information therefore it is vital to remove the abundant high MW proteins for classifications between tumour types. Utilising multiple size MW filters on the same sample allows for greater selectivity of specific MW regions and will demonstrate which biological molecules are important for the stratification between tumour types. These initial findings will help guide future directions for differentiation between multiple brain cancer types and will improve in understanding which molecular species are important for greater sensitivity and specificity of serum diagnostics.

(CLIRSPEC 1.6) Development of a hybrid confocal fluorescence and Raman endomicroscopy technique for real-time morphochemical tissue analysis.

Speaker: Mads S. Bergholt, MSc PHd - King's College London

Confocal laser endomicroscopy (CLE) has emerged as a promising tool for clinical diagnostics. This technique, however is inter-observer dependent and does not provide biomolecular information about the tissue. Here we present a simultaneous CLE and confocal Raman endoscopic modality based on a novel detection scheme for rejecting silica fiber interference. We show that this technique enables real-time microscopic visualization of tissue architecture and biomolecular characterization.

(EDU-LP) Topics in Education

(EDU-LP1) Promoting student engagement and meaningful learning in the classroom

Presenter: Renee Cole, PhD - University of Iowa

Discourse in the science classroom has been highlighted as an important way that students develop an understanding of scientific concepts. Analyzing student interactions and construction of knowledge and the increased adoption of active learning strategies to teach chemistry provide a unique opportunity to investigate how students develop understandings of fundamental concepts in chemistry, as well as the roles of curricular materials and instructor actions on student reasoning and conceptual growth. Analysis of classroom transcripts and videos provides evidence of the emergence of classroom social norms for reasoning as well as the impact of particular facilitation strategies on student interactions. The insights gained from this work have implications for how instructors can help scaffold student reasoning and promote productive discourse in chemistry classrooms.

(EDU-LP2) Supporting Educational Innovations by Providing Resources for High-Quality Assessment

Presenter: Regis Komperda, PhD - San Diego State University

Understanding the impact of educational innovations requires quality measurement of student outcomes. Chemistry educators looking for assessment instruments to measure student outcomes (e.g., tests, surveys, questionnaires, etc.) may choose to develop new instruments or search the literature to find an existing instrument that can either be used as-is or modified for a particular educational context. In the same way that bench chemistry research instruments are calibrated to ensure accuracy and precision of the data collected, research instruments used for chemistry education purposes must also be calibrated to ensure the data collected can be used to draw meaningful conclusions. This presentation will discuss best practices in finding or modifying assessment instruments as well as methods for evaluating the quality of data obtained from these instruments. These best practices are currently being incorporated into a new NSF-funded resource, the CHemistry Instrument Review and Assessment Library (CHIRAL). The CHIRAL project will aid chemistry educators by providing a searchable catalog of existing assessment instruments from the chemistry education literature along with a summary of evidence provided for the quality of data obtained from the instrument. The goals of CHIRAL include assisting in the selection of the most appropriate assessment instruments for a given context in order to provide support for the evaluation of educational innovations.

(EDU-LP3) Teaching Analytical Chemistry in the Time of Information Overload

Presenter: Katarzyna Slowinska, PhD - California State University, Long Beach

In the past, fast access to information was difficult and it was vital to be able to recall many facts without access to external sources. Currently, the availability of digital information is widespread, but the amount and complexity of information is overwhelming, thus sorting, evaluation and application of correct data is a vital skill for a student. Here I will discuss how to approach this problem in teaching analytical chemistry. The topic will include teaching, assessment, and evaluation of information source, application of general information to solving specific problems, and recognition of basic concepts and

data extraction from the "see of information". In addition, I will discuss the options for designing the assessment tools for testing these higher-level, according to Bloom's taxonomy, skills.

(EDU-LP4) Monolithic Spatial Heterodyne Raman Spectrometer (mSHRS): Advancement Towards Miniature Raman Spectrometers

Presenter: Abigail M. Waldron - University of South Carolina, Department of Chemistry and Biochemistry

Non-Presenting Author: Arelis Colón - University of South Carolina

Non-Presenting Author: Ashley N. Allen, PhD

Non-Presenting Author: J. Chance Carter, PhD - Lawrence Livermore National Laboratory

Non-Presenting Author: S. Michael Angel, PhD - University of South Carolina, Department of Chemistry and Biochemistry

The 2013 Planetary Decadal Survey recommends a high priority be placed on remote sensing technology with a focus on developing and maturing novel, crosscutting, low-mass/power sensors integrated into robust, low-cost system architectures. A spatial heterodyne Raman spectrometer (SHRS) is one such instrument that could fit these qualities. The SHRS is based on a spatial heterodyne spectrometer (SHS) which is a fixed grating interferometer that offers high spectral resolution and high light throughput in a small footprint. The resolution of the SHS is not dependent on a slit, and high resolution can be realized without a long optical path since it is not a dispersive device. Thus, the SHS can be used to make a very small Raman spectrometer with high spectral resolution and a large spectral range. The smallest SHRS, and most robust, for a given size diffraction grating, can be achieved by building it using monolithic construction techniques. A monolithic spatial heterodyne Raman spectrometer (mSHRS), where the optical components of the spectrometer are bonded to make a solid, one-piece structure, can be small, compact and stable, suitable for planetary spacecraft and rovers. We discuss the mSHRS and compare the stability, spectral resolution, spectral range, and signal to noise ratio to the bench top SHRS, composed of free standing optics.

(PP-LP1) Topics in Pharmaceutical Analysis and Process Analytical Part I

(PP-LP1.1) Raman Spectroscopy for Bioprocesses: How Hardware, Sampling and Data Analysis Decisions Drive Success

Presenter: Brian Marquardt, PhD - MarqMetrix Inc.

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.

(PP-LP1.2) ATR-FTIR Spectroscopy To Assess Protein A Affinity Resin Performance As A Function Of Spatial Location Within A Column

Presenter: James W. Beattie - Imperial College London

Non-Presenting Author: Bernadette Byrne - Imperial College London

Non-Presenting Author: Ruth C. Rowland-Jones - GSK Biopharm Process Research

Corresponding Author: Sergei G. Kazarian - Imperial College London Non-Presenting Author: Monika Farys - GSK Biopharm Process Research

Therapeutic Monoclonal antibodies (mAbs) are major biopharmaceuticals, accounting for 22% of the total FDA newly approved drugs between 2016-2018. mAbs can be used to treat a wide range of both cancerous and non-cancerous diseases. mAb treatments are expensive, averaging around $96,731 per annum per patient. Improvements to upstream mAb production have been made to reduce production costs. The industry favoured purification step of protein A affinity chromatography, although extremely efficient, accounts for over 50% of total mAb production costs with 1 L resin costing ca. $17K. This cost issue is exacerbated by protein fouling and protein A ligand leaching shortening the life span of the resin (50-200 cycles). Traditionally, methods such as static binding capacity assays are used to assess the maximum capacity and affinity of a mAb for the protein A ligand in a specific resin. This method lacks chemical information on what is causing any reduction in capacity of the resin. Attenuated total reflection Fourier transform infrared (ATR-FTIR) spectroscopy is a non-destructive and label-free method that provides insight into the secondary structure of proteins via the amide I & II bands. Here we utilised ATR-FTIR spectroscopy in combination with static binding capacity assay measurements to explore the ability of resin from different parts of an industrially used pilot-scale MabSelect Sure Protein A column (981 ml). A range of known quantities of mAbs were bound to the different protein A resin

samples and both 1) the flow-through was examined to provide an accurate assessment of the amount of material bound and 2) the resin was analysed with ATR-FTIR spectroscopy to quantify material bound and protein A ligand present. The results indicate a variation in overall resin capacity depending on the spatial location within a column, inlet samples showing the greatest reduction in mAb binding. All of the used resins showed a mark reduction of mAb binding compared to unused MabSelect Sure resin.

(PP-LP1.3) The Multivariate Process Paradigm

Presenter: Brian G. Rohrback, PhD - Infometrix, Inc.
Non-Presenting Author: Scott Ramos
Non-Presenting Author: Randy Pell

There is a distinction between the requirements of a laboratory-based R&D project and what needs to happen when R&D gives way to a process implementation. The focus must expand such that evaluations that were once restricted to designed experiments run on (usually) one instrument, now need to integrate with myriad process sensors and unit operation variability that is difficult to simulate in the R&D stage. Now the issue is to extract information from multiple, byte-dense data sources in a systematic way which, in turn, leads to improvements in decision making at all levels of the chemical, petrochemical, and petroleum industries. To accomplish anything in the Big Data space, we need to combine traditional approaches in statistics, database organization, pattern recognition, and chemometrics with some newer concepts tied to better understanding of data mining, neurocomputing, and machine learning. For industry to achieve the goals that this form of AI promises, we need to approach the issues with more than just words. This is a summary of a multi-company, multi-industry, chemical processing consortium, established eight years ago to re-evaluate how the calibration process for sensors and analyzers could be managed more efficiently. The focus spans optical spectrometers, chromatographs, and process sensors, independently and in combination. The idea is to enable a shift from current practices to approaches that take advantage of the computational power at our fingertips. It was critical to prioritize solutions that are non-disruptive, utilize legacy systems, and lessen the workload rather than layer on additional requirements. The result is a choice of tools available to consume the data and generate actionable, process-specific information. The analyzers in place, optical spectrometers in particular, represent the low-hanging fruit.

(PP-LP1.4) Use of Deep Neural Networks to develop truly diverse therapeutic antibody datasets

Presenter: Jeremy Shaver, PhD - Just - Evotec Biologics, Inc.
Non-Presenting Author: Tileli Amimeur
Non-Presenting Author: Randal Ketchem

We will present the use of our new Antibody Generative Adversarial Networks (Antibody-GANs) to create diverse antibody sequences that can advance the analytical methods used to study these molecules. Large molecule biotherapeutics such as antibodies exhibit a diverse range of biophysical behaviors that drive similarly-diverse needs in process development (e.g., purification and formulation) and behaviors in vivo (e.g., clearance and injection site sensitivities). While good analytical methods exist to interrogate biophysical behavior of antibodies, the data to develop and assess how these biophysical behaviors relate to process and in vivo behaviors are severely limited. Most analytical development and application studies focus on only small sets (handfuls or dozens) of antibodies, often

with highly-related molecules that severely underestimate the complicated inter- and intra-molecular relationships. This insufficient molecular diversity limits the ability to draw broader conclusions from the analytical results. The Antibody-GAN approach allows us to better sample sequence space and assures we understand how these analytical methods map to other behaviors.

(PP-LP1.5) Hybrid Model for Real-time Reaction Monitoring

Presenter: Ricardo Sousa, MA - Hovione
Non-Presenting Author: Joana Parker
Non-Presenting Author: Pedro Valente
Non-Presenting Author: Marco Reis

In the field of chemistry, the real-time monitoring of chemical reactions is very important to determine the reaction endpoint, to optimize yield and to avoid impurity formation. Process analytical technology (PAT) tools, such as spectroscopy sensors, are very attractive since they can provide real-time information about the reaction. This enables the concentration of analytes to be monitored in a continuous and nondestructive manner, thus eliminating the need for the use of labor-intensive offline analytical methods. Nevertheless, it can be challenging to determine a reaction endpoint with spectroscopy methods, since the reagents concentration may reach the method's limit of quantification (LOQ). Moreover, PAT alone cannot predict when a reaction will end before it happens. The combination of PAT with the process mechanistic knowledge has proved to yield better predictions. Kramer et al. used a Kalman filter to predict biomass and other analytes from near-infrared spectroscopy (NIR) and the kinetic relations between substrates [1]. In the present study, it was combined infrared (IR) spectroscopy and the reaction kinetic model to predict the concentrations of the reacting species with lower uncertainty. The authors analyze as a case study a second-order reaction with two reagents forming one insoluble product. A PLS model was calibrated to predict the concentration of the limiting reagent from IR spectra readings in real-time. No additional calibration effort was necessary, because the kinetic constant of the reaction kinetic model was continuously fitted during the reaction, based on the PLS predictions. The predictions of these two models were combined with the application of the Kalman filter. The resulting hybrid model decreased the prediction uncertainty. Finally, the kinetic model was applied to the Kalman filter predictions to estimate when the concentration of the limiting reagent would decrease below 0.1%. The reaction endpoint estimation error decreased below 10% before reaching the reaction half time. In conclusion, the hybrid model captured the physics of the reaction with a much greater degree of accuracy and enabled the estimation of the reaction endpoint.

(PP-LP2) Topics in Pharmaceutical Analysis and Process Analytical Part II

(PP-LP2.1) Confidence in annotating metabolites and exposome compounds in untargeted screening

Presenter: Oliver Fiehn, PhD - UC Davis

Non-Presenting Author: Yuanyue Li

Non-Presenting Author: Sajjan Mehta

Non-Presenting Author: Tobias Kind

Non-Presenting Author: Clayton Bloszies

Non-Presenting Author: Gert Wohlgemuth

Non-Presenting Author: Diego Pedrosa

Non-Presenting Author: Hiroshi Tsugawa

Non-Presenting Author: Paolo Bonini

Untargeted metabolomics, lipidomics and exposome compound screening gathers more than 6,000 MS/MS spectra in published studies. Companies provide lists with up to 2,000 annotated chemicals per study, while academic authors struggle to validate metabolite assignments that originate from open access tools such as GNPS, Mummichog or CFM-ID. A dizzying array of software tools, databases and algorithms claim to alleviate the burden, while it becomes increasingly subjective how authors claim the confidence for compound annotations. Chemical annotations by chromatography- or ion mobility-based mass spectrometry urgently needs solid, mathematics-based algorithms that give False Discovery Rates (FDR) in untargeted metabolomics, detailing the likelihood that given annotations may be false based on acquired data. In analytical chemistry, all types of information can be used to support structural annotations, ranging from retention times, collision cross sections, accurate masses, adduct formation, isotope abundances, mass spectral fragmentation under different collision energies, MS^n fragmentation trees, chemical derivatizations to biological background data such as the likelihood a specific compound may be present in a given organ or cell, based on literature or genetic information. Currently, no such algorithms exist, but first steps have been made. We here present studies that incorporate machine-learning based prediction of retention times in HILIC and RP chromatography, hydrogen/deuterium exchange reactions that inform on the presence of the number of acidic groups in unknown metabolites, and large-scale analyses of FDR ratios in orbital ion trap MS/MS fragmentations based on NIST17 and Massbank.us spectral libraries. We present entropy analysis as new concept in mass spectral matching, detail the accuracy of MS/MS matching across 39 similarity algorithms and give similarity thresholds based on collision energies and compound classes. We highlight the use of biological background databases such as bloodexposome.org and outline the benefits of high quality metabolome databases such as GC- and LC-BinBase with its public BinVestigate query tools.

(PP-LP2.2) MEMS-Based Mid-IR Spectral Sensing Solutions for Process Analytical Technology

Presenter: Yasser M. Sabry, PhD - Si-Ware Systems

Non-Presenting Author: Momen Anwar - Si-Ware Systems

Non-Presenting Author: Mohamed Al Haron - Si-Ware Systems

Non-Presenting Author: Islam Samir - Ain Shams University

Non-Presenting Author: Amr Ghoname - Ain Shams University

Non-Presenting Author: Ahmed Saeed - Si-Ware Systems

Non-Presenting Author: Mai Said - Si-Ware Systems

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Non-Presenting Author: Ahmed Fadeel - Si-Ware Systems

Non-Presenting Author: Botros George - Si-Ware Systems

Non-Presenting Author: Bassam Saadany - Si-Ware Systems

Non-Presenting Author: Tarik Bourouina - Si-Ware Systems

Non-Presenting Author: Diaa Khalil - Si-Ware Systems

Real or near real-time monitoring of chemical production and processes has a significant impact on process control aspects such as quality of products, level of safety, and impact on the environment and economic aspects such as reducing raw materials intake and energy consumption. This domain is known as process analytical chemistry (PAC) or process analytical technology (PAT). According to the recent process spectroscopy market reports, FT-IR spectroscopy is expected to emerge as the most lucrative technology in the market due to its distinguished features of ease of maintenance and high-quality output. The industrial applications include pharmaceutical, food and agriculture, polymer, chemical, oil and gas, metal and mining in addition to others. In the last decade, near-infrared (NIR) has started to be adopted in many segments as a near-line and on-line monitoring technique especially with the emergence of portable and handheld devices. However, some domains are not well supported by the NIR, such as oil and gas analysis. In this case, the mid-infrared (mid-IR) spectral range is a more powerful tool detecting the fundamental absorption lines of the material. In this talk, we shed light on NeospectraTM, a chip-scale microelectromechanical system (MEMS)-based FTIR spectrometer. The core engine is highly integrated comprising the self-aligned monolithic silicon MEMS chip; self-aligned micro-optics and a single photodetector in a tiny package. This architecture allows broad spectral range of operation limited by the photodetector, thanks to the excellent transmission characteristics of silicon and the achromatic reflective micro optics. NIR sensors and solutions based on this core technology are now commercially available in the wavelength range of 1350 nm to 2500 nm. Moreover, a spectrometer with a wavelength range spanning from 1500 nm to 4800 nm has been realized and various portable mid-IR spectral sensing solutions targeting different applications are being explored. The applications include gas leakage detection, air pollution monitoring including VOCs, analysis of natural/biogas, and lubricant oil degradation monitoring. It is believed that these spectral sensing solutions will not only revolutionize the analytical technology but also work as a catalyst enabling ubiquitous material analysis.

(PP-LP2.4) Taking pictures of your reactions

Presenter: Xiaoyun Chen, PhD - The Dow Chemical Company

Non-Presenting Author: Kshitish Patankar

Non-Presenting Author: Larive Matthew

Non-Presenting Author: Dan Roscioli

Non-Presenting Author: Michael Desanker

The ability to monitor your reactions in real time in situ brings many advantages to chemists and engineers, and is becoming widely adopted in both academia and industry. Generally an immersion probe or a flow cell is used to collect spectra in situ. Here we demonstrated the use of hyperspectral imaging to take pictures of reactions in situ. Polyurethane (PU) foaming is used as an example. A non-contact near-infrared (NIR) hyperspectral imaging (HSI) camera was used in this study to monitor PU foaming reactions. A design of experiment (DOE) was carried out to monitor the foaming of five foams prepared with three process variables: water content, mixing time, and catalyst levels. Spectral changes characteristic of the PU reactions were observed and clear difference in kinetics could be effectively extracted from such NIR HSI results. In addition chemical imaging of PU foams could be rapidly carried out.

(PP-LP2.5) One Shot Learning Using Bayesian Modeling for Metabolistes in CHO Cell Culture

Presenter: Rajeev Ram, PhD - Massachusetts Institute of Technology

Non-Presenting Author: Ningren Han

Non-Presenting Author: Zheng Li

Non-Presenting Author: Nili Persits

The collection of high quality Raman spectroscopy training data sets with reference measurements for multivariate regression algorithms can be a long, challenging, and resource-intensive process for many applications. Here, we present an analyte quantification algorithm without any requirement on training data - in contrast to established PLS approaches and emerging Neural Networks. A Bayesian modeling framework with Reversible Jump Markov Chain Monte Carlo has been developed and applied to the problem of metabolite quantification in CHO cell culture. We test our algorithm on experimentally collected spontaneous Raman spectroscopy datasets to validate its usage. The successful quantification of glucose concentration in a complex aqueous cell culture environment without any mixture training data suggests its promising potential for applications where training may not be feasible.

(AES-LP) Electric-field Driven Phenomena: Fundamentals, Technology Development and Applications

(AES-LP.1) Microchip Electrophoresis as an Analytical Tool to Study Neuroinflammatory Processes

Presenter: Susan M. Lunte, PhD - University of Kansas

Oxidative stress is involved in many neurological diseases including Alzheimer's Disease and traumatic brain injury. New tools capable of monitoring biomarkers of oxidative stress in vivo and in vitro can be used to investigate the cause and progression of these disease states. Microchip electrophoresis is a powerful tool for the analysis of biological samples. In particular, its ability to perform fast, efficient separations of multiple analytes in a single run makes it possible to monitor several biomarkers in a single sample with high temporal resolution. In addition, the small dimensions of the channels in the chip are compatible with the analysis of microdialysis samples and single cells. In this presentation, two applications of microchip electrophoresis (ME) for biochemical investigations will be presented. The first involves the development of ME-based methods for the detection of reactive nitrogen and oxygen species (RNOS) in macrophages and immune cells. This includes direct amperometric detection of RNOS as well as the evaluation of fluorescent reagents used for specific species. The second application involves the combination of microdialysis with microchip electrophoresis for near real-time continuous in vivo monitoring of biogenic amines and biomarkers of inflammation. The ultimate goal is to use these tools to investigate the role of oxidative stress in neurodegenerative disease.

(AES-LP.2) Nonlinear Electrokinetic Effects in Devices with Insulating Structures

Presenter: Blanca H. Lapizco-Encinas, PhD - Rochester Institute of Technology

In recent years there has been a significant progress on the development of microscale electrokinetic (EK) methodologies for the separation, sorting, enrichment, analysis and detection of a wide array of particles, ranging from macromolecules to parasites. However, to fully realize microscale EK techniques as established bioanalytical methodologies, the screening and selectivity capabilities of these powerful techniques still need further development. Nonlinear EK phenomena are enhanced in devices with a non-uniform electric field distribution. There are two common approaches for producing non-uniform electric fields in microfluidic devices: Electrode-based devices and insulator-based devices. The former has been the most employed method, there is a significant wealth of knowledge electrode-based devices, and numerous designs and electrode configurations have effectively been employed. However, some microelectrode systems may require expensive and complex fabrication method and their performance can be affected by fouling effects. In contrast, insulator-based devices offer an alternative by employing 3-dimensional (3D) insulating structures located between two external electrodes to produce non-uniform electric fields. These posts transverse the entire depth of the microchannel, creating a true 3D field distortion effect. Non-linear EK effects, such as electrophoresis (EP) of the second kind and dielectrophoresis (DEP) are present in those regions of higher electric field intensity. This work is focused on the use of non-linear EK effects for the manipulation and separation of particles. Microchannels made from PDMS, containing an array of insulating structures were employed. This study includes experimentation and mathematical modeling work aimed to identify the best microchannel configurations and operating conditions that can enhance particle separation with non-linear EK. An overview on the results obtained from several recent projects in our laboratory will be

presented, highlighting the distinct strategies employed to achieve particle enrichment and particle sorting in just a few minutes, while reducing system requirements in terms of applied electric potentials. Acknowledgements: The authors would like to acknowledge the financial support provided by the National Science Foundation (Award CBET-1705895).

(AES-LP.3) Direct-Laser-Writing-Enabled Microfluidic Devices for Cardiac Tissue Studies

Presenter: Alice E. White, PhD - Department of Mechanical Engineering, Boston University

Non-Presenting Author: Rachael K. Jayne - Department of Mechanical Engineering, Boston University

Non-Presenting Author: Cagatay Karakan - Department of Mechanical Engineering, Boston University

Non-Presenting Author: Kehan Zhang - Department of Biomedical Engineering, Boston University

Non-Presenting Author: Christos Michas - Department of Biomedical Engineering, Boston University

Non-Presenting Author: Pranjal Nautiyal - Department of Mechanical and Materials Engineering, FIU

Non-Presenting Author: Arvind Agarwal - Department of Mechanical and Materials Engineering, FIU

Non-Presenting Author: Kamil Ekinci - Department of Mechanical Engineering, Boston University

Non-Presenting Author: Christopher S. Chen - Department of Biomedical Engineering, Boston University

Over the last few decades, stereolithography has emerged as a leading additive-manufacturing technique. More recently, a high resolution (sub-micron) variant, direct laser writing (DLW) via two-photon polymerization (TPP), has become available. This advanced fabrication technique can be used to produce complex 3D microstructures out of polymer-based materials ranging from hydrogels to standard photoresists. It is a powerful prototyping method that enables researchers to iterate through many different designs that would otherwise be impossible to make using any other fabrication method. DLW is a particularly attractive approach for producing structures that can be used in tissue-engineering studies because it provides the necessary resolution to explore the complicated relationships between cells and their surroundings. The structure of the cellular microenvironment is a critical component of healthy tissue function, but fabrication constraints often limit the degree of design control available to researchers. The use of DLW to produce testbeds for fundamental cell studies will be presented. Important considerations for fabricating testbed structures, such as interfacing between different materials, will be addressed. I will describe how two-photon direct laser writing can be used as an enabling fabrication technique for 3D scaffolds with embedded induced pluripotent stem cell (iPSC)-derived tissues. Methods to introduce softness in structures made from intrinsically stiff materials are explored through incorporation of unique design features and variable fabrication parameters. To illustrate the efficacy of using DLW to facilitate cell studies, two examples will be described. First, a microfluidic-based platform for studying dynamic cardiac-microtissue behavior is designed and tested. Next, an in vitro cardiac model with 3D concave tissues that can recapitulate ventricular ejection within a microfluidic system is demonstrated. Our tissue constructs show fundamental established responses to well-characterized chemical compounds and pave the way for advanced in vitro cardiac models, that better resemble the in vivo hearts. ^ This work was supported by the Engineering Research Centers Program of the National Science Foundation under NSF Cooperative Agreement No. EEC-1647837 and the Boston University Photonics Center. RKJ acknowledges a fellowship from the Clare Boothe Luce Foundation. CM acknowledges the Predoctoral Fellowship from the American Heart Association.

(AES-LP.4) Electric-Field-Driven Microfluidics for Rapid CRISPR-Based Diagnostics and Application to COVID-19 Detection

Presenter: Juan G. Santiago, PhD - Stanford University

Non-Presenting Author: Ashwin Ramachandran - Stanford University

Non-Presenting Author: Diego Huyke - Stanford University

Non-Presenting Author: Eesha Sharma - Stanford University

Non-Presenting Author: Malaya Sahoo - Stanford University

Non-Presenting Author: Niaz Banaei - Stanford University

Non-Presenting Author: Benjamin Pinsky - Stanford University

The rapid spread of COVID-19 across the world has revealed major gaps in our ability to respond to new virulent pathogens. Rapid, accurate, and easily configurable molecular diagnostic tests are crucial during pandemics for early identification of infected patients to prevent global spread of new diseases. CRISPR biology offers new methods for rapid and accurate pathogen detection, and CRISPR-based diagnostic approaches are proving to be amenable as field-deployable solutions. In a basic form of this assay, the CRISPR-Cas12a enzyme complexes with a reconfigurable synthetic guide RNA (gRNA). The CRISPR complex between the Cas12a enzyme and gRNA then specifically recognizes target pathogen DNA in the sample. This molecular recognition modifies the Cas12-gRNA complex into an active form which thereafter indiscriminately cleaves reporter ssDNA molecules (fluorophore-quencher pairs), generating a fluorescent signal and indicating the presence of the target DNA. Despite their versatility and specificity, existing CRISPR-diagnostic methods suffer from the requirements of up-front nucleic acid extraction, large reagent volumes, and several manual steps—factors which prolong the process and impede use in low resource settings. We here combine on-chip electric-field control in combination with CRIPSR biology to directly address these limitations of current CRISPR-diagnostic methods. Notably, we have discovered that electric field gradients can be used to control and accelerate this CRISPR assay by co-focusing Cas12-gRNA, reporters, and target. We achieve an appropriate electric field gradient using a selective ionic focusing technique known as isotachophoresis (ITP) implemented on a microfluidic chip. The system uses no moving parts and consumes less than 100X the reagents of other CRISPR assays. Unlike previous CRISPR diagnostic assays, we also use ITP for automated purification of target RNA from raw nasopharyngeal swab sample. We combine this ITP purification with loop-mediated isothermal amplification and the ITP-enhanced CRISPR assay to achieve detection of SARS-CoV-2 RNA (from raw sample to result) in 30 min for both contrived and clinical nasopharyngeal swab samples. This electric field control enables a new modality for a suite of microfluidic CRISPR-based diagnostic assays.

(AES-LP.5) Thermally-Responsive Nanophases in Capillary Electrophoresis Separations

Presenter: Lisa Holland, PhD - West Virginia University

Non-Presenting Author: Cassandra Crihfield, Ph.D. - West Virginia University

Protein sieving, which is a fundamental tool in the biotechnology field, can be automated using capillary gel electrophoresis. The high viscosity and biocompatible linear gels required for capillary sieving must be replaced for each run using high pressures. Thermally responsive gels are easier to renew in the capillary as they can be repetitively switched between low and high viscosity solutions. A thermally-

responsive sieving gel was recently demonstrated to separate DNA, which is a larger biomolecule than proteins. This material required no synthesis as it was self-assembled from common phospholipids. Nanogels composed of dimyristoyl-sn-glycero-2-phosphocholine and 1,2-dihexanoyl-sn-glycero-3-phosphocholine exhibit thermally reversible viscosity within a 10 °C temperature change, forming a sieving matrix above 24 °C. Additionally, these nanogels are non-denaturing and have been demonstrated to preserve the activity of enzymes. In this report a phospholipid nanogel is used for the first time for capillary gel electrophoresis separations of proteins. The mobilities in buffer and nanogel demonstrated that 20-30% nanogel supports sieving of proteins ranging from 20-80 kDa. Capillary separations based on sieving rather than electrophoresis had similar precision in both area and migration time as well as similar separation efficiencies. However, the migration time increased with gel concentration. The nanogel was used for the analysis of proteins in human serum. Proteins in the sample were more effectively resolved and quantified with capillary sieving as compared to free solution capillary electrophoresis. This allowed for accurate quantification.

ON-DEMAND ORAL ABSTRACTS

(AAF-OD1) Nuclear Forensics

(AAF-OD1.1) Quantifying Particle Morphology to Process History for Nuclear Forensics

Presenter: Luther McDonald, IV - University of Utah

The analysis of critical nuclear signatures is imperative in mitigating future instances of illicit trafficking of nuclear materials. When a sample of nuclear material is interdicted, the goal is to identify the origin and process history of that material. Understanding the impact of nuclear signatures can improve the speed and quality of nuclear attribution, as well as potentially prevent further trafficking. The Nuclear Forensics and Attribution Act was passed in 2010 to fulfill this goal. Traditionally, parent-daughter isotope ratios have been used as a signature to identify the age and origin of nuclear material. To determine process history, however, added signatures are required. Recently, our research team has focused on quantifying the surface morphology and oxygen isotope ratios of U-oxides as nuclear forensics signatures. These studies have demonstrated morphological features of U-oxides are a product of processing parameters such as starting material, intermediate material, oxidation rates, precipitation conditions, and calcination history. In addition, we have shown that the production of U-oxides in atmospheric furnaces results in unique stable isotope ratios indicative of meteoric water at the processing location. Stable isotopes can even be used to identify if inert atmospheres are used for calcination, including the temperature at which the processing occurred.

(AAF-OD1.2) Uranyl Fluorides and Potential Applications in NonProliferation

Presenter: Jenifer Shafer, PhD - Colorado School of Mines
Non-Presenting Author: Kevin Pastoor
Non-Presenting Author: Mark Jensen
Non-Presenting Author: Glenn Fugate

Nuclear nonproliferation is an area that is constantly searching for new approaches to address the ever-present threat of nuclear weapons development. Uranyl fluoride (UO_2F_2) is an important molecule in the nonproliferation phase space since UO_2F_2 is an anthropogenic molecule and is a degradation product of uranium hexafluoride, UF_6. Uranium hexafluoride, a compound volatile at 56° C, is the only known uranium compound useful for isotopic enrichment in most commonly used gaseous diffusion and centrifugal contactor techniques. Uranium hexafluoride has no other industrial applications and the UO_2F_2 degradation product, formed when UF_6 is exposed to natural atmospheric conditions, is not produced significantly in any other anthropogenic processes. The uniqueness of UO_2F_2 to serve as a signature for uranium enrichment operations, particularly undeclared operations, has increased the interest in the general chemistry of UO_2F_2. This work will consider characterization of fundamental UO_2F_2 properties to assess eventual opportunities for selective dissolution and recovery of uranyl fluoride from more complex matrices.

(AAF-OD1.3) Spatially-Resolved Elemental Associations in Post-Detonation Debris

Presenter: David Weisz, PhD - Lawrence Livermore National Lab (USA)

Non-Presenting Author: Kim Knight

Non-Presenting Author: Tashi Parsons-Davis

Non-Presenting Author: Naomi Marks

Non-Presenting Author: Peter Weber

Non-Presenting Author: Peter Bedrossian

Compositional relationships measured in nuclear fallout debris can inform how device materials interact with other sources, such as environmental and structural source terms, during debris formation. Spatially-resolved measurements reveal microscale variations in activity and composition within single samples. Isotopic analysis of such features using nano-scale secondary ion mass spectrometry provides further insights into the behavior of device-related vapor and associations with elements representing environmental contributions. We use these data to characterize the scale of compositional heterogeneity in post-detonation debris. This work was performed under the auspices of the U.S. Department of Energy by Lawrence Livermore National Laboratory under Contract DE-AC52-07NA27344. This work was supported by the LLNL-LDRD Program under Project No. 18-ERD-003. LLNL-ABS-810267

(AAF-OD2) Food Forensics

(AAF-OD2.1) Design and application of field-deployable instrument for food safety applications

Presenter: Euiwon Bae, PhD - Purdue University

Non-Presenting Author: Hyun Jung Min

Non-Presenting Author: Iyll-Joon Doh - Purdue University

Non-Presenting Author: Xiyao Wang

Non-Presenting Author: Bruce Applegate

Non-Presenting Author: Amanda Deering

Non-Presenting Author: J. Paul Robinson - Purdue University

Conventional instruments used in the laboratory is regarded as a standard method of food safety analysis. However, there are increasing needs for on-site detection methods that can provide rapid test to result while maintaining certain level of accuracy and sensitivity. As an example of field-deployable instruments, we present smartphone-based instrumentation with three different optical modalities and its application in food analysis. Method presented here are spectrometry, colorimetry, and bioluminescence-based method. First method, which utilizes a custom-designed transducer for diffraction measurement provides optical absorption spectrum. An example of protein concentration estimation was shown with milk sample. Second method, which utilizes colorimetry from smartphone camera provides an objective and unbiased color analysis that can use machine learning and different color space for accurate analysis. Application was presented with quantitative readings of lateral flow assay for E.coli O157:H7 detection. Finally, bioluminescence-based method transforms the smartphone as a luminometer which was achieved by hardware enclosure and software algorithm. Application of bacteriophage-based E.coli O157:H7 detection from ground beef resulted in 10-12 hrs of time to detection.

(AAF-OD2.2) Food Safety and Quality Inspection using Macro-scale Raman Imaging and Spectroscopy Technologies

Presenter: Jianwei Qin, PhD - USDA Agricultural Research Service (ARS)

Non-Presenting Author: Moon S. Kim, PhD - USDA Agricultural Research Service (ARS)

Non-Presenting Author: Kuanglin Chao, PhD - USDA Agricultural Research Service (ARS)

Non-Presenting Author: Walter F. Schmidt, PhD - USDA Agricultural Research Service (ARS)

Non-Presenting Author: Byoung-Kwan Cho, PhD - Chungnam National University

Commercial integrated Raman systems generally perform imaging and spectroscopy measurements at subcentimeter scales. Such small spatial coverage cannot be used to evaluate food samples with large surface areas (e.g., tomato fruit and beef steak), which is not convenient for food experiments. Efforts have been made by researchers at the USDA Agricultural Research Service (ARS) to remedy the lack of macro-scale Raman chemical imaging (RCI) tools for food safety and quality research. Two point-scan RCI systems have been developed using 785 and 1064 nm point lasers, which are mainly used for measuring low- and high-fluorescence food samples, respectively. Each point-scan system uses a whiskbroom method for hyperspectral Raman image acquisition from food samples carried by a two-

axis positioning stage. A more efficient line-scan RCI system has also been developed using a 785 nm line laser to implement high-throughput RCI. A one-axis positioning stage is used to move the samples to accumulate hyperspectral data using a pushbroom method. Dispersive Raman spectrographs are used in both point- and line-scan systems, which can all be configured to backscattering RCI mode for food surface inspection and spatially offset Raman spectroscopy (SORS) mode for subsurface inspection. The 785 nm point-scan system has also been configured to implement gradient temperature Raman spectroscopy (GTRS), which is a patented technique that applies the precise temperature gradients to Raman spectroscopy measurement. The line-scan RCI technology has been patented and is currently in licensing process for potential future industrial uses. In-house developed LabVIEW software and MATLAB programs are used to fulfill functions for system control, hardware parameterization, data transfer, and spectral and image processing. The ARS macro-scale Raman technologies have found many practical food safety and quality applications. Examples of the RCI applications include detecting chemical adulterants mixed in food powders, mapping carotenoid content on carrot cross section, and inspecting fish bones in fillets. Examples of the SORS applications include nondestructive evaluation of internal maturity of tomatoes, detection of gelatin-encapsulated powders, and through-package inspection of foods and ingredients.

(AAF-OD2.3) Trends and Emerging Approaches for Authentication of Food Ingredients – The Handheld Spectroscopy Revolution

Presenter: Luis E. Rodriguez-Saona, PhD - The Ohio State University

Economic adulteration and counterfeiting of foods results on increased costs (recall, liability, withdrawals), lost revenue or market share, damaged brand, failed business or bankruptcy, and sometimes safety risks. Current methods for testing adulterants in foods are time-consuming, expensive, and labor-intensive, requiring complex procedures of sample treatment and well-trained technicians to operate expensive instrumentation. Portable instrumentation for use in out-of-lab applications are uniquely positioned for rapid on-site identification and non-destructive authentication of incoming ingredients because of its speed, ruggedness, compactness, ease of use and transportation. Vibrational spectroscopy (NIR, IR and Raman) provides rapid and cost-effective tools for effective food surveillance. This presentation covers the current state of research on applications of vibrational spectroscopy for authentication of high-value raw materials and detection of food contaminants. Testing done close to the original source and throughout the process would permit detecting adulteration before an ingredient has been diluted or combined with other ingredients. Powerful pattern recognition techniques can be used to screen materials and enable real-time control of the raw material stream curtailing the growing danger of food ingredient fraud due to the unconventional adulterants or substituted products used for these activities.

(AAF-OD3) Forensic Analysis in the Lab and at the Crime Scene

(AAF-OD3.1) Forensic Science R&D Funding Programs at the National Institute of Justice

Presenter: Gregory Dutton, PhD - National Institute of Justice

The National Institute of Justice (NIJ) is the research, development and evaluation agency of the U.S. Department of Justice. NIJ maintains a program of external funding for research and development in the forensic sciences which is a leading federal funder in this mission space. The portfolio 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 to ensure that the perpetrators of crime can be identified quickly, increasing public safety, and promotes 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 biological and chemical samples. Due to the unique circumstances of forensic evidence, there is an ongoing need for these analyses to be done on ever smaller, degraded, compromised or mixed samples. At the same time, increased backlogs in operational forensic laboratories create pressure to increase the speed and decrease the cost of analyses. These needs drive NIJ's continuing R&D investments in analytical chemistry and applied spectroscopy for forensic application. In this effort, NIJ strives to engage the research community to bring novel methods to solving forensic problems. An overview of NIJ's research and development portfolio will be presented, highlighting relevant examples in Trace Evidence (fibers, glass, paint, dust, etc.); Seized Drugs and Toxicology; and Forensic Biology. The scope and growth of NIJ's R&D portfolio since the influential 2009 NAS report Strengthening Forensic Science in the United States will be discussed, including measures of program impact and examples of notable projects. Information on the funding cycle and anticipated R&D funding opportunities will be presented.

(AAF-OD3.2) Practical Application of a Kinetic Model to Identify Evaporated Liquids in Fire Debris Samples

Presenter: Ruth Smith, PhD - Michigan State University

Non-Presenting Author: Victoria L. McGuffin - Michigan State University

Non-Presenting Author: Briana A. Capistran

The presence of ignitable liquid residues in fire debris samples can be indicative of an intentional, rather than accidental, fire. Debris samples are conventionally analyzed using gas chromatography-mass spectrometry (GC-MS) and the resulting total ion chromatogram (TIC) and relevant extracted ion profiles (EIPs) are compared to those generated from an in-house reference collection of ignitable liquids. To account for evaporative losses that occur during a fire, many reference collections also include TICs and EIPs of liquids evaporated to a few different levels. On-going work in our laboratory is focused on the refinement and application of a kinetic model to predict evaporation rate constants of compounds in ignitable liquids as a function of retention index. The kinetic model is applied to the chromatogram of the unevaporated liquid to predict TICs or EIPs corresponding to any evaporation level of that liquid. In this manner, extensive reference collections containing predicted TICs and EIPs corresponding to any evaporation level can be generated in a time- and resource-efficient manner. In this presentation, practical application of the kinetic model to identify evaporated ignitable liquids in fire

debris samples will be described. A modeled reference collection containing TICs and EIPs of 18 liquids corresponding to 9 evaporation levels was first generated. A series of single-blind samples (liquid identity and evaporation level unknown to analyst) were analyzed and compared to the modeled reference collection, using Pearson product-moment correlation (PPMC) coefficients as a measure of similarity. For each blind sample, the highest PPMC coefficients ($r > 0.97$) were observed for comparison to the predicted TIC and EIPs corresponding to the correct liquid and evaporation level. Subsequently, fire debris samples were collected from three large-scale burn cells, furnished to represent a typical apartment, and were analyzed following standard procedures. Given the presence of substrate interferences in these samples, the EIP reference collection showed the strongest correlation and enabled correct identification of the liquid used in each burn cell. Throughout this presentation, the practical application of the kinetic model to identify liquids present in fire debris samples, even in the presence of substrate interferences, will be highlighted.

(AAF-OD3.3) Investigative leads using LIBS and orthogonal methods in crime laboratories and in the field

Presenter: Tatiana Trejos, PhD - West Virginia University Department of Forensic and Investigative Science

Non-Presenting Author: Luis Arroyo, PhD - West Virginia University Department of Forensic and Investigative Science

Non-Presenting Author: Colby Ott, MS - West Virginia University

Non-Presenting Author: Courtney H. Vander Pyl, MS - West Virginia University Department of Forensic and Investigative Science

Non-Presenting Author: Korina Menking Hoggatt, MS - West Virginia University

Non-Presenting Author: Kourtney Dalzell, BSc. - West Virginia University

This study evaluated the use of Laser-Induced Breakdown Spectroscopy (LIBS) and Electrochemical Sensors as fast screening methods for assisting firearm-related investigations. The methods were assessed to identify GSR around bullet orifices in complex substrates that are often difficult to remove from the crime scene and would benefit from reliable onsite testing. Twenty-one glass, wood, and drywall pieces were shot from closed range and were chemically mapped using an optimized LIBS protocol. The proposed approached allowed easy transfer of GSR from the substrates and quick mapping of GSR markers around the bullet orifice. LIBS was also evaluated for chemical mapping and estimation of shooting distance estimations on fabric items using discriminant analysis. Fifty cotton-based- clothing samples were fired from different distance intervals, with a 9 mm pistol, and then covered in blood to simulate typical scenarios. The LIBS method demonstrated superior performance compared to physical examinations and color testing (100%, 50%, and 40% correct classification rates, respectively). Superior performance over conventional methods was observed, eliminating the need for chemical reagents for testing, limiting sample preparation and destruction, enhancing selectivity, and generating permanent spectrochemical chemical maps, and more objective results. Finally, Electrochemical sensors and LIBS were evaluated to detect inorganic and organic gunshot residues from the hands of over 600 shooters and non-shooters. Accuracy ranging from 95% to 98% was achieved when both methods are combined using machine learning classification tools. The analytical techniques are anticipated to offer novel case management alternatives in the field and at the crime laboratories.

(AES-OD1) Microfluidics for Electric-field Driven Analysis

(AES-OD1.1) 3D printing for Microfluidics

Presenter: Rosanne M. Guijt, PhD - Deakin University, Geelong, Australia

3D printing has rapidly evolved to become a first line custom manufacturing approach for prototyping, and increasingly for product manufacture of custom parts as well as milli and microfluidic devices. 3D printing is an umbrella term for a range of fundamentally different additive manufacturing techniques. For the fabrication of fluidic devices, digital light projection (DLP), fused deposition modeling (FDM) and inkjet based approaches are most popular. Advances in 3D printing are driven be by the desire to print faster, smaller and more functionally integrated devices, and occur at the interplay of hardware and material science. This means that, in addition to differences in fluidic performance inherent to the printing technique used, a sound understanding of the printing process is required to optimise printer performance using material properties. Functional integration is a theoretical strength of 3D printing as manufacturing approach, with opportunities at the printer hardware/material science interface. Driven by a desire for functionally integrated devices, focus here will be on porous materials, with uses in chemical analysis including their use for filtration and chromatographic processes. Recent advances at the hardware/material science interplay for will be discussed for different 3D printing approaches. The importance of the relationship between hardware and material science in DIgital Light Projection printing is further illustrated based on progress made in two recent research projects.

(AES-OD1.2) Computer vision and deep learning assisted electrophoresis as a point-of-care test for hemoglobin level, anemia, and sickle cell disease

Presenter: Umut Gurkan, PhD - Case Western Reserve University

Anemia affects a third of the world's population with the heaviest burden borne by women and children. Anemia leads to preventable impaired development in children, as well as high morbidity and early mortality among sufferers. Genetic hemoglobin (Hb) disorders, such as sickle cell disease, are among the major causes of anemia globally. Blood Hb level (in g/dL) is used as the main indicator of anemia, while the presence of Hb variants (e.g., sickle Hb or HbS) in blood is the primary indicator of an inherited disorder. Even though treatments are available for anemia and Hb disorders, screening, early diagnosis, and monitoring are not widely accessible due to technical challenges and cost, especially in low-and-middle-income countries. We hypothesized that computer vision and deep learning will allow accurate, reproducible blood Hb level prediction and anemia detection in paper-based Hb electrophoresis, which is a clinical standard test for Hb variant screening and diagnosis worldwide. To test this hypothesis, we developed the first integrated point-of-care anemia and Hb variant test: Hb Variant/Anemia (HbVA). I will present the feasibility of this new, computer vision and deep learning assisted diagnostic approach via testing 46 subjects, including individuals with anemia and homozygous or heterozygous sickle cell disease. I will demonstrate that HbVA computer vision tracks the electrophoresis process real-time and the deep learning neural network algorithm reproducibly predicts blood Hb concentration with a mean absolute error of 0.55 g/dL and a bias of -0.10 g/dL (95% limits of agreement: 1.5 g/dL) according to Bland-Altman analysis. Anemia determination was achieved with 100% sensitivity and 92.3% specificity with a receiver operating characteristic area under the curve (AUC) of 0.99. With the same test, subjects with sickle cell disease were identified with 100% sensitivity and specificity. Overall, I will show that computer vision and deep learning methods can be used to extract new information from standard electrophoresis, enabling, for the first time, reproducible,

accurate, and integrated blood Hb level prediction, anemia detection, and Hb variant identification in a single point-of-care test.

(AES-OD1.3) Enabling Proteomic Characterization of Embryonic Single Cells through Capillary Electrophoresis Mass Spectrometry

Presenter: Leena Pade, MS - University of Maryland, College Park

Corresponding Author: Peter Nemes - University of Maryland, College Park

Recent studies have demonstrated the importance of analyzing single cells to improve our understanding of various biological processes such as embryogenesis, cell signaling and tumorigenesis. Variability in expression of genes and proteins leads to cell to cell variation, however traditional analytical approaches use average cell measurements which fail to capture these differences. Thus, analysis of single cells can improve our understanding of cellular heterogeneity. The Nemes research group focuses on studying molecular mechanisms that underlie tissue differentiation during embryonic development using Xenopus laevis (frog) as our vertebrate model. We developed a minimally invasive method coupled with capillary electrophoresis electrospray ionization mass spectrometry (CE-ESI-MS) which allows temporal proteomic profiling of neural fated live embryonic cells. Analyzing live embryonic cells at single cell resolution will enhance our knowledge of gene and protein expression changes in dividing cells which give rise to neural tissue. Our method uses a microsampling approach in which we use pulled borosilicate capillaries to aspirate less than 10% of cell volume from live dorsal neural fated cell in a 16 celled embryo and its descendants at different stages of development. To enable ultrasensitive measurements, aspirates collected at different stages were analyzed using our custom-built CE-ESI set up coupled to hybrid orbitrap mass spectrometer (Q-Exactive Plus, Thermo Scientific). Using this approach, we evaluated changes in protein expression across neural-fated cells at four embryonic stages: 16-cell, 32-cell, 64-cell, and 128-cell stage. Across these 4 stages of development we quantified ~460 protein groups based on their label-free quantification (LFQ) intensities. Proteomic data from each stage was further evaluated for statistical significance. A hierarchical cluster analysis (HCA) demonstrated that cells cluster based on their developmental stage and display protein profiles specific for every stage. Interestingly, we also found an increase in cell to cell variation in proteomic profiles as development progresses. We now apply this powerful microsampling single-cell CE-ESI-MS approach to open new avenues in studying cell specific translational changes that can induce and alter cell differentiation and advance our understanding of how the proteome changes in a during development.

(AES-OD2) Electric-field Mediated Techniques for Biological Applications

(AES-OD2.1) CE-MS for de novo Monoclonal Antibody Sequencing and Other Peptide Analysis

Presenter: David DY Chen, PhD - U. of British Columbia

Non-Presenting Author: Jianhui Cheng

Non-Presenting Author: Lingyu Wang

Mass spectrometry is a powerful tool for de novo sequencing of novel proteins. Recent efforts in this area have mainly focused on liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS). Here we present an alternative method, capillary electrophoresis tandem mass spectrometry (CE-MS/MS), for sequencing novel monoclonal antibodies. Using less than 200 ng in total of tryptic digest sample in a triplicated measurement, CE-MS/MS with pH mediated focusing successfully sequenced mAb infliximab with 100 % sequence coverage and 100 % accuracy for the light chain and 96 % coverage and 93 % accuracy for the heavy chain. It was also demonstrated that CE-MS/MS gives comparable results, and in some cases, even better results, than LC-MS/MS when used as a stand-alone technique. A combined workflow using both CE-MS/MS and LC-MS/MS was also used to sequence a novel antibody, anti-CD-176, resulting in the first proposed sequence for this mAb. A CE-MS/MS workflow was developed and was shown to be complementary to LC-MS/MS workflows for sequencing novel mAbs. A flow-through microvial interface was used to connect a CE system to a Thermo Scientific Orbitrap Fusion Lumos mass spectrometer for peptide identification. The use of CE-MS/MS as an alternative to LC-MS/MS has at least two clear benefits. First, each CE-MS/MS run took less than 30 min, making it much less time consuming than traditional LC-MS/MS, which typically takes 50 min per run including the time needed for analytical column preconditioning. Second, individual CE runs required only 50-100 ng of sample, meaning that residual sample material left behind after LC injection can be used to generate a large number of complementary peptide identifications. This latter point also means that a novel antibody could potentially be sequenced using only about 200 ng of sampled material if a purely CE-based workflow were adopted. These results demonstrate that CE-MS/MS is perfectly suited to mAb sequencing because of its efficiency, sensitivity, and complementarity to LC-MS/MS.

(AES-OD2.2) General-purpose electrochemical sensor for small molecules, peptides, and proteins in clinically-relevant ranges

Presenter: Christopher J. Easley, PhD - Auburn University

Non-Presenting Author: Niamat Khuda

Non-Presenting Author: Asanka Gurukandure Gedara

Non-Presenting Author: Subramaniam Somasundaram

Owing to the low cost and adaptability of electrochemistry (EC) to point-of-care (POC) setups, the past decade has seen renewed interest in EC biosensors for the many biomarkers that are not EC-active, do not undergo measurable enzymatic conversion, or are not suitable for potentiometer [1,2]. While these sensors have the potential to significantly impact human health, most method development has drifted towards being target-focused (clinical targets such as small molecules, nucleic acids, peptides, and proteins [3]) and has lacked generalizability. Currently, the EC toolbox for potential POC analysis is a

conglomerate of methods or specially-targeted probes, thus there is a pressing need to develop an EC platform amenable to rapid, generalizable, quantitative readout of multiple classes of clinically relevant targets.

Recently, our group has begun to address this need by developing a DNA-nanostructure sensor architecture for general-purpose sensing [4]. Since this initial validation with biotechnology controls and with a small molecule immunomodulatory drug in human serum, we have now shown that the same system can be used to detect a peptide drug for diabetics, exendin-4. We also have preliminary evidence of sensor response to a marker of muscle damage, creatine kinase (CK), as well as a peptide hormone secreted by the pancreas, C-peptide. These data already fall within the clinically-relevant ranges, and future work should allow refinement of the dynamic ranges.

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.

References:
1. Turner, A. P., Chemical Society Reviews 2013, 42 (8), 3184-96.
2. Wilson, G. S.; Johnson, M. A., Chem. Reviews 2008, 108 (7), 2462-81.
3. Labib, M.; Sargent, E. H.; Kelley, S. O., Chem. Reviews 2016, 116 (16), 9001-90.
4. Somasundaram, S. E., C. J., J. Am. Chem. Soc. 2019, 141, 11721-11726.

(AES-OD2.3) Automated capillary isoelectric focusing-tandem mass spectrometry for qualitative and quantitative top-down proteomics

Presenter: Liangliang Sun, PhD - Michigan State University

Non-Presenting Author: Tian Xu

Top-down proteomics (TDP) aims to delineate proteomes in a proteoform-specific manner, which is vital for accurately understanding protein function in cellular processes. It requires high-capacity separation of proteoforms before mass spectrometry (MS) and MS/MS. Capillary isoelectric focusing (cIEF)-MS has been recognized as a useful tool for TDP in 1990s because cIEF is capable of high-resolution separation of proteoforms. The Lee and Smith groups performed pioneering cIEF-MS works for top-down characterization of proteins. Previous cIEF-MS studies concentrated on measuring protein's mass without MS/MS, impeding the confident proteoform identification in complex samples as well as the accurate localization of post-translational modifications (PTMs) on proteoforms. Herein, for the first time, we present automated cIEF-MS/MS-based TDP for large-scale delineation of proteoforms in complex proteomes. Single-shot cIEF-MS/MS identified 771 proteoforms from an Escherichia coli (E. coli) proteome consuming only nanograms of proteins. Coupling two-dimensional size exclusion chromatography (SEC)-cIEF, named "gel-free 2D-PAGE", to ESI-MS/MS enabled the identification of nearly 2000 proteoforms from the E. coli proteome. Label-free quantitative TDP of zebrafish male and female brains using the SEC-cIEF-MS/MS quantified thousands of proteoforms and revealed sex-dependent proteoform profiles in brains. We discovered several proteolytic proteoforms of pro-opiomelanocortin and prodynorphin with significantly higher abundance in male brains as potential endogenous hormone proteoforms. Multi-level quantitative proteomics (TDP and bottom-up proteomics) of the brains revealed that majority of proteoforms having statistically significant difference in

abundance between genders showed no abundance difference at the protein group level. This work represents the first multi-level quantitative proteomic study of sexual dimorphism of brain.

(AES-OD3) Electrokinetic Fundamentals

(AES-OD3.1) Proteins & Dielectrophoresis: A Surprising Deep History, New Theories and Data

Presenter: Mark A. Hayes, PhD - Arizona State University

Non-Presenting Author: Yameng Liu

Proteins are perhaps the most important yet frustratingly complicated and difficult class of compounds to analyze, manipulate and use. Analytical and preparative strategies for proteins have been pursued and modeled for nearly a hundred years, with great advances and success. Core to all of these studies is the separation, isolation, purification, and concentration of pure homogeneous fractions of a specific protein in solution. Processes to accomplish this useful solution include biphasic equilibrium (chromatographies, extractions), mechanical, bulk property, chemical equilibria, and molecular recognition. Ultimately, the goal of all of these is to physically remove all non-like protein molecules—to the finest detail: all atoms in the full three dimensional structure being identical down the chemical bond and bulk structure chirality. One strategy which has not been effectively pursued is exploiting the higher order subtle electrical properties of the protein-solvent system. The advent of microfluidic systems has enabled the use of very high electric fields and well defined gradients such that extremely high resolution separations of protein mixtures are possible. These advances and recognition of these capabilities has caused a re-evaluation of the underlying theoretical models and they were found to be inadequate. New theoretical descriptions are being considered which align more closely to the total forces present and the subtlety of differences between similar proteins. These are focused on the interfacial area between the protein and hydrating solvent molecules, as opposed the macroscale assumptions of homogeneous solutions and particles. This critical review examines all data which has been published that place proteins in electric field gradients which induce collection of those proteins, demonstrating a force greater than dispersive effects or countering forces. Evolving theoretical constructs are presented and discussed, and a general estimate of future capabilities using the higher order effects and the high fields and precise gradients of microfluidic systems is discussed.

(AES-OD3.2) A Novel Porous Flow-Through Electrochemical Sensors for Increased Selectivity and Sensitivity from PFAS to Biological Molecules

Presenter: Sagnik Basuray, PhD - New Jersey Institute of Technology

Non-Presenting Author: Yu Hsuan Cheng

Non-Presenting Author: Li Zhenglong

Non-Presenting Author: Lixin Feng - New Jersey Institute of Technology

Non-Presenting Author: Charmi Chande

Non-Presenting Author: Juliana Yang

The parasitic Debye double-layer capacitor hinders the detection of analytes on the electrode surface in electrochemical impedance spectroscopy-based electrochemical sensors. We provide a new nonplanar, interdigitated, flow-through, porous electrode sensor device called ESSENCE. This results in enhanced shear forces and increased convective fluxes that disrupt the diffusive process in the double layer in a microfluidic channel. This moves the double layer capacitor signal from low frequencies to higher frequencies making the device rapid with a high signal to noise ratio. A novel integration process allows us to switch the nanomaterials that make up the porous electrode quickly. The proposed biosensor,

ESSENCE, has four significant benefits over the current generation of electrochemical biosensors. (1) The electrode nanoporosity facilitates the development of shear forces of the order of a hydrogen bond, which significantly increases selectivity by mitigating non-specific adsorption. (2) Our preliminary results indicate that the interdigitated, nanoporous microelectrode design, results in a high signal to noise ratio (SNR), which is realized by increases in both sensitivity and specificity. As the ionic flux is confined to nanodomains due to nanostructured electrodes (nanoconfinement effects), it enables the ESSENCE technology to tremendously increase sensitivity and boost the signal so significantly that even the surface functionalizations of the electrodes can be resolved and characterized. (3) The nanoporous electrode architecture increases convective transport of the analyte of interest to the sensing element, thus overcoming diffusion limitations and reducing assay times. Additionally, the convection-mediated transport enhancement negates signal artifacts such as the parasitic double-layer capacitance, thus facilitating rapid, high resolutions characterization of the binding signal due to a significant reduction in noise. (4) Finally, given the ease of controlling shear force through flow rate, challenges related to sensitivity and device selectivity can be decoupled from each other (and each parameter investigated individually) using the shear force as a tuning design parameter. This decoupling allows us to mitigate the problems of biofouling, false positives, and false negatives, among others. ESSENCE is used to detect DNA, proteins, and small molecules like Perfluorooctanesulfonate. We show that we can detect PFAS below recommended EPA levels.

(AES-OD3.3) Hot-SWV: Combining AC Electrokinetics with Square Wave Voltammetry

Presenter: Aliaksei Boika, PhD - The University of Akron

Non-Presenting Author: Ariana Frkonja-Kuczin, PhD - The University of Akron

Non-Presenting Author: Josean Alicea-Salas - University of Puerto Rico

Non-Presenting Author: Netz Arroyo, PhD - Johns Hopkins University

Square Wave Voltammetry (SWV) is arguably the most sensitive electroanalytical technique. It employs a square-wave waveform superimposed on a linear potential sweep to effectively eliminate background charging currents. In the Boika lab, we are developing a novel modification to a conventional SWV that utilizes the electrothermal fluid flow (ETF) to further improve the sensitivity of the aforementioned technique. ETF is generated in an electrolyte solution by applying a high frequency alternating current (ac) waveform (ca. 100 MHz frequency, several Vrms amplitude) to a working microelectrode. As a result of this perturbation, the mass transfer and the kinetic rate of electrochemical reactions are enhanced, thus leading to a substantial improvement in the analytical signal. In this presentation, I will report on the most recent advancements in the development of hot-SWV. Both the theoretical and experimental findings will be presented. It is expected that the novel technique will be particularly useful for the analysis of ultra-low concentrations of environmental pollutants like lead.

(ATOM-OD1) Atomic Mass Spectrometry

(ATOM-OD1.1) Single particle ICP-MS to measure nano- and micro-particles in ice cores using quadrupole and time-of-flight mass spectrometers

Presenter: John Olesik, PhD - Ohio State University

Non-Presenting Author: Madeleine Lomax-Vogt - Ohio State University

Non-Presenting Author: Paolo Gabrielli - Ohio State University

Non-Presenting Author: Aja Ellis - Ohio State University

Non-Presenting Author: Luke Monroe - Carnegie Mellon University

Non-Presenting Author: Garret Bland - Carnegie Mellon University

Non-Presenting Author: Ryan Sullivan - Carnegie Mellon University

Measuring continuously on a sub-ms time scale allows ICP-MS signals from individual nano-particles and micro-particles to be observed. ICP-Quadrupole MS provides element selective detection of nano- and micro-particles while ICP-TOFMS provides a complete mass spectrum from each particle. We will discuss some of the capabilities and limitations of single particle ICP-MS (spICP-MS) as well as potential concerns related to particle suspension stability, incomplete vaporization of microparticles in the ICP, dynamic range of both signal intensities and number concentration and calibration. We will discuss these as well as comparing spICP-MS measurements with quadrupole versus time-of-flight mass spectrometers. Most of the applications of spICP-MS to date have measured engineered nanoparticles of known (expected) composition (Ag, Au, ZnO, etc) where element selective detection is sufficient. However, natural particles, for example mineral particles, can have a wide variety of chemical compositions. Therefore, a complete mass spectrum is needed from each individual nano- or micro-particle. Ice cores provide a time-resolved history of past climate that can be gleaned from bubbles of trapped atmospheric gases, biological components (algae, bacteria, spores, DNA remnants) and elements present in the melted solution or as particles. Almost all of the elemental analysis of particles in ice cores has been done by dissolving the particles in an acidic solution (although dissolution may not be complete for some particles) and measuring the bulk elemental concentrations. This provides data on the sum of all elemental masses but cannot distinguish particles of different compositions. spICP-MS can measure the elemental composition of individual particles. We will show some initial spICP-MS measurements of nano- and micro-particles trapped in recent and ancient ice from the Alps and from Antarctica and discuss the analytical considerations and challenges involved.

(ATOM-OD1.2) Perspectives On Pulsed Glow Discharge Mass Spectrometry For In-Depth Profile Analysis Of Nanolayers.

Presenter: Jorge Pisonero, PhD - University of Oviedo

Non-Presenting Author: Nerea Bordel, PhD - University of Oviedo

Non-Presenting Author: Jonatan Fandiño - University of Oviedo

Non-Presenting Author: Cristina Gonzalez-Gago - University of Oviedo

Development and characterization of challenging materials (e.g. photovoltaic cells, production of high wear-resistant coatings, hard-disks, Ni and Co super-alloys, etc.) is highly demanded in the different sectors of the manufacturing industry. In particular, multiple innovative materals are based on the used

of nano-coatings (single and multiple layers). Therefore, the ability to accurately analyse and characterise these materials is essential for quality control applications and for the development of new products and processes. Atomic spectrometry techniques have long been used for direct elemental chemical characterization. For instance, Secondary Ion Mass Spectrometry, Auger Electron Spectroscopy, or X-Ray Photoelectron Spectroscopy, are techniques that provide very valuable information about the chemical composition of the surfaces/coatings. However they also have some major drawbacks, such as high operating costs, complex sample pre-treatment and handling, low sample throughput and/or severe matrix effects that result in difficult quantification procedures. Other techniques, such as those base on laser ablation or laser induced plasma (e.g. LA-ICP-MS, LIBS, LAMS), provide high spatial resolution and high sensitivity but their depth resolution capabilities are restricted to a few tens or hundreds of nanometers. In order to overcome some of these drawbacks Glow Discharge Mass Spectroscopy (e.g. GD-TOFMS) is proposed as a complementary methodology that provides an ideal solution for fast and accurate bulk and layer analyses. Here, we evaluate and critically discuss the advantages and limitations of this technique, the recent progresses, and the demanding features.

(ATOM-OD1.3) New Possibilities Of Single Cell-ICP-ToF-MS For Ecotoxicological Assessment

Presenter: Marcus von der Au, M.Sc. - BAM Berlin

Non-Presenting Author: Olga Borovinskaya - TOFWERK AG

Non-Presenting Author: Claudia Büchel - University of Frankfurt - Institute of Molecular Biosciences

Corresponding Author: Björn Meermann, n/a - Federal Institute for Materials Research and Testing (BAM)

Diatoms are located at the bottom of the food chain. Thus, toxicological relevant metals taken up by diatoms can possibly accumulate within the food web and cause harmful effects. Diatoms are a common test system in ecotoxicology. Toxicological effects weaken the growth of algae which is by default investigated by means of fluorescence detection - diminished fluorescence compared to a non-exposed control group indicates an effect. On basis of the expose concentration as well as obtained fluorescence data potential threshold exceedance in e.g. surface waters is assessed. However, this approach does not allow for the determination of "real" accumulated metal concentration in diatoms. Common approaches are based on microwave assisted digestion and elemental analysis via e.g. ICP-MS, ICP-OES or AAS. But, with regard to low absolute metal-content in algae this strategy is only feasible in case of availability of a high biomass. To tackle this problem, alternative, complementary approaches are highly needed. Within the last years, sp-ICP-MS for nanoparticle as well as single cell analysis turned out as a powerful technique to analyze metal contents as well as size distributions on broad size range (nano- to low micrometer scale). But, common ICP-MS systems do not allow for multi-element detection within single particle/cell events. Thus, simultaneous MS detection devices are needed - just recently, ICP-ToF-MS experienced a revival. Within our previous work, we developed an automated sample introduction system based on a HPLC system on-line with single particle-ICP-MS, which allowed for ionic background separation and single algae analysis. However, for unambiguous tracing several fingerprint elements and multielement analysis in single algae (diatoms) is needed. Thus, we coupled our previous setup on-line to ICP-ToF-MS. Test diatom species were exposed to test substances (Zn) as well as nanoparticles (FeNPs). The developed setup allowed for a fast, automated and multielement analysis in single diatoms. Furthermore, we combined our approach with multivariate data assessment - multielement detection of characteristic fingerprint elements allowed for an unambiguous diatom

tracing. Clustering of diatoms according to metal exposure concentration levels was enabled. Our approach is a new potential tool in ecotoxicological testing.

(ATOM-OD2) Nanoparticles and Microscopy

(ATOM-OD2.1) Platforms for Nanoparticle Characterization Via Glow Discharge Optical Emission Spectroscopy Elemental Mapping

Presenter: Gerardo Gamez, PhD - Texas Tech University

Non-Presenting Author: Kevin Finch, n/a - Texas Tech University

Non-Presenting Author: Yue She, n/a - Texas Tech University

Non-Presenting Author: Aldo Hernandez, n/a - Texas Tech University

Nanoparticle (NP) use is prevalent in many different fields, from catalysis to fuels to medicine, and several others. Given the advantages they afford, NPs span a wide breath of applications that go from therapeutics and diagnostics to disinfection and UV protection. Thus, methods are required for quantitative elemental mapping (EM) of NPs not only to assess their desired properties but also to monitor their environmental impact and toxicological effects. Nevertheless, typical elemental mapping techniques suffer from limited sample throughput due to the common pixel-to-pixel data collection schemes. On the other hand, glow discharge optical emission spectroscopy (GDOES) has demonstrated ultra-high throughput elemental mapping capabilities when operated under higher pressures/pulsed-power conditions and coupled with hyperspectral imaging techniques. Here, the development of a new method for GDOES EM of NPs will be presented. The possibility of using different relevant platforms will be explored, including glass slide microarrays and hollow cathode arrays on Cu plates. Relevant analytical figures of merit (FOM) will be reported. The effect of NP characteristics (e.g. nanoparticle size) on the FOM will be investigated.

(ATOM-OD2.2) A System for the Quantification and Classification of Metal-Containing Nanoparticles

Presenter: Alexander Gundlach-Graham, PhD - Iowa State University

Non-Presenting Author: Kamyar Mehrabi - ETH Zurich

Non-Presenting Author: Ralf Kaegi - Eawag

Non-Presenting Author: Detlef Günther - ETH Zurich

Non-Presenting Author: Alexander Lancaster - Iowa State University

Non-Presenting Author: Stasia Harycki - Iowa State University

The use of engineered nanoparticles (ENPs) in various industries and commercial products continues to expand. Likewise, as global population shifts to urban centers, the prevalence of anthropogenic incidental NPs (INPs), e.g. NPs produced from automotive breaking, tire wear, or industrial and automotive combustion processes, is becoming an increasingly important to human health outcomes. However, without characterizing and quantifying the abundance of ENPs and INPs in various environmental compartments, researchers cannot effectively model and predict the impact of ENPs and INPs on environmental processes or human health. To this end, single-particle Inductively Coupled Plasma Mass Spectrometry (sp-ICP-MS) has emerged as a powerful technology for the detection and quantification of metal-containing NPs at low (environmentally relevant) particle-number concentrations. A limitation of most sp-ICP-MS setups is the inability to quantify multi-element NPs and mixtures of NPs with variable elemental compositions. This limitation is particularly pronounced for the measurement of NP types (such as Ti-, Fe-, Zn-, Ce-containing NPs) that exist as naturally occurring

nano-minerals or nano-colloids, in addition to ENP and INP forms. Here, we discuss how the use of Inductively Coupled Plasma Time-of-Flight Mass Spectrometry (ICP-TOFMS), combined with online microdroplet calibration, can be used to deliver high-throughput and accurate single-NP measurements. Further, we investigate the use of multi-element signatures of individual NPs to classify and differentiate NP types. Such classification could enable the accurate simultaneous quantification of analyte metals present as naturally occurring NPs, ENPs, and INPs. Together, we report the development of an automated, high-throughput, sp-ICP-TOFMS quantification strategy for the untargeted analysis and unsupervised classification of NP mixtures.

(ATOM-OD2.3) RISE Microscopy: Correlative Raman & SEM Imaging

Presenter: Joachim Koenen - WITec Instruments Corporation

Non-Presenting Author: Ute Schmidt

Non-Presenting Author: Olaf Hollricher

The characterization of composite materials greatly benefits the combination of different analytical methods. The interconnection of data from separate methods can deliver the comprehensive understanding often thought. When using different analysis techniques on one and the same sample, the measurement workflow can be accelerated by combining several analytical methods in one instrument. In this contribution, we present a new microscope solution along with a novel operating concept that combines 3D confocal Raman imaging with other nano-analytical techniques such as epifluorescence, profilometry, AFM, or SNOM. This combination of analytical techniques allows for the identification of cell components in biomedical studies, the characterization of phase separations in polymer blends and their wetting behavior on various substrates, or the visualization of optical properties of materials with resolution below the diffraction limit. RISE microscopy is a established correlative microscopy technique which combines confocal Raman Imaging and Scanning Electron (RISE) Microscopy within one integrated microscope system. SEM (Scanning Electron Microscopy) equipped with various accessories and detectors (SE – secondary electrons, BSE – backscattered electrons) and with microanalysis tools (EDS, CL) is a powerful tool for scientific inquiry, providing information on morphology, elemental composition and crystallography. Confocal Raman imaging of the same composite sample area reveals the chemical composition as well as polymorphisms, stress states and anisotropies. The aim of this contribution is to describe and highlight the unique features of such combined scientific analysis instruments, based on examples from composite materials.

(ATOM-OD3) LIBS

(ATOM-OD3.1) Detailed study of spectral features obtained from LIBS and Raman spectroscopy

Presenter: Jozef Kaiser, Prof - Brno University of Technology

Non-Presenting Author: Ivana Chamradova

Non-Presenting Author: Daniel Holub

Non-Presenting Author: David Prochazka

Corresponding Author: Pavel Porizka, PhD - Brno University of Technology

Investigation of samples is getting complex when providing structural and chemical analysis. The chemical analysis itself may be obtained via the utilization of various techniques giving diverse, yet complementary information. Their combined utilization is not trivial when considering sample preparation, sampling, data collection, and processing. In our work, we focus on elaborate data processing to provide robust data analysis and not using machine learning tools as black boxes. This demands a straightforward connection of machine learning to the data sources (e.g., spectroscopy, plasma physics, analytical chemistry) for efficient feature extraction and visualization. We have selected a series of polymer materials characterized by complex spectra datasets obtained by using various spectroscopic methods (LIBS and Raman spectroscopy). We demonstrate a step-by-step algorithm for polymer classification using individual spectroscopic datasets as well as their combination. The robustness of our classification algorithm is given in terms of overall accuracy.

(ATOM-OD3.3) LIBS imaging: a brief overview of breakthrough applications

Presenter: Vincent Motto-Ros, PhD - Institut Lumiere Matiere

Non-Presenting Author: Florian Trichard

Non-Presenting Author: Frédéric Pelascini

The imaging capability of laser-induced breakdown spectroscopy (LIBS) has a high potential in various domains in-cluding biology, industry, geology and medicine. This approach can be distinguished by its ease in use, multi-elemental capability, detection of light elements, as well as operation at ambient conditions. This is furthermore the only all-optical technique providing space-resolved elemental information with ppm-scale sensitivity and µm-range resolution. These advantages, make LIBS imaging very attractive to be used in research laboratories for rou-tine investigations. However, advanced technological solutions must be found for this application since elemental imaging requires high sensitivity, sharp spatial resolution, high speed of acquisition as well as the ability to process a huge quantity of data. In this presentation, we will summarize the recent progresses made in the Light and Matter Institute and the Cetim Grand-Est concerning the implementation of the LIBS imaging. In particular, different examples of applications will be shown with the aim of illustrating the specificities and the great potential of LIBS imaging. Different perspectives will be finally discussed.

(ATOM-OD4) Automation and Machine Learning in LIBS

(ATOM-OD4.1) Machine-learning supported LIBS elemental imaging: a potential tool for automatic pathologic analysis of cancer tissues

Presenter: Xiaohui Li, PhD - Harbin Institute of Technology

Non-Presenting Author: Xue Chen - Harbin Medical University Cancer Hospital

Non-Presenting Author: Yao Zhang - Harbin Institute of Technology

Non-Presenting Author: Guodong Yao - Harbin Medical University Cancer Hospital

Non-Presenting Author: Aichun Liu - Harbin Medical University Cancer Hospital

Non-Presenting Author: Xin Yu - Harbin Institute of Technology

In this talk, we present multi-elemental mapping of paraffine-embedded human breast cancer tissues using laser-induced breakdown spectroscopy (LIBS). Both ductal carcinoma and lobular carcinoma in different clinical stages were investigated. Thin slices (~5 micrometer) of biopsies were prepared and fixed onto glass substates. LIBS spectra were measured following a grid scan on the biopsies, with spatial resolution of 100 micrometer by 100 micrometer. Distributions of major elements, including Ca, K, Mg and Na, were obtained. Machine learning methods including principal component analysis and cluster analysis were used to realize automatic discrimination of malignant and non-malignant regions. Using conventional histological staining results as the reference, the accuracy of the cluster analysis was evaluated. The results demonstrated a general accuracy of around 70%, indicating that the machine-learning-supported LIBS elemental mapping could serve as a potential tool for automatic pathologic analysis of cancer tissues.

(ATOM-OD4.2) Automated LIBS Analysis of Molten Metal Composition in a Primary Aluminum Smelter

Presenter: Kristjan Leosson, PhD - DT Equipment

Non-Presenting Author: Sveinn Hinrik Gudmundsson

We have applied laser-induced breakdown spectroscopy for the analysis of molten aluminum during primary production and casting, using both portable and stationary liquid-metal LIBS analyzers. An automated stationary analyzer has been used, in conjunction with robotic sampling, for analyzing metal from transport crucibles. Element concentrations down to ppm levels have been monitored, with typical concentrations of impurity elements of interest in primary aluminum ranging from 1-3000 ppm. For many of the trace elements investigated, the repeatability of LIBS measurements on liquid metal matches or surpasses that of laboratory analysis of solid samples, where chemical segregation increases the measurement variance within a single process sample. The smelter environment provides many challenges to operating sensitive equipment, including alumina dust, corrosive fumes, high temperatures and strong magnetic fields. The fully automated stationary LIBS analyzers, however, allow smelters to remove human operators from the hazardous tasks of manually collecting liquid metal and casting samples for subsequent off-line laboratory analysis. This enables much more frequent sampling without added cost or increased safety risk to personnel. Portable analyzers, while still requiring a human operator to extract samples of liquid metal from production cells, result in significant time savings and

real-time feedback to potroom control, with the time elapsing from sample extraction to completion of a full trace-element analysis being around 45 seconds.

(ATOM-OD4.3) LIBS and Raman Spectroscopy Integration with Advanced Machine Learning Methods to Analyze Complex Samples

Presenter: Prasoon K. Diwakar, Ph.D. - South Dakota School of Mines and Technology

Non-Presenting Author: Sofia Pozsonyiova

Non-Presenting Author: Romila Pradhan, PhD - Purdue University

Non-Presenting Author: Samuel E. Kessinger, n/a - South Dakota School of Mines and Technology

Non-Presenting Author: Christian Leckband

Non-Presenting Author: Kamtung Chen

Non-Presenting Author: Jon Kellar

Non-Presenting Author: Daniel Diaz, PhD - University of Arizona

Non-Presenting Author: David Hahn - University of Arizona (USA)

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 including conflict minerals in conjunction with Raman spectroscopy. These complex samples result in generation of intricate spectral results hindering the identification of samples with certitudes. The key to overcoming this challenge can be achieved by analyzing LIBS data along with Raman using advanced machine learning models and simulations that lead to sample identification, classification and pattern recognition. An advanced hybrid machine learning approach as well simulation has been developed and applied on LIBS and Raman spectra for classification and identification of complex samples including conflict minerals and future direction of data analysis and application will be presented.

(ATOM-OD5) Atomic Student Awards

(ATOM-OD5.1) Elemental and Isotopic Analysis with Solution Cathode Glow Discharge Mass Spectrometry

Presenter: Asaf Harel - Rensselaer Polytechnic Institute

Non-Presenting Author: Garett M. MacLean, MS - Rensselaer Polytechnic Institute

Non-Presenting Author: Malina Helling - University of Münster

Non-Presenting Author: Montwaun Young

Non-Presenting Author: Jessica Hellinger - Rensselaer Polytechnic Institute

Corresponding Author: Jacob T. Shelley, PhD - Department of Chemistry and Chemical Biology, Rensselaer Polytechnic Institute

Mass Spectrometry (MS) is a powerful analytical tool for the detection of atomic or molecular analytes, which separates ionized species based on their mass-to-charge (m/z) ratios. Due to its selectivity and low limits-of-detection, MS has long been used for elemental analysis of metals in the environment and elsewhere. When performing such analyses, the ionization step is one of the more critical processes. However, popular ionization approaches for this task, such as Inductively Coupled Plasma (ICP) and Secondary Ion MS (SIMS), are expensive and can require large amounts of power or resources to operate. Recently, our group has been exploring the use of solution-cathode glow discharge (SCGD) as an ionization source for MS with broad utility. Originally developed for optical-emission spectroscopy (OES), SCGD operates by forming an atmospheric-pressure glow discharge between a tungsten anode and a flowing, conductive sample solution (i.e. the cathode) when a high voltage is applied to the anode. This plasma produces ionized analytes directly from the solution, which are detected, separated and sampled by the mass analyzer. The apparatus is compact and operates at atmospheric conditions, making the SCGD a versatile ionization source with the capability to detect a wide range of elemental, organic, and biological analytes. In this study, SCGD was coupled with a Q-Exactive mass spectrometer for elemental and isotopic analysis of metal and metalloid species such as uranium, lead, silver, and cadmium. Through analysis of metal solutions in nitric acid, metrics such as limits-of-detection (LODs) and linear dynamic range were experimentally determined. The LODs for some elements were in the hundreds of parts-per-quadrillion range with a linear working range of at least four orders of magnitude. Isotope-ratio measurements of uranium were performed with the SCGD that resulted in precise and accurate measurements of ratios between 234U, 235U, 236U, and 238U in depleted and natural abundance samples. The capabilities of SCGD-MS for uranium analysis were found to be on par with multimillion-dollar multicollector ICP-MS instruments.

(ATOM-OD5.2) Bone Under The Analytical Eye: Isotopic Analysis – From Anthropological Studies of Archaeological Humans to Modern Biomedicine

Presenter: Anika Retzman, Dr. - Chair of General and Analytical Chemistry, Montanuniversität Leoben

Non-Presenting Author: Johanna Irrgeher, Dr. - Department General, Analytical and Physical Chemistry, Chair of General and Analytical Chemistry, Montanuniversität Leoben, Austria

Non-Presenting Author: Michael Wieser, PhD - Physics and Astronomy, University of Calgary

Corresponding Author: Thomas Prohaska, Prof. Dr. - Department General, Analytical and Physical Chemistry, Chair of General and Analytical Chemistry, Montanuniversität Leoben, Austria

Incrementally grown bone of living things incorporates time-resolved chemical information of the environment and diet. The isotopic composition of numerous elements (e.g. H, C, N, S, Sr, Pb and Nd) can therefore provide information about the health status, diet and residential changes. Moreover, variabilities of isotopic composition caused by imbalances in the regulation of elements in metabolic processes have therefore a potential to be applied as diagnostic marker (e.g. Ca isotopes for changes in bone mineral balance). The variability of incorporated isotopic signatures are often small and historical samples are affected by contaminations from the repository material (e.g. diagenesis). For reliable protocols, a set of crucial parameters (procedural blank, spectral and non-spectral interferences, instrumental isotopic fractionation, recovery) have to be investigated as they significantly affect the metrological quality of the reported data. In the present work, the isotopic systems of Sr, Pb and Nd with their unique features related to trace residence changes of humans were investigated. Anthropological interpretations require a large number of samples. Therefore, a robust automated simultaneous analyte/matrix separation was developed, allowing the determination of precise Sr, Pb and Nd isotopic ratios from Ca-rich matrices using multi collector inductively coupled plasma mass spectrometry (MC ICP-MS) [1,2]. Further, a combined chemical imaging approach of hyperspectral imaging and laser ablation (LA) ICP-MS/MC ICP-MS visualizing mineral, molecular, elemental and isotopic, distributions in archaeological bone was developed to evaluate diagenetic alteration of bones with the aim to assess biogenic Sr isotopic composition [3]. Additional investigations were devoted to assessing the variation of stable Ca isotopic composition in biological tissues (i.e., bone, blood, urine). A low-level Ca isotopic analysis method using DGA Resin for Ca/matrix separation and double spike multi collector thermal ionization mass spectrometry (DS MC TIMS) was developed. The Ca isotopic composition could be obtained in samples containing down to 1000 ng Ca by lowering procedural blanks down to 5 ng, providing sufficiently low uncertainties to resolve natural variations in biological tissue. [1] A. Retzmann, Anal. Bioanal. Chem., 2017 https://doi.org/10.1007/s00216-017-0468-6. [2] T. Zimmermann, Spect. Chim. Acta B, 2019 https://doi.org/10.1016/j.sab.2018.11.009. [3] A. Retzmann, Anal. Bioanal. Chem., 2019, https://doi.org/10.1007/s00216-018-1489-5.

(ATOM-OD5.3) Tracing The Pathway Of Anthropogenic Gadolinium From Surface To Drinking Waters By Means Of Automated Speciation Analysis Methods

Presenter: Marcel Macke, MSc - University of Münster, Institute of Inorganic and Analytical Chemistry

Non-Presenting Author: C Derrick Quarles, Jr., PhD - Elemental Scientific, Inc.

Corresponding Author: Uwe Karst, Prof. Dr. - University of Münster, Institute of Inorganic and Analytical Chemistry

In the past decades, magnetic resonance imaging (MRI) has emerged as an important and frequently applied tool in clinical diagnosis. To improve MR signal and image contrast intravenous administration of paramagnetic gadolinium-based contrast agents (GBCAs) is required. Because free Gd(III) is highly toxic, polyaminocarboxylic acid ligands are used to create thermodynamically stable chelate complexes that are known to be excreted fast and unmetabolized via the kidneys. Due to their chemical nature, these compounds pass through wastewater treatment plants almost unaffected. In this way, up to 50 tons of Gd are released into the environment every year, which can also end up in drinking waters. Nevertheless, the behaviour of GBCAs in aqueous systems and especially the long-term toxicological effects on plants, animals and humans are largely unknown. Powerful methods of speciation analysis are required to monitor the distribution and fate of GBCAs in environment and drinking water systems. For this project, a fully automated single platform system for total metal analysis and syringe-driven

chromatography in combination with inductively coupled plasma-mass spectrometry (ICP-MS) was used to determine several Gd species in water samples. A method based on anion-exchange chromatography (IC) was developed to achieve the separation of the polar substances, whereas the hyphenation to quadrupole-based ICP-MS led to a highly sensitive element specific detection. Furthermore, the use of an automated inline-dilution function allowed a fast-external calibration from single stock standards to determine total Gd and GBCA concentrations. The developed IC/ICP-MS method enables a fast separation of several commonly administered contrast agents in less than ten minutes, which is a significant improvement of analysis time in comparison to previously published methods and allows a rapid monitoring of even large sample numbers. Limits of detection in the low pmol/L range turned out to be sufficient for the detection and quantification of GBCAs in environmental samples without prior sample enrichment. To trace the pathway of GBCAs from their point of release via surface waters into municipal drinking water systems the method was finally applied for speciation analysis of several water samples that were systematically obtained from surface and drinking waters around the city of Münster.

(ATOM-OD5.4) Exploration of Microelectromechanical Systems (MEMS) Devices as Adaptive Optics for Temporal Gating in Laser-Induced Breakdown Spectroscopy and Laser Ablation Molecular Isotopic Spectrometry

Presenter: Kelsey L. Williams - The State University of New York at Buffalo

Non-Presenting Author: George Chan - Lawrence Berkeley National Laboratory

Corresponding Author: Steven J. Ray - The State University of New York at Buffalo

Laser-induced breakdown spectroscopy (LIBS) and laser ablation molecular isotopic spectrometry (LAMIS) are time-dependent laser spectroscopy techniques that require temporal gating for optimum results. Typically, intensified charge coupled devices (ICCDs) are used to perform the temporal gating but are costly and fragile pieces of instrumentation. When ICCDs are not available or, in the case of hand-held devices, unrealistic, the signal is dependent on either mechanical choppers or background subtraction. These methods, although useful, may be insufficient for a given experiment or result in error in spectral readout. Here, we have demonstrated that a digital micromirror array (DMMA) is a cost-effective, robust device capable of temporally gating on the timescale of LIBS and LAMIS events, and further, spatially filter the image of a laser-induced plasma. The device is compact and lightweight, making it a suitable candidate as a temporal gating/spatial filtering device for hand-held devices. The DMMA is a MEMS device comprised of 1 million, micro-scale, individually controllable, aluminum mirrors that sit atop electrodes that tilt mirrors $\pm 12°$. Though the device has proven useful, it suffers from a truncation of the accessible wavelength range due to a hermetically sealed, glass layer that protects the micromirror array. Due to both its limitations and its capabilities, we have begun to explore the use of alternative MEMS devices for temporal gating of time-dependent experiments.

(ATOM-OD6) Applications of Atomic Spectroscopy

(ATOM-OD6.1) The Added Power of Combined LA-ICP-MS and LIBS for Food and Agricultural samples: A Summary of Applications Including Elemental Data for C, N, F and P Measurements for Soils, Food and Beyond

Presenter: Alan Koenig - Applied Spectra, Inc.

Non-Presenting Author: Jhanis J. Gonzalez, PhD - Applied Spectra, Inc. / Lawrence Berkeley National Laboratory

Non-Presenting Author: Charles Sisson - Applied Spectra, Inc.

The interaction of solid (or even liquid) material with high energy laser light such as what is done for laser ablation ICP-MS (LA-ICP-MS) analyses produces a laser induced plasma. This plasma is the basis for laser induced breakdown spectroscopy (LIBS) and provides complimentary and additional elemental and perhaps even molecular information that is not available in from just the LA-ICP-MS. Results using this combination of LA-ICP-MS and LIBS will be presented for a variety of food and agricultural products including data for important elements such as C, N, F and P as well as metals detected by either LIBS, LA-ICP-MS or both. Since LIBS records spectral information from every laser pulse, it is quick and easy to produce both 2 and 3 dimensional elemental maps. A number of methodologies are already in place to process this spectral information for multi-variate comparison or discrimination as well as for classification. This presentation will summarize advantages of tandem LA-ICP-MS and LIBS for food and agricultural samples.

(ATOM-OD6.2) Leica THUNDER Imager Cryo CLEM

Presenter: Jen Lee, PhD - Leica Microsystems

Recent revolutionary developments in the field of cryo electron microscopy (EM) revealed cellular mechanisms in subnanometer resolution. Unfortunately, it is very challenging to identify and localize structures of interest in the cryo TEM alone. To facilitate this, cryo workflows have been established integrating cryo light microscopes for sample assessment and target identification. The THUNDER Imager EM Cryo CLEM is a cryo light microscope used to identify and mark fluorescent structures in vitrified cellular samples for easy retrieval in the subsequent cryo EM preparation steps. The THUNDER technology employs the innovative Leica method of Computational Clearing to eliminate the out-of-focus blur that can occur with widefield observation. The result is crisp, haze-free images for more precise identification of cellular target structures.

(ATOM-OD6.3) Next-gen Planetary Exploration Technologies Using LIBS and Related Techniques

Presenter: Pablo Sobron, PhD - Impossible Sensing

Non-Presenting Author: Evan Eshelman

Non-Presenting Author: Kirby Simon

Next-gen technologies for planetary exploration using spectroscopic approaches will boast integrated drilling/coring/caching, imaging, and laser spectroscopic mapping systems. Such architectures deliver three game-changing advantages in lander/rover based planetary exploration: a) unprecedented

analytical capabilities – in-situ, coregistered high-resolution imaging and LIBS, Raman, and fluorescence, mapping of cores and excavated subsurface material, b) minimization of the resources and complexity required to perform subsurface science analyses – no need for core processing and delivery systems and robotic arm movement between the rock and an instrument onboard of the rover, and c) possibility for novel mission architectures – coring + analysis + caching capabilities are offered within single, highly modular arm-mounted instruments. These advantages cannot be overestimated: planetary subsurface environments are key scientific targets but remain technologically challenging – e.g. NASA's MSL and ESA's ExoMars require complex sample acquisition, processing, and handling systems prior to analysis. Integrated sampling + analysis tools change paradigms in space exploration: they bring an instrument to the subsurface, as opposed to bringing subsurface samples to an instrument. Our team at Impossible Sensing has designed, developed, and integrated key enabling subsystems for these applications; critically evaluated their performance; and demonstrated the feasibility of our novel system architecture and detection approach. We developed a breadboard of a lander-mounted instrument suitable for remote in-situ science investigations; demonstrated the ability of the breadboard to perform novel, LIBS, Raman, and fluorescence astrobiological analysis in landed spacecraft; and generated science-driven requirements for advanced versions of our instrument; and commenced a systematic LIBS+Raman+fluorescence survey of planetary analog materials. At the conference we will review analyses demonstrating [1] instrument sensitivity to the presence of trace organic compounds through the spectral signatures of: CH, CN, CO, OH molecules (Raman); C and H atomic emissions (LIBS); b-carotene and chlorophyll in multiple spots; other bio-related materials included degraded carbon (kerogen), scytonemin, alanine, and other amino acids, and [2] mapping/imaging capabilities, including semi-quantitative maps of elemental and molecular abundances from Raman, LIBS, and fluorescence measurements. From spectral calculations, we estimate that the lowest organic abundance detected was ~100 ppb.

(ATOM-OD7) Nuclear and Semi-conductor Applications

(ATOM-OD7.1) Absorption Spectroscopy of U and UO at Elevated Temperatures in Reactive Environments

Presenter: Nick Glumac, PhD - University of Illinois urbana champaign (USA)

Non-Presenting Author: Emily Weerakkody

Emission spectroscopy is widely used as a LIBS diagnostic for uranium species under simulated fireball conditions. Here we apply broadband absorption spectroscopy to investigate ground states of U, U+, and UO in a time-resolved fashion in LIBS plumes. These measurements allow quantification of ground state concentrations of each species and are used to test and validate high temperature uranium oxidation models. A broadband pulsed xenon source provides a short pulse of light which is delayed with respect to the laser pulse. A high dispersion spectrograph records a series of individual U and U+ lines from which both ground state number density of the neutral and ion species and temperature can be obtained. Though the resolution is coarse (~9 us), temporal decay of concentration and temperature in the plume over the period of 10 – 100 us can be well quantified. These profiles are obtained as a function of oxygen content in the ambient atmosphere over the 0 to 20% range. The measurements provide a sensitive test of the oxidation rates of U and U+ under elevated temperatures, and the data can be compared to models employing explicit kinetics. In addition to the atomic species, UO is observed in absorption as well, and its absorption depth is quantified as a function of time, though estimation of number density is more difficult due lack of knowledge of energy level and spectroscopic parameters for the transitions of UO;however, the high resolution spectra obtained here may be helpful in testing evolving models.

(ATOM-OD7.2) Ultra-low level Determination of Difficult Elements in Solvents including Phosphorus, Sulfur, Silicon, and Uranium-236 by ICP-QQQ with MS/MS mode

Presenter: Bert Woods, Dr - Agilent Technologies

The semiconductor world is increasingly trying to drive device size smaller and with that comes the need for lower and lower detection limits in process chemicals. Solvents like Ethyl Lactate , PGMEA, PGME, and Isopropanol play a key role in the semiconductor manufacture process and thus the need for lower detection limits in these materials. Traditionally single quad ICPMS has struggled with doing low levels of Sulfur, Silicon and Phosphorus in these materials due to low ionization potentials and carbon based interferences. The ICP-QQQ with MS/MS technology alleviates these hurdles by removing these interferences and showing superior detection limits for these elements in these solvents.

(ATOM-OD7.3) What Levels of Detection are Needed Today in Semiconductor Industry?

Presenter: Ewa Pruszkowski, PhD - Perkin Elmer

For many decades, the semiconductor industry has been designing new devices that are smaller, faster and consume less power than their predecessors. To maintain this trend, the critical features of these devices must become smaller and have fewer defects. Smaller diameters of chip's features require that all liquid chemicals and solid materials used in semi processes should have less and less contaminants. Inductively coupled plasma mass spectrometry (ICP-MS) traditionally has been an indispensable

analytical tool for quality control because of its ability to rapidly determine high number of analytes at the ultra-trace (ng/L or lower) levels in various process materials and chemicals. Traditionally, analyses are performed using conventional, hot plasma conditions in the Standard and DRC (Dynamic Reaction Cell) modes. However, a few elements with a low ionization potential measured in cold plasma mode yield lower background due to more efficient removal of spectral interferences. A multi-quad quad technology and the Triple Cone Interface incorporated in the NexION 5000 are also components instrumental in further lowering BECs (Background equivalent Concentrations) and improving DLs (Detection Limits). In this presentation the hardware innovations will be discussed and their influence on DLs and BECs needed today by semi industry will be shown. Examples of analysis of various chemicals would be demonstrated.

(ATOM-OD8) LIBS Frontiers and Fundamentals

(ATOM-OD8.1) Laser Ablation Inductively Coupled Plasma Mass Spectrometry Enhanced by Metallic Nanoparticle (NELA-ICPMS)

Presenter: Annarosa Mangone, PhD - University of Bari

Non-Presenting Author: Lorena C. Giannossa, PhD - University of Bari

Non-Presenting Author: Vincent Gardette - University of Bari

Non-Presenting Author: Marcella Dell'Aglio, PhD - CNR-NANOTEC

Non-Presenting Author: Alessandro De Giacomo, PhD - University of Bari

A methodology to enhance the sensitivity of LA-ICPMS up to 1 order of magnitude with respect to the conventional LA-ICPMS, without any changes of the experimental set up (i.e. laser parameters or gas carrier composition), is proposed. It is based on the surface plasmon resonance phenomenon as a result of metallic nanoparticles (NPs) deposited on the surface of the sample. Different kinds of metallic NPs (AuNPs, AgNPs, PtNPs) and substrates (metallic and dielectric -Cu, Cu-based alloys, Ti, glass, Si-) were tested. The results show that enhancement depends on both dropped nanoparticles (kind, concentration and size) and sample tested (investigated element and matrix). Metallic elements show enhancement in both conductive and dielectric matrices, although the better results are obtained on conductive matrix. Different elements show different enhancement in the same matrix, as well as the same element shows different enhancement in different matrices. Differences in morphology and depth of the craters produced by the laser pulse in the presence and in the absence of NPs, as well as the different size and composition of laser-generated particles allow to attribute to a different laser-substrate interaction the observed enhancement. In particular, NPs induce locally more efficient ablation below the ablation threshold, that leads to the formation of smaller laser-generated particles, consisting of target material aggregated around NPs, that exhibit better transport/vaporization efficiency, thus enhancing signals for metallic samples. NPs do not contaminate the sample irreversibly because, after a very limited number of laser shots, they are completely removed from the sample surface. The method developed allows to obtain the same intensity signal as traditional LA-ICPMS by strongly reducing the number of laser pulses on samples, making the technique more suitable for analyses in which negligible destructivity and/or determination of surface-distribution patterns of very thin layers without underlying contamination are demanded. Moreover, it can be particularly useful to cut down isobaric 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. The undoubted strength of this approach is represented by its simplicity, affordability and fast performance.

(ATOM-OD8.2) Measuring Dynamics of Laser-Ablated Mass via Absorption Spectroscopy under Different Cover Gases

Presenter: Jonathan A. Merten, PhD - Arkansas State University

Non-Presenting Author: Erin Nicholas

Non-Presenting Author: Anna Anders

Non-Presenting Author: Aaron Hopson

Non-Presenting Author: Jackie Brees

A relatively novel technique, pseudocontinuum source atomic absorption spectroscopy (psCS-AAS) is used to absolutely quantify the mass of atomized titanium in a cooling laser-ablation plasma. Knowledge of the absorption oscillator strength and maps of the line-of-sight absorption of a pulsed OPO probe, are combined to measure the mass contained in the ground and metastable terms at times <10 microseconds post-ablation. The distribution of mass across these terms provides insight into the dynamics of the atomic state distribution function at times later than the typical LIBS emission study. This measurement of ablated mass is distinct from crater measurements in that it is dynamic and distinguishes between atomized and spalled/splashed material. Mass measurements under helium and neon are presented. Limitations of the technique (including linear dynamic range) are discussed in the context of the Beer-Lambert law and absorption kinetics. Titanium's rich spectrum provides exceptional experimental flexibility and is used to verify linear dynamic range with different experimental configurations.

(ATOM-OD8.3) Detection of Gold in Ore Samples by LIBS in the Sub ppm Range

Presenter: Mohamad Sabsabi, PhD - National Research Council Canada

Non-Presenting Author: Paul Bouchard

Non-Presenting Author: Aissa Harhira

Non-Presenting Author: Josette El Haddad

Non-Presenting Author: Alain Blouin

The Laser-Induced Breakdown Spectroscopy (LIBS) technique is a form of atomic emission spectroscopy of a plasma plume induced by laser on the material to be analyzed. This gives LIBS an advantage of interrogating samples at-a-distance and analyzing the material without contact, independently from the nature of the sample, thus making it suitable for in-the-field and real-time analysis of any type of materials, whether in the solid, liquid, slurry or gas phase. LIBS has advanced over the last 50 years to become a successful emerging technology for numerous chemical analysis applications. The advent of new compact components (laser, spectrometer, and detector) makes the technology more accessible in terms of robustness, low cost, analytical performances to deliver its benefits for real time analysis. The miniaturization of LIBS equipment has opened new opportunities to perform real time measurements and responds to emerging needs under conditions in which conventional techniques cannot be applied, in particular for the detection of carbon, precious metals and low Z elements. In this talk, we will present some analysis for the reproducibility of the LIBS measurements versus non-homogeneity of the sample and its optimization for analytical aspects. We will present a critical analysis of the LIBS sampling in order to improve its uses for real time measurements of gold and precious metals in ore samples. We will explain how it is possible to detect gold in the few tens of ppb and discuss these aspects in terms of robustness, analytical performance and comparison to conventional techniques.

(BIM-OD1) Topics in Bioanalysis I

(BIM-OD1.1) Non-invasive Determination of Depth of Inclusion in Ex vivo Tissues using Deep Raman Spectroscopy

Presenter: Sara Mosca, PhD - RAL,CLF,STFC

Non-Presenting Author: Priyanka Dey, PhD, MRSC - University of Exeter

Non-Presenting Author: Francesca Palombo, PhD - University of Exeter

Non-Presenting Author: Nick Stone, PGDip, MSc(Dist), MSc(Dist), MBA, PhD, CSci, FSAS, FIPEM, FRSC - University of Exeter

Corresponding Author: Pavel Matousek, Professor - Science and Technology Facilities Council

In a clinical context it is beneficial to identify both the chemical information and the depth of a buried object in biological tissues. For example, the in vivo identification and localisation of a cancer lesion located deep inside biological tissues could potentially facilitate more accurate spectroscopic diagnosis or improve the effectiveness of subsequent treatments. Here we demonstrate the use of spatially offset (SORS) and transmission Raman (TRS) spectroscopy for non-invasive depth prediction of an inclusion, made up of surface-enhanced Raman scattering (SERS) labelled nanoparticles (NPs), buried inside ex-vivo porcine tissues. The concept exploits the differential attenuation of two Raman bands of the inclusion due to their different absorption by surrounding tissue matrix to retrieve depth information. The relative degree of the Raman band intensity changes are directly related to the path-length of Raman photons travelling through the medium thereby encoding also the information on the depth of the object within the tissue. The calibration model for depth prediction is created using data only from external measurements carried out in SORS and TRS configurations. Monte Carlo simulations of the photon propagation in the two different geometries confirm the relationship between the spatial offset and the phonon path length inside the tissues. These approach was tested and evaluated for predicting the depth of the SERS NPs, within an up to 40 mm slab of ex-vivo porcine tissue yielding an average root mean square error of prediction of total depth of 6.7 % for TRS and 11 % for SORS. Our results pave the way for future non-invasive deep Raman spectroscopy in vivo enabling to localize cancer biomarkers for early disease diagnosis and targeted treatments.

(BIM-OD1.2) Optical Guidance in Neurosurgery

Presenter: Darine Abi Haidar, HDR - Paris Diderot University

Non-Presenting Author: Hussein Mehidine

Non-Presenting Author: Bertrand Devaux

Standard of care in the management of brain tumors primarily consists of achieving maximal safe resection, while preserving eloquent brain regions. To date, this has been accomplished by neuronavigation guided by anatomical and even functional imaging. However, such imaging modalities have only been able to assist the surgeon in accurately highlight the bulk tumor and possibly identifying solid tumors that have infiltrated into adjacent areas within the surgical field. Diffusive tumors however, are still elusive to the surgeon and are the main cause of recurrence. The diagnostic gold standard to discriminate between normal and diseased tissue of with sparse tumor cells is histopathological analysis of a biopsied sample, a time-consuming technique prone to miss-sampling errors with no possibility to

provide real-time diagnosis. Therefore, replacing conventional pathological examination with a slide-free and label free technique capable of providing accurate intraoperative diagnosis in real-time will positively impact patient outcomes. We have addressed this critical need by developing a non-invasive multimodal nonlinear endomicroscope that allows real-time optical biopsy. It will provide immediate information for diagnostic use without removal of tissue and will assist the surgeon in forming the optimal strategy for resection. This instrument will combine several means of contrast. Parallel to the instrumentation development, we are currently improving our understanding of the various optical features measured by multimodal optical imaging pertaining to different biomolecules. This endeavor will allow us to create a database on the optical signatures of the diseased and control brain tissues.

(BIM-OD1.3) Detection of blood stream parasites using portable ATR-FTIR spectroscopy

Presenter: Bayden Wood, PhD, PhD BSc. (Hons) FRACI CChem FRSC - Monash University
Non-Presenting Author: Phil Heraud, PhD - Monash University
Non-Presenting Author: Kamila Kochan, PhD - Monash University
Non-Presenting Author: David Perez-Guaita, PhD - Technical University Dublin
Non-Presenting Author: Supti Roy, PhD - Monash University
Non-Presenting Author: Miguela Martin, BSc(Hons) - Monash University
Non-Presenting Author: Anja Anja Ruether, PhD - Monash University
Non-Presenting Author: Rebekah Duffin, PhD - Monash University
Non-Presenting Author: Philip Andrews, PhD - Monash University

There are over 300 parasitic worms and 70 species of protozoan that infect human. Parasites generally trigger varying degrees of changes within their hosts including inflammation, tissue damage and cellular degradation. According to the World Health Organization (WHO), the world's leading topical diseases are namely; malaria, schistosomiasis, filariasis, African trypanosomiasis, and leishmaniasis are caused by blood-borne parasites and are responsible for substantial morbidity and mortality worldwide. The most notable and deadliest parasites are Plasmodium spp., responsible for malaria infections putting a child to death every two minutes. The unique chemistry of parasites makes them ideal targets for spectroscopic diagnostics. Vibrational imaging techniques can play an important role in characterizing the chemistry of the pathogen and its effect on the human host. Here we show the application of vibrational and Raman imaging to characterize the chemical composition of four deadly parasites including: 1) Plasmodium falciparum in red blood cells using AFM-IR imaging, 2) the liver fluke (Opishorchis viverrini), which causes Cholangiocarcinoma, a bile duct cancer that originates in the bile duct epithelium using focal plane array imaging, 3) Babesia bovis parasites, which present a serious and significant health concern for the beef and dairy industry, using Raman and AFM-IR imaging and, 4) Leishmaniasis, caused by protozoan parasite Leishmania, which is endemic in 98 countries and territories. The high-quality images reveal distinct marker bands associated with the parasite that can be used as biomarkers for diagnosis and monitoring disease progression.

(BIM-OD2) Engineering Materials for Plasmonics and Plasmon-Driven Processes

(BIM-OD2.1) Uniting Top-down and Bottom-up Strategies for Tailoring Plasmonic Nanostructures

Presenter: Jennifer S. Shumaker-Parry, PhD - University of Utah

Non-Presenting Author: Cady Lancaster

Non-Presenting Author: Wallis Scholl

Non-Presenting Author: Matthew Ticknor

Top-down fabrication and bottom-up synthesis of metal nanoparticles provide different benefits for producing plasmonic substrates for surface-enhanced spectroscopy applications. As one example, gold nanostars synthesized in solution produce some of the strongest signal-enhancements in plasmonics due to the large number of sharp features per particle. However, the tendency for the high-aspect ratio features to undergo structural changes and the prevalence of particle aggregation in different solution conditions present challenges for research as well as applications of these particles. As an alternative to solution-based synthesis, top-down fabrication methods have been used to produce plasmonic structures with sharp features with greater stability in different environments and elimination of aggregation issues. These advantages tend to be at a cost of decreased sensitivity as compared to particles like nanostars. We have developed the synthesis of gold nanomites on gold nanotriangles by combining top-down nanosphere lithography and a one-pot solution-based synthesis. We studied the nucleation and growth of the nanomite features using scanning electron microscopy and UV-Vis absorption spectroscopy. The effects of temperature, reaction time, pH, and reagent concentration provide a basis for adjusting growth conditions to achieve a high density of nanomites on the nanotriangles. Fabricated gold nanostructures with different shapes and sizes could be hosts for the nanomites making this hybrid bottom-up/top-down approach a highly versatile route to tunable plasmonic substrates with a high density of hot spot regions for plasmon-enhanced spectroscopy applications.

(BIM-OD2.2) Plasmon-Mediated Chemistry of Plasmonic/Poorly Plasmonic Hybrid Nanoparticles

Presenter: Michelle Personick, PhD - Wesleyan University

Plasmon-assisted reactions can be used to achieve activity and selectivity that is not possible using classic thermal reactions in both catalysis and materials synthesis. We report the use of a plasmon-mediated approach to overcome key challenges in the synthesis of bimetallic nanomaterials, including the acceleration of kinetically slow metal ion reduction and the prevention of undesired galvanic exchange processes. Excitation of the plasmon resonance of silver nanoparticle cores with visible light illumination drives the plasmon-assisted oxidation of a weak reducing agent, citrate, which in turn generates electrons that reduce platinum ions onto the silver cores. With this approach, we are able to synthesize silver core-platinum satellite architectures with platinum localized at the tips of bipyramids or triangular nanoprisms, as well as core-shell structures with tunable platinum coverage. Notably, the core-satellite architecture is not currently accessible using thermal synthetic approaches. These core-satellite materials are particularly promising for applications in plasmon-assisted catalysis because they localize a catalytically active metal, platinum, at the tips of a plasmonically active silver nanostructure. This work represents the first use of the plasmon-assisted oxidation of citrate to reduce metals other than

silver, and this approach has the potential to be extended to other catalytically active but poorly plasmonic secondary metals as well as other plasmonically active core materials.

(BIM-OD2.3) Plasmonic Hot Electron Dynamics in the Steady State

Presenter: Matthew Sheldon, PhD - Texas A&M University

There is significant interest in photochemical and optical energy conversion processes that occur under low intensity CW illumination. This is especially true in energy science where a variety of chemical reactions are envisioned to be powered by sunlight, e.g. solar fuel production, water splitting, or CO_2 reduction. The dynamics of electrons in this regime of low intensity CW excitation is poorly understood because of a lack of suitable measurement strategies, in comparison with more established ultrafast, high intensity, pump-probe, time-resolved spectroscopies. Our laboratory has recently developed a spectroscopic technique that provides an unprecedented picture of non-equilibrium electronic processes that occur during relatively low power CW optical excitation. Our strategy takes advantage of an anti-Stokes Raman signal that is due to a direct interaction between photons and electrons. Unlike more familiar Raman signals which result from the coherent scattering of photons by specific vibrational modes, this electronic Raman signal gives rise to a broadband anti-Stokes spectrum that is characteristic of the entire energetic distribution of electrons during steady state illumination. To date, our experiments have measured the electronic behavior of plasmonic nanostructures under different environmental conditions and with differing chemical adsorbates. We have established that it is possible to quantitatively determine the characteristic non-equilibrium temperature of a sub-population of photo-excited electrons that have yet to thermalize with the environment. The technique also measures the size of this sub-population, and therefore the lifetime of electrons in this sub-population, as well as the magnitude of electron coupling to different relaxation pathways through coupling to molecules. Simultaneously, the technique provides quantitative measure of the phononic (or vibrational) temperature during photo-thermalization. When direct comparisons to established TA results can be made, for example by determining the electron-phonon coupling constant of the metal, there is excellent agreement. However, because these spectral features are characteristic of the steady state behavior of the sample, many of the insights are quite different from what is provided by ultrafast TA decay signals, informing the nanoscale design of metal nanostructures for use in solar photochemistry.

(BIM-OD3) Chemical Imaging

(BIM-OD3.1) Gathering a boatload of information in multi-dimensional imaging with plasmonic gold nanoparticles

Presenter: Ning Fang, PhD - Georgia State University

Non-Presenting Author: Xiaodong Cheng

Non-Presenting Author: Kuangcai Chen

Non-Presenting Author: Bin Dong

We have developed single particle orientation and rotational tracking (SPORT) for visualizing rotational dynamics of anisotropic plasmonic gold nanorods in live cells. The current effort aims to "reinvent" multi-dimensional optical imaging with gold nanorods to approach dynamic cellular processes with unprecedented details. Here, "dimension" can refer to different types of signals, colors, polarizations, and spatial dimensions including translation and rotation. Biological processes usually involve multiple species and their chemical and physical responses to each other; it requires a multi-dimensional observation of the same sample simultaneously to completely understand the process. We have integrated the new techniques that require point spread function (PSF) engineering into one system/device. Multi-dimensional imaging for rotational tracking enables reliable 3D rotational tracking without angular degeneracy to enable the visualization of left- or right-handed twisting motions in live cells. Furthermore, the deep neural networks (DNNs) based methods are being developed to offer excellent resistance to interfering background and noises. The applications of multi-dimensional imaging are demonstrated through the studies of dynamic processes involved in nanoparticle-based drug delivery. The biological processes include how relevant nanomachines assemble near the cargo, enclose the cargo into a pit, remove it from the cell membrane in a complete clathrin-mediated endocytosis, and transport the cargo inside the cell.

(BIM-OD3.2) Ultrafast Raman spectroscopic probes of plasmon-molecule energy transfer

Presenter: Renee Frontiera, PhD - University of Minnesota

Plasmonic materials show great promise as highly selective photocatalysts, however their efficiency is currently limited by a lack of understanding of the relevant mechanisms of light to chemical energy conversion. Here we describe our use of ultrafast SERS to probe contributions of hot electron transfer, heating, and vibrational energy transfer on timescales relevant to photocatalysis. Ultrafast SERS is a pump-probe spectroscopic technique designed to monitor transient changes in adsorbate molecules following plasmon excitation. In order to understand processes contributing to plasmon-driven chemical conversion, we probe a variety of analytes, looking for effects of carrier transfer, as well as vibrational and electronic energy transfer. Specifically, we probe plasmon to molecule carrier transfer on the picosecond timescale by quantitating the growth of radical molecular species. We probe the effects of temperature by monitoring both Stokes and anti-Stokes Raman intensities to determine the amount of vibrational kinetic energy in molecules, finding that heating is not a major contributor to plasmonic photocatalysis. Finally, we track vibrational energy transfer, finding evidence for long-lived coherent effects, with potential applications for coherent control of photo-driven reactions.

(BIM-OD3.3) Nonlinear optical imaging with mid-infrared light

Presenter: Eric O. Potma, PhD - University of California, Irvine
Non-Presenting Author: Dave Knez
Non-Presenting Author: Adam Hanninen
Non-Presenting Author: Richard Prince
Non-Presenting Author: Dmitry Fishman

Imaging with spectroscopic contrast based on molecular vibrations is one of the most informative forms of chemical mapping. The strongest light-matter interaction that facilitates the probing of molecular vibrations is the absorption of an IR-photon by the molecule, which leads to the resonant excitation of a given vibrational mode. This principle is used in IR-based microscopy, producing images with genuine spectroscopic contrast, allowing label free imaging of a wide range of biomedically relevant samples. However, IR microscopy suffers from several technical hurdles, among which the low spatial resolution and the unavailability of affordable IR cameras. In recent years, some of these hurdles have been overcome with the help of nonlinear optical interactions. In this presentation, we will show that nonlinear optics with mid-IR light enables chemical selective imaging with high 3D resolution. In addition, we will present mid-IR images collected with a regular Si-based camera, made possible by the nonlinear optical response of the camera chip itself.

(BIM-OD4) Topics in Bioanalysis II

(BIM-OD4.1) Shape, Decoration, and Plasmonic Properties of Magnesium Nanoparticles

Presenter: Emilie Ringe, PhD - University of Cambridge

Non-Presenting Author: Jeremie Asselin

Non-Presenting Author: Christina Boukouvala

Non-Presenting Author: Elizabeth Hopper

Non-Presenting Author: John S. Biggins

Non-Presenting Author: Quentin Ramasse

Localized surface plasmon resonances have attracted much attention due to their ability to enhance light-matter interactions and manipulate light at the sub-wavelength level. 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, is one of the most abundant elements in earth's crust, and is fully biocompatible, making it an attractive framework for plasmonics. This talk first discusses the hexagonal, folded, and kite-like shapes we modelled using Crystal Creator, our Wulff-based shape generation code for twinned nanoparticles. Nanoparticles found in colloidal syntheses are then presented and match well with predictions. Then, the optical response of Mg nanoparticles is overviewed, highlighting Mg's ability to sustain localized surface plasmon resonances across the ultraviolet, visible, and near-infrared electromagnetic ranges. The various resonant modes of hexagons, leading to the highly localized electric field characteristic of plasmonic behavior, are presented numerically and experimentally. The evolution of these modes and associated field from hexagons to the lower symmetry folded structures is then probed, again by matching simulations, optical, and electron spectroscopy data. Lastly, results demonstrating the opportunities and challenges related to the high chemical reactivity of Mg are discussed, including surface oxide formation and galvanic replacement as a synthetic tool for bimetallics.

(BIM-OD4.2) Multi-Contrast Spectroscopy for Tumor Diagnosis and Therapy

Presenter: Michael Schmitt, PhD - Friedrich-Schiller University Jena, Germany

Non-Presenting Author: Jürgen Popp, PhD - 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

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The recent progress in the development of high intensive ultrashort laser sources has also revolutionized microscopy by utilizing non-linear optical phenomena to create a higher microscopic contrast. It emerged to be very advantageous to combine several non-linear spectroscopic contrast mechanisms in a multimodal approach. In this contribution it will be shown that multo-contrast nonlinear imaging, using different spectroscopic methods such as coherent Raman scattering (CARS, SRS), two-photon excited

autofluorescence (TPEF), multi-photon excited fluorescence lifetime imaging (MPE-FLIM) and second harmonic generation (SHG), represents a powerful tool for the label-free characterization of the molecular composition of biological tissue. Among others we highlight the potential of a combined CARS/SHG/TPEF approach to reliably assess tumor tissue and the success of an operation directly in the operating theatre. For a clinical application, this approach was transferred into a compact and portable microscope. In order to further extend the applicability of this multimodal microscopy approach for in vivo tissue screening, different endospectroscopic probe concepts are also presented. Furthermore, it will be shown that the presented CARS/SHG/TPEF multimodal imaging approach can be combined with laser tissue ablation for tissue specific laser surgery. The specific detection of malignant tissue during curative surgery is the most important precondition for complete tumor removal. Finally, we will highlight the potential of non-linear multimodal imaging to visualize cold atmospheric plasma (CAP)-induced changes in tissue for reaching a new quality level of CAP application in medicine via online monitoring of wound or cancer treatment. Acknowledgment: Financial support of the EU, the ”Thüringer Ministerium für Wirtschaft, Wissenschaft und Digitale Gesellschaft”, the ”Thüringer Aufbaubank”, the Federal Ministry of Education and Research, Germany (BMBF), the German Science Foundation, the Fonds der Chemischen Industrie and the Carl-Zeiss Foundation are greatly acknowledged.

(BIMOD-4.3) Detection of a MicroRNA Cancer Biomarker Using a Fluorescent Smart Probe

Presenter: Sulayman A. Oladepo, BSc, MS, PhD - King Fahd University of Petroleum & Minerals

Non-Presenting Author: Abdulmalik Aminu - King Fahd University of Petroleum and Minerals

A fluorescent hairpin smart probe has been designed for the sequence-specific detection of miRNA-21, a cancer biomarker. The smart probe is a nucleic acid probe with a stem-loop structure. The loop is perfectly complementary to the target sequence of miR-21 while the stem consists of self-complementary strands. One end is terminated with a fluorescent dye and multiple guanine moieties are placed on the other end to serve as quenchers. In the absence of the target sequence, the fluorophore and the guanine quenchers are in close proximity and fluorescence is thereby quenched. However, in the presence of the target sequence, the smart probe spontaneously hybridizes with the target while at the same time undergoing a conformational change that forces the fluorophore and the guanine quenchers apart. The hybridization and attendant conformational change lead to high fluorescence signal. This fluorescence signal was correlated with miR-21 concentration and it is also specific for the target sequence. Therefore, the smart probe is able to discriminate between the target of interest and mismatch sequences. Using this smart probe, we obtained limit of detection (LOD), limit of quantitation (LOQ) and sensitivity values that are consistent with previous data on nucleic acids hairpin probes.

(CHEM-OD1) Advances in Calibration

(CHEM-OD1.1) Automatic Selection of Models Updated to New Sample and Measurement Conditions Using Unlabeled Samples

Presenter: John Kalivas, PhD - Idaho State University

Non-Presenting Author: Robert C. Spiers - Idaho State University

Multivariate calibration is a mechanism for modeling a mathematical relationship between sample spectra and the corresponding analyte concentration profile allowing for inexpensive real time analysis. A particularly important issue in chemometrics is calibration model maintenance where model updating is required to adapt the model from the original (primary) sample and measurement conditions (sample and measurement matrix effects or covariance shifts, etc.) spanned by the primary calibration samples to new (secondary) conditions. Otherwise, a complete secondary condition-based recalibration is needed if the secondary conditions are too different from the primary conditions. A constraint limiting model updating is being able to update to the secondary conditions in an unsupervised setting (using secondary samples without analyte reference values (unlabeled samples)). Another significant issue is selecting a final prediction model relative to the multiple tuning parameters typically involved with model updating. Specifically, existing methods of model updating and selection require analyte values from the secondary sample set. Presented are semi-supervised model updating schemes that only use unlabeled secondary samples to update with. Models are selected to predict the same secondary samples used to update with (transductive updating). Model selection is based on a consensus approach of prioritizing model diversity while simultaneously conserving prediction similarity. Presented are updating methods and the selection process showing that updated models are formed and selected with consistent low prediction errors across four diverse spectral datasets.

(CHEM-OD1.2) Self-optimizing Support Vector Elastic Nets

Presenter: Zewei Chen, MS - Clippinger Laboratories, Department of Chemistry and Biochemistry, Ohio University

Corresponding Author: Peter B. Harrington, PhD - Ohio University

Chemometrics is widely used for quantitative and qualitative problems in analytical chemistry. An automated chemometrics method facilitates scientists to exploit the advantages of chemometrics. Supervised models that minimize bias (i.e., error in the estimates) tend to overfit the data when the number of objects or spectra is less than the number of uncorrelated measurements or variables. Regularization is a strategy to overcome this problem. The elastic net (EN) that uses L1 and L2 regularizations can minimize data overfitting and select important variables simultaneously. By using the support vectors, a faster algorithm of EN is achieved and referred to as the support vector elastic net (SVEN). To maximize the performance of SVEN, the constants for the L1 and L2 regularizations should be optimized. A novel support vector elastic net that automatically determines the two key regularization constants, i.e., λ for L2 regularization and t for L1 regularization, was developed and referred to as the self-optimizing support vector elastic net (SOSVEN). Response surface modeling (RSM) and bootstrapped Latin partitions (BLPs) are incorporated into SOSVEN to optimize the two regularization parameters. Responses to the set of design points over the ranges of the two factors are evaluated with an internal BLP evaluation using the calibration data. A 2-dimensional interpolation with a cubic spline fits the response surface to determine the best condition that gives the best-estimated response. The

developed SOSVEN compared favorably with two parameter-free chemometrics methods, super partial least squares regression (sPLSR) and super support vector regression (sSVR) for calibration of cannabidiol (CBD) concentrations in hemp oil samples using near¬–infrared spectroscopy. The SOSVEN and sSVR had similar root mean squared errors of validation, i.e., 6.6 and 6.7 mg/mL, respectively and performed significantly better than 7.0 mg/mL given by sPLSR. Other than the robust performance, the SOSVEN also has the desirable advantage of yielding simpler models by removal of irrelevant variables.

(CHEM-OD1.3) Good Practice in Class Modelling

Presenter: Raffaele Vitale, PhD - Université de Lille

In the last decades, so-called multivariate modelling approaches for classification (also known as one-class classifiers) have been extensively resorted to, in the domain of analytical chemistry and, more specifically, in that of chemometrics, for addressing a large number of issues mainly related to foodstuff origin authentication, quality control and process monitoring. Contrarily to the more popular discriminant methods, which directly focus on the dissimilarity among samples belonging to different categories, the basic principle of modelling techniques is that classification rules are derived from data (e.g., spectra, chromatograms, etc.) collected on specimens of individual target classes. Consequently, the objects under investigation are not necessarily assigned to one and only one of these classes (as in a discriminant scenario), but can also be accepted by multiple or none category models: decision making becomes, therefore, particularly flexible, which renders such techniques well-suited to tackle complex or asymmetric classification problems, typical in the aforementioned contexts of study. Still, in spite of the fundamental difference between discrimination and modelling, practitioners tend to overlook and underestimate the advantages and benefits the latter may provide and abuse the former in situations where its application would not be recommended (for instance, when categories are highly unbalanced, extremely variable, ill-defined or underlain by several distinct subgroups of samples). Given these very common misunderstandings, the present communication is conceived as a broad tutorial of modelling classification in applied sciences and aims at shedding light on the three principal aspects of its good practice: when, why and how to employ it. These points will be covered throughout a comprehensive overview of probably the best-established and most widely exploited chemometric modelling strategy: Soft Independent Modelling of Class Analogy (SIMCA). Along with a detailed illustration of the SIMCA algorithmic scheme, the ideal conditions for the utilisation of one-class classifiers as well as the implications resulting from it will be outlined in detail. Moreover, an interactive demonstration of a novel graphical interface implemented for guiding potential users across the steps of SIMCA model calibration, optimisation and validation will be given together with real-world pragmatic examples from the fields of chemistry, biology and biomedicine.

(CHEM-OD2) Chemometrics Opportunities in Food Security

(CHEM-OD2.2) Application of Chemometric Methods to Food Authentication Problems

Presenter: Mengliang Zhang, PhD - Middle Tennessee State University

Non-Presenting Author: Jianghao Sun - Food Composition and Methods Development Laboratory, BHNRC, ARS, USDA

Non-Presenting Author: Ping Geng - Food Composition and Methods Development Laboratory, BHNRC, ARS, USDA

Non-Presenting Author: Pei Chen - Food Composition and Methods Development Laboratory, BHNRC, ARS, USDA

Spectral fingerprinting combined with chemometric data analysis can be used to profile chemicals and discriminate between food materials. One of the important applications of the combined technique is to test the authenticity of the food and prevent potential counterfeit materials. In this presentation, several food-related examples will be discussed, and the application of different chemometric strategies used in these examples will be introduced. Overall the combination of spectral fingerprinting with chemometric analysis enhanced the ability to distinguish chemical differences between food materials. The results from our study indicate that chemometrics with various instrumental analysis techniques has excellent potential for the application of improving food quality and food security.

(CHEM-OD2.3) Descattering Autoencoder for Fast Mie Scatter Correction of Infrared Microscopy Spectra

Presenter: Eirik Almklov Magnussen, MSc - Norwegian University of Life Sciences

Non-Presenting Author: Johanne Solheim

Non-Presenting Author: Uladzislau Blazhko, MSc - NMBU

Non-Presenting Author: Valeria Tafintseva

Non-Presenting Author: Kristin Tøndel

Non-Presenting Author: Kristian Liland

Non-Presenting Author: Simona Dzurendova

Non-Presenting Author: Volha Shapaval

Non-Presenting Author: Christophe Sandt

Non-Presenting Author: Ferenc Borondics

Non-Presenting Author: Achim Kohler, PhD - Norwegian University of Life Sciences

Mie scattering can severely distort infrared microscopy spectra of cells and tissues. The Mie Extinction Extended Multiplicative Signal Correction (ME-EMSC) algorithm is the state-of-the-art pre-processing technique and has been shown to be able to recover pure absorbance spectra from highly scatter-distorted measured spectra. However, the algorithm is computationally expensive, and the correction of large infrared images may require hours of computations. We suggest a deep convolutional Descattering Autoencoder which is trained on a set of spectra corrected with ME-EMSC and which can perform Mie scatter-correction of FTIR images in a fraction of the time required by the ME-EMSC. The speed gain of

the Descattering Autoencoder could allow for pre-processing images in near real-time and thereby enabling such images to be used by medical professionals in clinics. As an example we consider a dataset of FTIR images of oleaginous filamentous fungi grown at different growth media is considered. The spectra of fungi grown under different conditions showed high variability in the chemical features. Thus, different reference spectra were used to initialize the ME-EMSC correction algorithm to ensure optimal correction. Thereafter, one Descattering Autoencoder was trained on data corrected with the different reference spectra. Upon validation on an independent test set, it was shown that the Descattering Autoencoder learned from the ME-EMSC corrected spectra how to correct scatter-distorted spectra, and implicitly which reference spectrum to use for the correction. We demonstrate that the spectra corrected with the Descattering Autoencoder contain the same chemical signals as the ME-EMSC corrected spectra. In terms of speed, robustness and noise-levels, we show that the Descattering Autoencoder outperforms the ME-EMSC algorithm. In particular, our approach is very useful for correcting hyperspectral images since it can be used to correct spectra with large variability in chemical signals and it, therefore, yields informative hyperspectral images even when we have very different chemical constituents in the image.

(MOLEC-OD1) New Components and Systems for Mid-IR Sensing

(MOLEC-OD1.1) Mid-infrared supercontinuum lasers – a powerful new tool for vibrational spectroscopy

Presenter: Markus Brandstetter, PhD - Research Center for Non Destructive Testing - RECENDT GmbH

Non-Presenting Author: Ivan Zorin - Research Center for Non Destructive Testing - RECENDT GmbH

Non-Presenting Author: Robert Zimmerleiter - Research Center for Non Destructive Testing - RECENDT GmbH

Non-Presenting Author: Paul Gattinger - Research Center for Non Destructive Testing - RECENDT GmbH

Non-Presenting Author: Alexander Ebner - Research Center for Non Destructive Testing - RECENDT GmbH

Supercontinuum lasers (SCL) have been rapidly progressing into the mid-infrared spectral region in recent years. Due to their unique properties they open up new possibilities for laser-based mid-infrared spectroscopy in many areas. SCLs unite high brightness and spatial coherence with extremely broadband spectral coverage in the mid-infrared spectral region up to 16 µm wavelength, achieved by a single device. Thereby, they exceed thermal sources and even challenge quantum cascade lasers, as SCLs also cover the spectral range around 3 µm, where powerful quantum cascade lasers lack broader availability. In this contribution we present an overview of the currently available hardware and its analytical possibilities in mid-infrared spectroscopy. Initial limitations imposed by high intensity noise levels of SCLs have been overcome in the meantime, paving the way for their use in demanding applications. Selected experimental results obtained with commercially available SCLs in different measurement configurations are shown. Among them chemical detection of an explosive precursor at standoff distances and diffraction-limited chemical mapping of polymer films and red blood cells in reflection geometry. Furthermore, we introduce this advanced light source as a replacement for conventional thermal emitters in a Fourier Transform Infrared (FTIR) spectrometer. The analytical performance of the SCL based FTIR spectrometer was tested and compared with its conventional configuration employing a thermal emitter. The obtained results show a four-times-enhanced detection limit due to the extended path length enabled by the high brightness of the laser.

(MOLEC-OD1.2) Towards In-situ Measurements Of The Protein Secondary Structure Based On Mid-IR Lab-on-a-chip Quantum Cascade Technology

Presenter: Borislav Hinkov, PhD - TU Wien

Non-Presenting Author: Florian Pilat - TU Wien

Non-Presenting Author: Laurin Lux - TU Wien

Non-Presenting Author: Benedikt Schwarz - TU Wien

Non-Presenting Author: Hermann Detz - TU Wien

Non-Presenting Author: Aaron M. Andrews - TU Wien

Non-Presenting Author: Bettina Baumgartner - TU Wien

Non-Presenting Author: Bernhard Lendl, Prof. Dr. - Technische Universität Wien

Non-Presenting Author: Gottfried Strasser - TU Wien

The rise of quantum cascade technology led to novel concepts and devices for chemical sensing in the mid-IR spectral region. Especially, the invention of quantum cascade lasers (QCLs) in 1994 [1] sparked a complete new field of research, investigating this new type of optoelectronic devices and their applications. After more than two decades, QCLs and their counterpart, the quantum cascade detector (QCD), can be designed to address any wavelength between $3 - 12$ µm while showing high performance operation. This allows e.g. tailoring suitable single-mode emitting devices addressing the strong fundamental absorption features of most molecules [2]. Nowadays, especially the field of liquid sensing has attracted much attention. This is due to significant QC device improvements, which e.g. allows the measurement of protein secondary structures in aqueous solution, outperforming state-of-the-art bulky FTIR-systems [3]. In addition, novel devices developed in our lab support same-wavelength emitting and detecting active regions, paving the road towards monolithically integrated lab-on-chip sensors [4]. In this work, we present the detection of changes in the secondary structure of Bovine Serum Albumin (BSA), when using such type of monolithic devices. By carefully designing the active region and on-chip connecting QCL and QCD via a plasmonic waveguide, we can realize highly sensitive and versatile lab-on-a-chip devices. Their key features are a very small footprint (~25 mm3), with a small and tailorable, but still highly sensitive, interaction section, suitable for microliter scale liquid detection. We present two types of experiments: a) measuring residual water concentrations in isopropyl alcohol within a homemade microfluidic cell (~60 µl probe volume) or in-situ. And b) the analysis of temperature-induced changes in the secondary structure of BSA. The later experiment is performed in-situ by submerging our chip into the liquid. This shows the robustness and suitability of our devices for in- and on-line measurements, where only small probe volumes are available, contrasting sampling techniques, including their delayed response. [1] Faist et al., Science 264, 553-556, 1994. [2] Szedlak et al., Opt. Engineering 57, 011005, 2018. [3] Schwaighofer et al., Sci. Rep. 6, 33556, 2016. [4] Schwarz et al., Nat. Commun. 5, 4085, 2014.

(MOLEC-OD1.3) Broadband Integrated Waveguide Sensor for trace analysis in the fingerprint Mid-IR range

Presenter: Nuria Teigell Beneitez, Dr. - Photonics Research Group, INTEC, Ghent University-imec

Non-Presenting Author: Bettina Baumgartner - TU Wien

Non-Presenting Author: Jeroen Missinne - CMST, ELIS, Ghent University-imec

Non-Presenting Author: Bernhard Lendl, Prof. Dr. - Technische Universität Wien

Non-Presenting Author: Gunther Roelkens, Prof. - Photonics Research Group, INTEC, Ghent University-imec

Advances in mid-IR sources, detectors and integrated photonic circuits (PIC's) allow the design and fabrication of novel, highly integrated sensor systems. In this contribution we present a sensing platform for mid-IR laser spectrometers based on novel Germanium-on-Silicon waveguides incorporating dedicated optics for coupling the laser beam in and out of the PIC. Opposed to classical ATR systems, the light interaction in the PIC takes place over the whole length of the waveguide in contact with the sample, leading to higher effective path lengths and thus to potentially higher sensitivities. Furthermore, to enrich the analyte in the evanescent region, the Germanium-on-Silicon waveguides were coated with a well defined and chemically modified mesoporous silica. By means of micro-lenses etched on the Si

substrate and broadband grating couplers fabricated on the PIC, the developed sensor chip can easily be interfaced to a collimated broadband laser beam and a mid-IR detector. A dedicated micro-flow cell was used as to provide passive mechanical alignment to the chip with respect to the optical ports of the laser spectrometer and to deliver the samples to the sensing region. The mesoporous coating covering the Germanium-on-Silicon waveguides serves two purposes. First, it reversibly adsorbs contaminants on its surface and thereby concentrates them in the region probed by the evanescent wave, thus increasing analyte absorption for a given concentration. On the other hand, it isolates the evanescent field from the water stream significantly reducing the water background absorption. In this work, we targeted the 6.5 μm-7. 5 μm spectral region using a MEMS based EC-QCL source and we used aqueous BTX's solutions to evaluate the sensing performance for trace detection of apolar analytes. Different concentrations of toluene and benzene in water were measured showing almost instantaneous sensor response due to fast analyte diffusion into the mesoporous structure of the coating. When flushing the flow-cell with distilled water a rapid decrease in analyte specific absorption was observed, confirming full and complete sensor regeneration. The LODs were found to be 7ppm for toluene and 1ppm for benzene despite still rather high for noise floors of 0.02 and 0.05 AU, respectively.

(MOLEC-OD2) Nanoscale IR I

(MOLEC-OD2.1) IR Nanopolarimetry of Anisotropic Materials and Thin Films

Presenter: Karsten Hinrichs - ISAS e.V.

Non-Presenting Author: Timur Shaykhutdinov

Infrared (IR) spectroscopic techniques in linear optics are often used as tools for identification of functional molecular groups or bonds via specific vibrational absorption bands. Polarimetric measurements are required for a detailed analysis of structured or anisotropic materials. [1,2] Thereby in nanopolarimetric AFM-IR spectra as well as in classical far-field IR reflection spectra of thin isotropic and uniaxial films the s-polarized measurements resemble the material specific absorption properties parallel to the sample surface. For p-polarized spectra of thin inorganic and organic films the situation is more complex. In this case the absorption and related bands can be manipulated by the incident radiation-induced electric fields in direction normal to the sample surface and if applicable also by anisotropic absorption indices. [3,4] Support by EFRE 1.8/13 is acknowledged. [1] K. Hinrichs, T. Shaykhutdinov, Appl. Spectroscopy 72, 817-832 (2018); [2] K. Hinrichs, T. Shaykhutdinov, C. Kratz, A. Furchner, J. Vac. Sci. & Technol. B 37 (2019); [3] T. Shaykhutdinov, A. Furchner, J. Rappich. K. Hinrichs, Mid-infrared nanospectroscopy of Berreman mode and epsilon-near-zero local field confinement in thin films, Opt. Mater. Express 7 (2017) 3706-3714; [4] K. Hinrichs, J. Rappich, T. Shaykhutdinov, Phys. Status Solidi B 257, 1900490 (2020).

(MOLEC-OD2.2) Analytical models for practical technology to transform Atomic Force Microscopy – Infrared (AFM-IR) Spectroscopic imaging measurements

Presenter: Seth M. Kenkel, PhD - University of Illinois Urbana-Champaign

Non-Presenting Author: Rohit Bhargava - University of Illinois Urbana-Champaign

Contact mode Atomic Force Microscopy (AFM) combined with Infrared (IR) spectroscopy (AFM-IR) is widely used to measure signals proportional to far-field infrared absorption. Confounding factors such as spatial- and time-varying cantilever responsivity effects, however, limit the chemical accuracy and noise of AFM-IR instruments and confound routine use by a wide audience. Here we first present rigorous theory-based analytical models that explain the recorded data in typical measurements and provide insight into instrument design. Led by theoretical predictions, we implement two innovations in correcting responsivity effects and controlling noise. Both innovations are economically deployable on existing instruments, greatly improving both the accuracy and noise in AFM-IR measurements. These improvements can enable wide adoption while providing consistent measurements under diverse conditions, making this exciting technology more robust and useful.

(MOLEC-OD3) SERS, TERS, and Surface Plasmon Resonance

(MOLEC-OD3.1) Development of Ultrasensitive SERS Sensors Using Au Nanoparticles-anchoring Nanodimple Substrates

Presenter: Jaebum Choo, PhD - Chung-Ang University

Non-Presenting Author: Hajun Dang

Non-Presenting Author: Namhyun Choi

Electromagnetic enhancement effects through localized surface plasmon resonance greatly amplify the incident light intensity when target molecules are positioned in the vicinity of tiny nanogaps. Therefore, it is important to position target molecules at plasmonic nanogaps for the generation of hot spots. Au or Ag colloidal nanoparticles, synthesized by bottom-up methods, were popularly used for this purpose. In this case, target molecules should be placed at nanogaps for the amplification of incident light. However, it is difficult to precisely control their aggregation condition in solution phase. Consequently, irregular plasmonic signals were obtained in many cases due to non-uniform nanogap formations. Two-dimensional nano-templates consisting of metal nanostructures have been also applied to enhance the optical properties of plasmon resonance. In this top-down approach, photolithography, e-beam and fast ion beam techniques have been extensively utilized for the fabrication of regular nanostructures with a gap of 20-100 nm. However, these methods have also limitations such as high cost and low throughput. To resolve the problems in the bottom-up and top-down approaches, a nano-dimples array internalized with AuNPs has been developed in this work. Precise nanogaps could be generated by internalizing AuNPs in each well of nano-dimples arrays. Using FDTD simulation, it was confirmed that the electromagnetic field is greatly enhanced by the nanogaps in nano-dimple arrays. In this presentation, the plasmonic properties of AuNPs internalized nano-dimples arrays will be introduced.

(MOLEC-OD3.2) Evaluation of Proteinase Activity using Surface Enhanced Raman Scattering to Aid Cancer Detection

Presenter: Sian Sloan-Dennison, PhD - University of Strathclyde

Non-Presenting Author: Karen Faulds, PhD - University of Strathclyde

Corresponding Author: Duncan Graham, BSc Hons, PhD, CChem, FRSC, FRSE, FSAS - University of Strathclyde

In the UK, one person is diagnosed with cancer every two minutes and close to half of these cases are diagnosed at a late stage. It has been well established that early detection and treatment is vital for successful control of the disease. Therefore, by detecting biomarkers that are known to be indicative of cancer when they first present at a detrimental level, treatment can be administered earlier, which will significantly reduce the likelihood of the cancers' progression. Although there are many potential biomarkers that can be investigated, matrix metalloproteinase (MMP) enzymes are particularly useful for cancer prognosis. This is due to the increased level of multiple MMPs located at tumor sites which exert their activity to facilitate tumor cell invasion and metastasis by degrading the extracellular matrix, modulating cell adhesion and bioactivating molecules. It is important to identify if multiple MMPs are present, and their concentration, as metastasis routes will vary depending on a specific MMPs activity. A single MMPs activity is conventionally detected using fluorescent resonance energy transfer (FRET)

probes. However as multiple MMPs are responsible for tumor metastasis, a multiplex assay is preferred. As this is challenging with fluorescence, surface enhanced Raman scattering (SERS) has recently been investigated in a number of different formats to detect a selection of MMPs simultaneously and sensitively. In this paper we demonstrate that SERS nanoparticle probes can be used to detect MMP activity. By designing SERS probes which incorporates a Raman reporter, MMP cleavable peptide and protecting polymer element, the SERS response of the probe can be monitored before and after MMP activity and the change related to the concentration of MMP in both solution and in a tumor environment. By pairing Raman reporters with cleavable peptides that are MMP specific, multiple MMP dependent SERS probes can be designed and the relevant MMPs activity detected together in a multiplex assay. By applying this approach, the detection of MMP activity earlier and at clinically relevant concentrations can be achieved, aiding in identification and treatment of cancer.

(MOLEC-OD4) SORS and SEDRS

(MOLEC-OD4.1) Increasing SORS Specificity to the Lower Layer: New Spectral Processing and an Application to Noninvasive Bone Assessment in Mice

Presenter: Andrew J. Berger, PhD - University of Rochester

Non-Presenting Author: Christine Massie, BS - University of Rochester

Non-Presenting Author: Keren Chen, PhD - University of Rochester

Transcutaneous estimation of a bone's Raman spectrum is complicated by the type I collagen in the overlying soft tissue being spectroscopically identical to that in the bone. In previous studies of murine tibiae, we have developed a method for decomposing transcutaneous Raman spectra into three components: estimates of the bone spectrum and the overlying soft tissue spectrum (each built from measured spectral libraries) and the inevitable spectral residual. Here, we demonstrate the value of retaining that residual when spectra are submitted to multivariate regressions to predict bone properties. We compare the results of partial least squares regressions performed using either a transcutaneous SORS spectrum, a best estimate of the bone contribution alone, or a "top-layer-subtracted" (TLS) spectrum that contains the sum of the bone estimate and the residual. When the bone library is limited in scope, we observe superior prediction of two standard bone metrics (volumetric bone mineralization density and maximum torque) using regression models based upon TLS spectra, implying that the spectral residuals from the fitting process contain useful information. This chemometric approach is not limited to layered samples, and it could have broad applicability in situations where comprehensive spectral libraries are difficult to acquire. In the case studied here, subtracting off just the estimate of the top layer's spectrum produced better results than retaining just the estimate of the bone.

(MOLEC-OD4.2) Surface Enhanced Deep Raman Spectroscopy (SEDRS): Quantification of Physical properties at depth, advances and pitfalls

Presenter: Ben Gardner, BSc(Hons) PhD MRSC - University of Exeter

Non-Presenting Author: Nick Stone, PGDip, MSc(Dist), MSc(Dist), MBA, PhD, CSci, FSAS, FIPEM, FRSC - University of Exeter

Non-Presenting Author: Pavel Matousek, Professor - Science and Technology Facilities Council

Deep Raman Spectroscopy (DRS), the grouped term for spatially offset Raman spectroscopy (SORS) and Transmission Raman (TRS), has shown great promise in the biomedical domain; especially for its potential in areas such as non-invasive diagnostics combined with therapeutic interventions (theranostics). The capabilities and complexities of what is possible using these techniques has steadily increased over the past decade, especially with the introduction of labelled nanoparticles allowing Surface Enhanced Raman Spectroscopy (SERS) to be combined with DRS (SEDRS), to allow deeper and specific signal recovery, as well as targeting specific physical properties / chemical moieties (pH, temperature, glucose sensing and neurotransmitters) to name but a few. Here we highlight the latest advances in SEDRS, looking at multiple simultaneous signal recovery including accurate depth localisation; as well as new developments in non-invasive temperature sensing and modulation i.e. heating and the experimental complications associated with monitoring and validation of this process.

(MOLEC-OD4.3) Time-gated spatially-offset Raman spectroscopy

Presenter: Ioan Notingher, PhD - University of Nottingham

Non-Presenting Author: Christopher Corden - School f Physics and Astronomy, University of Nottingham

We investigated the use of time-gated and spatially-offset Raman spectroscopy based on spectral multiplexing detection to obtain sub surface molecular analysis and imaging for both fluorescing and non-fluorescing samples. A multiplexed spectral detection was implemented based on a digital micromirror device (DMD) to enable fast acquisition of time-gated signals using a single element detector1. This approach allows 3D Raman mapping of optically-turbid materials, by using sample raster scanning in the lateral x-y plane and time-of-flight for the axial z-direction. We show that sub-millimetre resolution molecular depth mapping was achieved with dwell times on the order of seconds per pixel. For samples eliciting strong auto-fluorescence backgrounds, time-gating Raman spectroscopy was combined with spatially offset Raman spectroscopy (SORS). Using defocusing micro-SORS approach, both fluorescence and Raman signals from the surface layers were further suppressed, which enhanced the Raman signals from the deeper sublayers containing the pigment. These results demonstrate that time-gated Raman spectroscopy based on multiplexed detection, and in combination with micro-SORS, is a powerful technique for subsurface molecular analysis and imaging, which may find practical applications in medical imaging, art and cultural heritage, forensics, and industry. References 1. Christopher J. Corden, Dustin W. Shipp, Pavel Matousek, Ioan Notingher, Fast Raman spectral mapping of highly fluorescing samples by time-gated spectral multiplexed detection, Optics Letters 43(23), 5705-5708 (2018)

(MOLEC-OD5) Higher energy UV and NIR

(MOLEC-OD5.1) Investigation for Electronic States of Lithium Ion Complexes using ATR-FUV Spectroscopy

Presenter: Yusuke Morisawa, PhD - School of Science and Engneering, Kindai University

Li salts and poly ethylene glycols (PEGs) are well-known to form complex between Li+ and oxygen atom at ether group of PEGs. Li ion complexes got knee interest for safe and high-performance electrolyte for batteries and electronic devices. Recently, aqueous electrolyte with high concentration of Li+, hydrate-melt, has been expected to have great potential to improve not only performance but also cost. Our investigation directed to give the information about electronic transitions in far ultraviolet (FUV) region of these electrolytes. In this study, changes of electronic states were observed with variation of anions, cations and concentrations. Variations of the spectra were discussed by comparing experimental data and simulation spectra by time dependent density functional theory (TD-DFT). PEGs have 3 transitions due to those from non-bonding electron at oxygen atoms (n) to Rydberg orbitals in FUV. In the Li+-PEGs complex, these transitions shift to higher energies since n orbits which have coordination bond become stable. In the aqueous solution we also have been observed blue shift of first electronic transition of water. According to the FUV spectra, , all the water coordinates with Li+ in the highest concentrated aqueous solution as predicted in the first principles molecular dynamics study.

(MOLEC-OD5.2) DUV Raman spectroscopy for probing the structure and stability of protein aggregates

Presenter: Igor K. Lednev, PhD - University at Albany, SUNY

There is a great need for a simple and time-efficient tool for evaluating the activity of therapeutic proteins. Probing the structural integrity of proteins in pharmaceutical formulations is the most promising and straightforward approach. The application of Raman spectroscopy for this purpose will be discussed in this presentation. Specifically, deep UV Raman spectroscopy with excitation below 200 nm has been shown to be a powerful tool for characterizing the protein secondary and tertiary structure by probing the conformation of the polypeptide backbone and the local environment of aromatic amino acids. Near-IR Raman spectroscopy is uniquely suitable for probing proteins disulfide bonds, which play a significant role in stabilizing a native physiologically active form of proteins. We have recently investigated the behavior of disulfide bonds during the formation of amyloid fibrils, protein aggregates associated with various neurodegenerative diseases. For example, the secondary and tertiary structure of insulin changes dramatically as a result of its fibrillation in vitro. An α-helical protein is converted into mainly β-sheet form and yet all three disulfide bonds of native insulin remained intact during the aggregation process, withstanding scrambling. Polarized Raman spectroscopy showed strong orientation of disulfide bonds in insulin fibrils, indicating their association with the fibril core. We have recently found that Lysozyme disulfide bonds undergo significant rearrangements in the presence of hydrogen sulfide. Raman bands corresponding to disulfide (RSSR) vibrational modes in the 550−500 cm−1 spectral range decrease in intensity and are accompanied by the appearance of a new 490 cm−1 band assigned to the trisulfide group (RSSSR) based on the comparison with model compounds. The presented evidence indicates that hydrogen sulfide causes the formation of trisulfide bridges, which destabilizes lysozyme structure, preventing protein fibrillation. As a result, small spherical aggregates of unordered protein form, which exhibit no cytotoxicity by contrast with lysozyme fibrils.

(MOLEC-OD5.3) Advances in Portable NIR Spectroscopy for Phytopharmacy and Food Quality

Presenter: Christian W. Huck, Mag.Dr. - Leopold-Franzens-Universität

It is commonly accepted to divide the fieldable spectrometers (i.e. deployable in-the-field, in contrast to benchtop instrumentation, that is only applicable in a laboratory setting) into transportable (e.g. deployable on field while mounted in a car), portable in 'suitcase' format (>4 kg of total equipment weight) and handheld (<1kg) ones. This presentation unveils that in general, the capability for on-site, rapid chemical/physical analysis, minimally invasive measurement, and optimization towards the use by an untrained personnel offered by these techniques form an outstanding value of particular importance especially for the analysis of food, phytopharmacy including medicinal plants. Searching for "portable near-infrared spectroscopy" in ISI Web of Science database (https://apps.webofknowledge.com) results in 239 publications since 2005 with increasing tendency. However, first it is necessary to summarize the essentials of portable NIR spectroscopy, the instrumental basis and the applicability of the latest generation of handheld NIR spectrometers. In the second step, it is essential to set up a powerful strategy for critical evaluation of instruments performance related to the individual scientific demand. Otherwise several projects might fall, due to non efficient optimization/evaluation plans. Therefore, two-dimensional correlation spectroscopy (2D-COS) offers an essential screening tool for monitoring the individual dynamics of an analytical system. Quantum chemcial calculations and simulations are a major milestone in order to get a deeper understanding of the spectral pattern. For the evaluation of systems sensitivity, multivariate determination of lower limits of detection (LOD) and quantitation (LOQ) applying Kennard-Stones and Duplex algorithm enables objective technical and applicable insight details. Finally, the most suitable evaluation of a spectrometers performance is its application, taking the above evaluation/optimization strategy into consideration. For this reason, the suitability of different types of portable NIR spectrometers will be discussed for phytopharmacy and food quality analysis. From this presentation technical and application limits and advantages should become clear and future trends and outlook can be concluded therefrom. Therefore, restrictions in the current applicability of these techniques, as well as challenges yet to be addressed are highlighted.

(MOLEC-OD6) Nanoscale IR II

(MOLEC-OD6.1) Near-field IR detection enables nanoscale computational staining

Presenter: Georg Ramer, MSc PhD - Technische Universität Wien

Non-Presenting Author: A. Catarina V. D. Santos, MSc - Technische Universität Wien

Non-Presenting Author: Bernhard Lendl, Prof. Dr. - Technische Universität Wien

Non-Presenting Author: Rosa Heydenreich, MSc - Technische Universität Wien

Non-Presenting Author: Christian Derntl, Dr. - Technische Universität Wien

Non-Presenting Author: Robert Mach, Prof. Dr. - Technische Universität Wien

Non-Presenting Author: Astrid R. Mach-Aigner, Prof. Dr. - Technische Universität Wien

The photothermal induced resonance technique (PTIR, also called AFM-IR) coupled with broadly tunable mid-IR laser light sources gives access to nanoscale spatially resolved chemical information via well established spectra-structure correlations of mid-IR spectroscopy. By using an atomic force tip for near field detection, this method can provide spatial resolution on the order of 20 nm laterally. This ultra-high spatial resolution has led to an ever increasing range of applications of PTIR, ranging from archeology, over material science (2D materials, material interfaces, polymer science) to biology and biomedical applications. As applications of PTIR and with them the spectra that are recorded grow ever more complex, tools are required to aid spectroscopists in extracting information from them. Such methods - unsupervised and supervised chemometric methods - are well established for far-field imaging but have seen only tentative use in near-field imaging. To the best of our knowledge, supervised regression methods have not been applied to PTIR data before, mainly due to the challenge of gathering reference data as required for supervised methods. Here, we describe our approach to the challenging task of establishing a multivariate calibration for quantitation of a specific group of proteins inside an indivual microorganism. This allows the determination of protein abundance (i.e. computational staining) at the spatial resolution of PTIR via hyperspectral nanoscale imaging.

(MOLEC-OD6.2) Extraction of local charge carrier densities with s-SNOM in presence of phonon resonances

Presenter: Thomas Taubner, PhD - RWTH Aachen University

Non-Presenting Author: Martin Lewin - RWTH Aachen University

Non-Presenting Author: Lena Jung - RWTH Aachen University

Non-Presenting Author: Julian Barnett - RWTH Aachen University

Scattering-type scanning near-field microscopy (s-SNOM) allows for nanoscale resolved IR spectroscopy of soft and solid matter by probing the characteristic response of molecular vibrations, collective crystal lattice vibrations (phonons) and free charge carriers in doped semiconductors around their plasma frequency. The latter two lead to strongly enhanced phonon- or plasma (near-field) resonances between the tip and sample, enabling the ultra-sensitive probing of crystal lattice and electronic properties, respectively. While in many previously investigated systems the electronic (plasma) response was mainly unaffected by the phonons, in this overview talk I will now address the characteristic interplay of phonon resonances with the free charge carrier response in three prototypical systems, where phonons and free carriers are either hosted in the same material, in two different

materials or at the interface between two phonon-resonant materials. In the first system, the resistively switching Oxide (SrTiO3, short STO), we demonstrate how the weak signal of free charge carrier outside the plasma resonance can be enhanced by the intrinsic phonon response of STO due to plasmon phonon coupling. This enables us to quantify the charge carrier density along grain boundaries and individual defects in doped STO ceramics [1]. In the second system, modulation-doped Silicon nanowires, we show how the phonon response of a thin native oxide layer modifies the Drude-response of the free charge carriers and show a new evaluation method which allows for quantitative charge carrier profiles despite the presence of the oxide resonances [2]. Finally, we show how to access the free carrier response of a 2D electron gas at the interface of two phonon-resonant oxides (STO and LAO) by s-SNOM spectroscopy and advanced modelling [3]. References: [1] M. Lewin, et al. "Nanospectroscopy of Infrared Phonon Resonance Enables Local Quantification of Electronic Properties in Doped SrTiO3 Ceramics." Adv. Funct. Mater. 2018, 28, 1802834. [2] L. Jung, et al. "Quantification of Carrier Density Gradients Along Axially Doped Silicon Nanowires Using Infrared Nanoscopy". ACS Photonics 2019, 6, 1744–1754. [3] J. Barnett et al. „Phonon-enhanced near-field spectroscopy to extract the local electronic properties of buried 2D electron systems in oxide heterostructures", submitted (2020)

(MOLEC-OD6.3) Nano-polaritonics in twisted vdWs heterostructures

Presenter: Guangxin Ni, PhD - Florida State University

Interlayer coupling in atomic van der Waals heterostructures plays a rather unique role in controlling their optical and electronic properties. The character of the interlayer coupling can be manipulated by a particular stacking arrangement of the proximal layers and by adjusting the orientation of the neighboring planes. The latter method is known to trigger the long-range periodic modulations referred to as twisted moiré superlattices. The presence of periodic moiré patterns enables further fine tuning of the electronic band structure and yielding rich insights into the electronic phenomenon. This has been manifested in graphene/hexagonal boron nitride (G/hBN) moiré patterns, twisted bilayer graphene structures as well as twisted hBN crystals. Using nano-infrared optical microscopy we have experimentally studied the collective excitations in these twisted moiré structures. We analyzed these soliton networks and obtained the local electrodynamical characters based on the infrared active polaritonics at the nanoscale. Reference: G. X. Ni et al., "Fundamental limits of graphene plasmonics", Nature 505, 190 (2018). S. S. Sunku et al., Photonic crystals for nano-light in moire graphene superlattices. Science 362, 1153 (2018). G. X. Ni et al., Soliton superlattices in twisted hexagonal boron nitride. Nature Communications 10, 4360 (2019). G. X. Ni et al., "Plasmons in graphene moire superlattices", Nature Materials. 14, 1217 (2015).

(MOLEC-OD7) Topics in Molecular Mass Spectrometry

(MOLEC-OD7.1) Chemical Reactions and IM-MS to Differentiate Isomeric Controlled Substances

Presenter: Christopher D. Chouinard, PhD - Florida Institute of Technology

Non-Presenting Author: Samuel W. Maddox - Florida Institute of Technology

Non-Presenting Author: Stine S.H. Olsen - Florida Institute of Technology

Non-Presenting Author: Diana Velosa - Florida Institute of Technology

Non-Presenting Author: Aurora Burkus-Matesevac - Florida Institute of Technology

Mass spectrometry-based methods have been a cornerstone in testing for the use of performance enhancing drugs (PEDS) in sport for several decades. Established gas chromatography (GC) and liquid chromatography (LC) methods coupled to MS allow routine detection, even at exceedingly low concentrations, of compounds banned by the World Anti-Doping Agency (WADA), including anabolic androgenic steroids (AAS). A significant challenge, however, lies in the detection and identification of novel or "designer" steroids that have not yet been classified on the Prohibited List. Research in our group focuses on the development of methods that can allow for unambiguous structural identification of unknown steroids using ion mobility-mass spectrometry (IM-MS) and structurally selective chemical reactions. This presentation will detail our work in both solution- and gas-phase chemical reactions including ozonolysis, UV-catalyzed photoaddition, and the Paternò-Büchi reaction. We have recently demonstrated the utility of solution-phase ozonolysis for improving resolution of steroid epimers testosterone and epitestosterone using a low-cost mercury lamp to produce 185 nm UV radiation. (Maddox SW, et al. J Am Soc Mass Spectrom, 2020, 31, 411-417) Our work has expanded to include a range of endogenous steroid isomers, important in studies on developmental disorders, and controlled anabolic steroids such as androstenedione, methyltestosterone, nandrolone, and stanozolol. We will present the results of performing the aforementioned reactions on these and other controlled substances as a rapid method for improving resolution and structural assignment using IM-MS.

(MOLEC-OD7.2) Laser Desorption Postionization vs. Secondary Ion Mass Spectrometry for Imaging of Organic Biomarkers in Geological Samples

Presenter: Luke Hanley, PhD - University of Illinois Chicago

Non-Presenting Author: Raveendra Wickramasinghe - University of Illinois at Chicago

Non-Presenting Author: Michael Pasterski - University of Illinois at Chicago

Non-Presenting Author: Anton Ievlev - Oak Ridge National Laboratory

Non-Presenting Author: Matthias Lorenz - Oak Ridge National Laboratory

Non-Presenting Author: Igor Veryovkin - University of Illinois at Chicago

Non-Presenting Author: Fabien Kenig - University of Illinois at Chicago

Multidimensional gas chromatography mass spectrometry (MS) has been used to detect organic biomarkers within bulk extracts of geological samples [1]. However, it is difficult to rule out the possibility of organic contamination in such studies due to the loss of spatial information during sample preparation. MS imaging and other spatially resolved sampling methods can overcome these

shortcomings by identifying organic biomarkers within intact geological samples and correlating such MS data with petrographic and fluorescence images. Secondary ion mass spectrometry (SIMS) imaging is currently the premier method for identifying organic biomarkers within geological samples [2], but suffers from extensive ion fragmentation that can limit molecular identification. A novel MS imaging strategy is described here that seeks to improve upon SIMS imaging of organic biomarkers within geological samples. Femtosecond laser desorption postionization mass spectrometry (fs-LDPI-MS) employs 800 nm, ~75 fs laser pulses for ablation of neutral organic molecules from a thin prepared slice of a geological sample, followed by postionization using 157 nm (7.9 eV) laser pulses and subsequent detection by a time-of-flight mass analyzer [3]. The high lateral resolution of fs-LDPI-MS imaging and its ability to detect molecular species with lower fragmentation relative to SIMS allows for high resolution MS-imaging and identification of isolated sample features. Multimodal imaging of geological samples with high organic content is conducted with Bi ion ToF-SIMS, fs-LDPI-MS, petrographic, and fluorescence imaging. SIMS and fs-LDPI-MS specifically are compared for their relative ability to identify organic biomarkers confined to micron-sized regions within intact geological samples.

 References: [1] G.T. Ventura, et al. (2007) Proc. Nat. Acad. Sci. U.S.A., 104, 14260. [2] V. Thiel and P. Sjövall (2011) Annu. Rev. Earth Planet. Sci., 39, 125. [3] L. Hanley, et al. (2019) Annu. Rev. Anal. Chem., 12, 225.

(MOLEC-OD8) Raman Imaging/Microscopy

(MOLEC-OD8.1) Imaging molecular orientation in 3D using Raman and IR microscopy

Presenter: Young Jong Lee, PhD - NIST

Non-Presenting Author: Shuyu Xu

A non-tomographic analysis method is proposed to determine the 3D angles and the order parameter of molecular orientation using polarization-dependent infrared (IR) and Raman spectroscopy. Conventional polarization-based imaging approaches provide only 2D-projected orientational information of single chromophores or vibrational modes. The newly proposed method concurrently analyses polarization-dependent absorption profiles of two non-parallel vibrational modes. Here, I will present hyperspectral image data from a semicrystalline poly($\square$-caprolactone) (PCL) film acquired by polarization IR microscopy. The results clearly show how the 3D angles and the order parameter are determined for every pixel. Based on the 3D orientation images of the polymer chains, I will discuss the molecular structure of the spherulite and its deformation under mechanical strain.

(MOLEC-OD8.2) Compressive Raman Imaging: Fast and Few

Presenter: Hilton B. de Aguiar, PhD - Physics Department, Ecole Normale Supérieure/Paris

Raman imaging is recognized as a powerful label-free approach to provide contrasts based on chemical selectivity. Nevertheless, Raman-based microspectroscopy has drawbacks mostly due to its inherent overwhelming data size, which slows imaging speeds, and non-trivial post-processing, in particular for non-specialists in vibrational spectroscopy. In parallel, compressive sensing has developed as a paradigm shift approach in signal processing: one can computationally reconstruct accurate information from highly undersampled data. Following the compressive sensing spirit, compressive Raman microspectroscopy has emerged as a potential approach to speed up the imaging and concomitantly simplify the post-processing analysis. In this contribution, I will discuss the concepts and assumptions in compressive Raman, in particular showing the fastest spontaneous Raman bio-imaging to date.

(MOLEC-OD9) Raman for Security

(MOLEC-OD9.1) Determination of saffron quality and authenticity using TLC-Raman with data fusion.

Presenter: Haochen Dai, MS - University of Massachusetts Amherst, USA

Corresponding Author: Lili He

Non-Presenting Author: Qixiang Gao

Saffron, one of the most expensive spices around the world, is highly vulnerable to economic adulteration in its powder form. Plant materials and artificial colorants are commonly used as adulterants to reduce saffron concentration as well as maintaining color strength in powdered saffron products. However, current analytical methods cannot test samples accurately onsite. Therefore, the objective of this study is to develop a fast screening and quantification method combining thin-layer chromatography (TLC) and Raman spectroscopy for saffron powder analysis, including quantifying the main colorant crocin content and identifying possible adulterants out of a lab setting. The performance of data fusion of improving the classification accuracy is also investigated. A droplet of aqueous extract of saffron powder was deposited on a TLC plate, exhibiting a uniform yellow round pattern on the TLC plate. The content of crocin was analyzed using the ISO 3632 method and Raman spectroscopy separately. A quantitative model based on Raman spectra was established and validated using partial least square (PLS) with R of 0.9955 and 0.9929 for calibration and validation of the PLS plot, respectively. Possible adulterants such as powders of safflower, turmeric powder, red 40 (allura red), and yellow 5 (tartrazine) were mixed with saffron powder at various degrees. Their water extracts exhibited unique patterns under bright ambient light and UV light that can be discriminated from that of pure saffron samples through visual observation and principal component analysis (PCA) based on the L*a*b* values. . Combining TCL pattern data and Raman spectroscopy, a data fusion method was developed via PLS-DA model to improve classification accuracy. Subsequently, PLS regression models were built for the quantification of each adulterant using the L*a*b* value, Raman data, and the fused data, respectively. This study demonstrates the potential practical application of this method in rapid analysis of saffron quality and adulteration. This project was complete at Umass Raman, IR and XRF core facility. Please contact our core facilities website (https://www.umass.edu/ials/raman-ir-xfr-spectroscopy) if you are interested in Raman, IR,and XRF analysis.

(MOLEC-OD9.2) The Spectroscopic Crime Clock! Determine the Time Since Deposition of Bloodstains using Vibrational Spectroscopy.

Presenter: Alexis Weber, MS - University at Albany, State University of New York

Non-Presenting Author: Igor K. Lednev, PhD - University at Albany, SUNY

Blood traces are commonly found at crime scenes and can provide substantial information about the crime and individuals involved. Determining the time since deposition (TSD) of bloodstains could be important for crime scene investigations. First, TSD could help to estimate the time of the crime. Second, if crime scenes contain multiple sets of bloodstains, the TSD determined for individual bloodstains 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 have shown to be 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 hemoglobin in ambient conditions. Hemoglobin is commonly affected by heated conditions. Thus, other components of bloodstains were investigated to determine if their degradation can be used to estimate the TSD of peripheral bloodstains. Before developing a complete regression model for determining the TSD of peripheral blood, one must first understand the biochemical changes that are occurring within a bloodstain as it dries. Fluorescence spectroscopy was used to capture changes that occurred over first 24 hours after deposition of peripheral blood. Knowing how the relative concentration of the endogenous fluorophores varies over time, provides a fundamental understating of what reaction mechanisms occur within bloodstain as the samples age. The observed spectral changes and their kinetics will be discussed.

(MOLEC-OD10) Biomedical Raman

(MOLEC-OD10.1) Brillouin Microscopy to Probe the Viscoelastic Properties of Tissue Phantoms

Presenter: Michelle Bailey, MPhys - University of Exeter

Non-Presenting Author: Noemi Correa - University of Exeter

Non-Presenting Author: Martina Alunni Cardinali - University of Perugia

Non-Presenting Author: Silvia Caponi - Istituto Officina dei Materiali del CNR, c/o University of Perugia

Non-Presenting Author: Timothy Holsgrove - University of Exeter

Non-Presenting Author: C. Peter Winlove - University of Exeter

Non-Presenting Author: Nick Stone, PGDip, MSc(Dist), MSc(Dist), MBA, PhD, CSci, FSAS, FIPEM, FRSC - University of Exeter

Non-Presenting Author: Daniele Fioretto - University of Perugia

Corresponding Author: Francesca Palombo, PhD - University of Exeter

Brillouin microscopy (BM) is an all-optical, contactless technique providing information on micro-elasticity and viscosity through the scattering of light from acoustic waves, or phonons. In biomedical sciences, BM applications have ranged from the measurement of live cells, to tissues and biofluid models showing potential for diagnosis of pathology. Despite the demonstrated capabilities of BM to generate contrast for detection of cellular and tissue features and abnormalities, the full significance of BM signals in biological matter is yet to be established. Here, we studied tissue-mimicking hydrogels made of gelatin (denatured type-I collagen from bovine skin), with tuneable physical and mechanical properties depending on composition, to elucidate the origin of BM signals. Gelatin hydrogels were studied over a range of concentrations using BM, Raman microscopy, ultrasound elastography, compressive testing and refractometry, and effects of temperature and water content were investigated. Most striking was the observation of a glass transition controlled by change in polymer concentration, denoted by a sigmoidal evolution of the Brillouin frequency shift and a maximum in linewidth. This corresponds to a dramatic slowdown of the structural relaxation process, ubiquitous to colloidal systems and, for the first time here, observed with BM in biological-based systems. Thus, the technique gives a unique insight into the viscoelastic properties of tissue phantoms spanning a wide range of physical states, from the highly hydrated, to the solid-like phase. As simple models for a host of biological systems, hydrogels provide a platform to investigate the effect of hydration and crosslinking in collagen based systems and set the basis for BM in biomechanics and clinical settings.

(MOLEC-OD10.2) Raman Spectroscopy for Intraoperative Margin Analysis in Breast Conserving Surgery

Presenter: Thomas J E Hubbard, MBChB MRCS PGC - University of Exeter

Non-Presenting Author: Angela Shore, PhD - University of Exeter

Non-Presenting Author: Douglas Ferguson, MB BS FRCS (Eng) MS FRCS (GenSurg) - University of Exeter Medical School

Corresponding Author: Nick Stone, PGDip, MSc(Dist), MSc(Dist), MBA, PhD, CSci, FSAS, FIPEM, FRSC - University of Exeter

Introduction Re-operation for positive pathological margins in breast conserving surgery is needed for 20% of patients; intraoperative margin analysis (IMA) may resolve this issue which has been identified as needing resolution. High Wavenumber Raman Spectroscopy (HWNRS) is a method of vibrational spectroscopy that can rapidly assess changes in lipid, protein and water in biological specimens; changes that may differentiate between normal and cancerous breast tissue. We present a HWNRS system for future IMA. Methods A probe with 785nm laser and InGaAs camera measured breast phantoms of gelatin, water and soyabean oil; pork stained with haemoglobin or blue dye and fresh frozen breast specimens obtained with ethical approval from Exeter Clinical Research Facility Tissue Bank (CRF Ref: CRF320). Water/total spectral peak area ratios (W/TAR) were calculated. Plots vs water concentration had gradient calculated by first order polynomial. Statistical testing by unpaired t-test, significance level $p<0.05$. Results W/TAR to water concentration had a linear relationship in gelatin phantoms of varying water concentrations of 85-95% water (gradient = 0.46; Root Mean Square Error (RMSE) = 0.001) and soyabean oil phantoms of water concentrations 40-95% (gradient = 1.24; RMSE = 0.031) demonstrating ability to predict water concentration and differentiate between microenvironments. Signal acquisition was unaffected by dyes and W/TAR between pork stained with haemoglobin (mean= 0.82; SD 0.04) and patent blue dye (mean=0.85; SD 0.01) was not significantly different ($p>0.05$). In human breast tissue (n=50) the W/TAR between normal (mean=0.25; SD 0.18) and tumour containing (mean =0.75; SD 0.24) breast tissue samples were significantly different ($p<0.0001$). Conclusions Our HWNRS system utilising a 785nm laser excitation and InGaAs camera can differentiate W/TAR at physiological concentrations, overcome potential confounding optical issues in the surgical environment and can differentiate between normal and tumour containing tissue in human breast tissue. Further work is needed to validate findings in fresh breast tissue and to establish a measurement protocol in a clinical environment at relevant timescales. This system has the potential to perform IMA.

(MOLEC-OD10.3) In-vivo Evaluation of the Interplay Between the Biochemicals in Preterm and Term Birth Models

Presenter: Rekha Gautam, PhD - Department of Biomedical Engineering, Vanderbilt University

Non-Presenting Author: Jennifer L. Herington, PhD - Department of Pediatrics, Vanderbilt University Medical Center

Non-Presenting Author: Jackson Rogers - Department of Pediatrics, Vanderbilt University Medical Center

Non-Presenting Author: Wilson R. Adams - Department of Biomedical Engineering, Vanderbilt University

Non-Presenting Author: Laura E. Masson - Department of Biomedical Engineering, Vanderbilt University

Non-Presenting Author: Naoko Boatwright - Department of Pediatrics, Vanderbilt University Medical Center

Non-Presenting Author: Jennifer Bateman - Department of Biomedical Engineering, Vanderbilt University

Non-Presenting Author: Christine M. O'Brien, PhD - Department of Biomedical Engineering, Vanderbilt University

Non-Presenting Author: Jeff Reese, MD - Department of Pediatrics, Vanderbilt University Medical Center

Corresponding Author: Anita Mahadevan-Jansen, PhD - Department of Biomedical Engineering, Vanderbilt University

Preterm birth (PTB) is defined as labor before 37 weeks of gestation. It affects approximately 1 out of every 10 births in the United States, leading to high rates of mortality. The events leading to PTB are poorly understood and a complete understanding of the mechanism requires non-invasive, multiplex methods that can provide information about the onset of labor. Raman spectroscopy (RS) is based on the inelastic scattering of light by molecules and in turn provides the molecular fingerprint of the target samples. Here, we used in-vivo RS to investigate infection-induced (LPS) or progesterone withdrawal-induced (RU486) remodeling and compared this to term remodeling in wild type (WT) mice. Raman spectra were acquired every two hours until the delivery on day 19 of pregnancy for WT term mice and on day 15 of pregnancy post LPS/PU486 injection for PTB mice. The measurements were taken using a portable, in-vivo Raman system with a ball-lens fiber optic probe from the cervix of 10-12 mice for each model. LPS treated mice displayed significantly different spectra over the course of labor compared to the WT and RU486 mice in regions of the spectrum primarily associated with protein conformation (817, 939, 1657 cm-1), proteoglycans (1340, 1367 cm-1), lipids (1300 cm-1), blood (1212, 1545 cm-1) and carotenoids (1156, 1520 cm-1). The spatial distribution of these biochemical changes was visualized using ex-vivo Raman maps. The protein structural changes observed in Raman data were also confirmed ex-vivo using second-harmonic generation and two-photon fluorescence for collagen and elastin respectively. Our results suggest that the changes in Raman spectra can help decipher the mechanism of cervical remodeling necessary for parturition. Overall, this study demonstrates the potential of RS as a non-invasive, in-vivo modality to understand the cervix remodeling, thus guiding future human pregnancy studies, facilitating early intervention, and improving reproductive and neonatal outcomes.

(PP-OD1) Solving Industrial Problems

(PP-OD1.1) ABB PGC1000 Fields of Application

Presenter: James Humphreys - ABB Inc

AN EVOLVED SOLUTION to the COMPLEX PROBLEMS OF GAS CHROMATOGRAPHS AS APPLIED TO PROCESS MEASUREMENTS AND INSTALLATIONS. The most important aspect of maximizing the production of the process industry, is ensuring precise, stable measurements using analytical technology. Gas chromatographs are commonly used for their reliable, robust measurements and data. Placement of these systems must be strategic regarding sample point and distance to the gas chromatograph. Applying gas chromatographs to support critical process measurements results in an industry standard practice of engineering high cost integrated systems solutions. The exorbitant number of requirements and cost needed to shelter, install, support and maintain these traditional systems continues to grow as fast as the industry itself. Targeting improvement of the design of gas chromatographs is the fundamental key in removing the necessity of fully housing, while also reducing all supply consumption and maintenance. The PGC1000 solves all these problems the industry has traditionally faced with the installation and support of gas chromatographs through an evolved design. The shelter less, modular and compact design of the PGC1000 allows for lower cost of installation, maintenance and reduction of utility supply and overhead. A field proven design with thousands of applications in all climate conditions globally, the PGC1000 has reduced all aspects of the traditional requirements of gas chromatographs. Not only reducing overhead for the purchase of the systems, but virtually eliminating the need for full analyzer shelters, while also reducing consumption of carrier gases, power supplies and maintenance without the demand of instrument air! I have personally worked in almost every aspect of the upstream, midstream and downstream production and processing industries. Through my experience of servicing, installing and supporting system designs, I have experienced all the challenges that come with understanding the importance of implementing critical process measurements in all these fields. The ABB PGC1000's design lends itself to be the most simplistic, trouble free, plug and play gas chromatograph on the market. The PGC1000 from install to start up, then on to maintenance and support, delivers a cost effective and compact platform that requires minimal training for operation.

(PP-OD1.2) Automated Data Processing to Understand Formulation Stability and Support Rapid NIR Model Development

Presenter: Patrick Wray, MEng, PhD - BMS

An ever increasing number of new active pharmaceutical ingredients (APIs) exhibit inherently poor bioavailability. To solve this problem, amorphous solid dispersions, salts and other advanced formulations are often employed. Maintaining the stability of these formulations is key to preserving the enhanced bioavailability characteristics. It is therefore very important to monitor their stability when placed on storage at conditions of elevated humidity and temperature. Vibrational spectroscopy is a powerful chemically specific tool, which can be used to do this with a high level of sensitivity. In the early stages of formulation development this can be a challenge as there are often very limited supplies of material with which to work and the form into which the API is most likely to convert is often unknown. This means that it is not possible prepare by building models for identifying and quantifying form changes as they occur. Stability studies often contain a large number of samples spread over a

range of conditions and time points, making manual checking laborious and prone to error. This work presents an automated method, which can be used to rapidly check for form changes which occur. Samples are analysed and compared across a range of time points and storage conditions to identify those in which changes occur. Based on this the data is then passed on to further automated routines which can be used to start identifying the nature of the form change and begin to quantify it. This work can be used to reveal the edges of failure for formulations and facilitates subsequent rapid quantitative model development by guiding the determination of appropriate processing parameters for development of PLS models.

(PP-OD1.3) Rapid Simultaneous Identification of Mineral Contents and Thermal Maturity in Oil Shale with High-Speed Raman Imaging

Presenter: Mohammed Ibrahim, PhD - Thermo Fisher Scientific

Oil shale is a fine-grained sedimentary rock with considerable compositional variations that contains a solid mixture of organic chemical compounds called kerogen. Oil shale was formed from the organic debris millions of years ago. When heated by natural geothermal heat these shales produce liquid organic products by thermal decomposition. With the conventional oil resources being quickly depleted, there has been a great upsurge on the exploration and production of shale oils in the US and the around the world. One of the traditional methods for identifying shale oil deposits is vitrinite reflectance (or VR), which uses light reflectance as an indicator of thermal maturity in hydrocarbon source rocks. However, this technique has several disadvantages, including analysis being subject to human error, low or absent reflectance values in some of the oil shale deposits, etc. Raman microscopy can provide a method for measuring thermal maturity of the kerogen in oil shale, as the Full Width at Half Maximum (FWHM) values of the kerogen Raman G- band are correlated with its thermal maturity: Increase in the thermal maturity increases structural order throughout the carbon network, thus decreasing the G band FWHM. In this study, we analyzed several oil shale samples from Eagle Ford Shale (Texas) with a the Thermo Scientific DXRxi Raman imaging microscope, and demonstrated that Raman imaging can identify, with high speed and accuracy, the presence of different minerals, including pyrite, calcium carbonate (calcite) and others. Knowledge of the presence or absence of these minerals in oil shale is essential for efficient oil extraction in drilling operations by the new technique of hydraulic fracturing (fracking). We also demonstrated that Raman imaging can be used for fast identifying thermal maturity of the kerogen in the oil shale.

(PP-OD2) PAT in the Biopharmaceutical Industries and Industrial IR

(PP-OD2.1) In Situ IR Study on Polyurethane Reactions

Presenter: William Wang, PhD - Lubrizol

Non-Presenting Author: Joseph Prata - Lubrizol

Non-Presenting Author: Sinan Li - Lubrizol

Non-Presenting Author: Nitin Sharma - Lubrizol

Non-Presenting Author: Ted Clifford - Lubrizol

The application of in situ IR spectroscopy has been widely used in industry for decades. The method can provide more insightful information on the reaction, which includes the reaction kinetics and possible intermediate(s) involved. Such information drives innovation in process modification and optimization. At Lubrizol Advanced Materials, polyurethane is frequently used in Life Science products and in situ IR monitoring enables us to have a better understanding of its reaction. In this talk, two applications will be discussed: one on the use of in situ IR spectroscopy to determine the reaction endpoint by examining the residual isocyanate level; the other on the use of in situ IR to optimize mixing method in a polyurethane reaction.

(PP-OD2.2) Analysis of Stressed Proteins using FTIR, Raman and DLS Methodologies

Presenter: John M. Wasylyk, PhD - Bristol-Myers Squibb Co.

Non-Presenting Author: Robert Wethman

Non-Presenting Author: Ming Huang

The discovery, development and production of protein-based pharmaceuticals are has increased greatly over the last 15 years. Monoclonal antibodies, antibody-drug conjugates, and derivatized bio-active proteins are all making their mark as therapeutics agents. Stability studies are key to understanding the limitations of production, transportation and administering of these drugs. As a result, the ever expanding role that spectroscopy can play on monitoring changes in the secondary structures of protein-based drugs and is paramount to understanding the requirements need to maintaining a stable proteins. We utilize infrared spectroscopy, Raman spectroscopy and dynamic light scattering technologies in order to track the stability of protein during the development lifecycle as well as in scale-up. The information gained through applying techniques in early and mid-stage development provides valuable process knowledge as we gear for large scale manufacturing. We will cover studies using these techniques to evaluate stability and set limits on parameters required in order to maintain structural integrity and biological activity.

(PP-OD3) Pharmaceutical Investigations

(PP-OD3.1) Atomic Spectroscopy: A Critical Aspect Of Pharmaceutical Investigations

Presenter: Lydia Breckenridge, PhD - Bristol Myers Squibb

Non-Presenting Author: Sharla Wood, PhD - Bristol-Myers Squibb

Foreign matter investigations during pharmaceutical development and commercial manufacturing are instigated when extraneous material of unknown source is discovered in drug product or the components used to formulate a drug product (for example, the drug substance or excipients). Accurately characterizing and identifying the material is critical in order to ascertain the source, and therefore remediate, the contamination. Additionally, knowing the composition of the foreign matter can facilitate quality assurance decisions relating to patient safety and product quality. When foreign matter is deemed to have an inorganic component that cannot be characterized by more traditional techniques used in initial forensic assessments (ex. FTIR spectroscopy) a variety of atomic spectroscopy techniques can be useful. The pharmaceutical atomic spectroscopy lab compiles a wealth of techniques that are suitable for foreign matter analyses regardless of the sample presentation (isolated, powder, tablet etc.), its solubility or the quantity. Samples amenable to direct dissolution or microwave digestion can be analyzed by ICP-AES or ICP-MS for both quantitative and qualitative inorganic analysis. In other cases, for instance samples of limited quantity or where the foreign matter specks are embedded in tablets, solid analysis techniques such as laser ablation, LIBS or XRF may be more appropriate. This presentation will highlight some specific examples where various atomic spectroscopy techniques have been used to provide critical information towards resolving foreign matter investigations. Emphasis will be on more novel solid analysis techniques such as laser ablation and XRF.

(PP-OD3.2) Investigating the Suppliers: Reagent Quality

Presenter: Anna Luczak, PhD - BMS

Corresponding Author: John M. Wasylyk, PhD - Bristol-Myers Squibb Co.

Non-Presenting Author: Ming Huang

Non-Presenting Author: Robert Wethman

Non-Presenting Author: Anna Luczak

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 chemical companies to meet the specific needs of their customers. As a result, this increased pressure can sometimes impact the quality of the reagents. The impact may be due to issues in the synthetic process, reagent packaging, reagent storage, and shipment. 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 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 reagent prior to utilization in laboratory and plant settings.

(PP-OD3.3) Pharma Forensics: Protecting Patients from Substandard Products

Presenter: Jeremy Peters - BMS

Non-Presenting Author: Ravi Kalyanaraman, PhD - Bristol-Myers Squibb

Non-Presenting Author: Scott Huffman - Bristol-Myers Squibb

Non-Presenting Author: Brendon Lyons

Non-Presenting Author: Brittany Handzo

Non-Presenting Author: Mark Wang

In the pharmaceutical industry, patients are at the focus of everything we do. Ensuring that every product provided to patients meets the critical and stringent standards intended is of paramount importance. What happens, though, when products do have defects, particularly foreign and particulate matter? Pharma Forensics utilizes a wide a array of spectroscopic techniques to evaluate and characterize foreign materials; and provide useful insight to various cross-functional stakeholders, enabling root cause analysis and effective corrective and preventative action. These techniques include light microscopy, IR microscopy, Raman microscopy, and SEM/EDS. The combination of morphological, chemical, and elemental analysis provides robust data sets to determine potential sources and root causes for foreign matter defects. Recent foreign matter cases involving formulated drug product tablets demonstrate this ability. Hundreds of tablets containing multiple foreign matter types were observed. The combination of these spectroscopic techniques enabled the characterization of the organic, inorganic, and metallic foreign matter present. These data were then used to determine how these materials could be introduced during the numerous process steps, as well as scope and impact of the issue. The end results enabled to effective release of multiple batches, assurance of patient safety, and corrective actions to ensure prevention of future defects.

(PP-OD4) Pharmaceutical Analysis

(PP-OD4.1) COVID-19 and other Viral Treatments: the Role of VCD Spectroscopy in Expediting the Process

Presenter: Rina K. Dukor, PhD - BioTools, Inc.

As the World battles the Pandemic of COVID-19, pharmaceutical companies rushed to discover treatments and develop vaccines. As it takes years to develop any new medications, one of the routes is to re-purpose some of the known antivirals and to use similar modalities to develop new molecules. There are about 100 anti-viral drugs on the market and more than half are chiral! The four FDA-approved treatments for Influenza are chiral! Chirality plays a critical role in pharmaceutical development. On a 'positive' side the chiral molecules are more effective with less side effects but on the negative side - chiral drugs are harder to develop and produce because making one form of the molecule is simply harder and requires more caution, more testing and thus more development time. Vibrational Circular Dichroism, VCD, is playing an important role in development of chiral drugs, including anti-virals, by helping expedite time in R&D, manufacturing and quality control. VCD has become the choice technique for determination of absolute configuration without a long and laborious crystallization. Absolute configuration is a critical attribute required by regulatory agencies for drug approval. In this presentation, we will discuss the anti-viral treatments and how VCD is helping expedite time to market.

(PP-OD4.2) Transmission Raman Spectroscopy (TRS) in Pharmaceutical Analysis: Current applications and discussion on needs for future technological development

Presenter: Claudia Corredor, PhD - BMS

The purpose of the present study was to investigate the feasibility of developing a fast non-destructive at-line transmission Raman spectroscopy (TRS) method for core tablet potency and content uniformity (CU) as part of a real-time release testing (RTRt) control strategy. One of the challenges of the current portfolio is to successfully implement a PAT control strategy for high potent products (with drug loads <1%). Raman and TRS are alternatives for PAT control of high potent products where spectroscopic solutions (such as NIR or LIF) are not optimal.

(PP-OD4.3) Imaging as a tool for problem-solving in Pharmaceutical Industry

Presenter: Venkata N K Rao Bobba, PhD - Bristol Myers Squibb

Spectroscopic imaging provides a non-destructive approach for various problem-solving solutions in the scientific field. Segregation during the pharmaceutical process of blending affects the critical quality attribute blend of uniformity. The study also emphasizes the effects of key variables in the process such as the number of revolutions of the blender on the blending process, blend components, and vessel fill volume etc. The process of segregation has been well studied with higher drug loadings, so understanding the process of segregation is of utmost importance to obtain a uniform blend for low drug load (<1%) formulations during the process of blending. Hence, chemical imaging has been applied as a tool to study the process of segregation. Chemical imaging provides both spectral and spatial information of the various pharmaceutical excipients within the formulation. Therefore, chemical imaging also helps to understand if the root cause of the segregation process occurs because of a specific

excipient present within the formulation. The tableting process can provide challenges with powder accumulation at the punches or cause defects on the tablet surface. Understanding which of these excipients causes the hindrance to the process is critical. This imaging approach can be used for identifying the excipients that adhere, by providing chemical images of the components sticking to the tableting tool. Chemical imaging provides clarification on the root cause of variation in the uniformity of blends and moreover, serves as an investigational tool to detect the excipients during the process. This knowledge will enhance the ability of formulation scientists to understand the process.

POSTER ABSTRACTS

Art/Archaeology/Forensics

A Workflow for the Forensic Identification of Psychoactive Plant Types Featuring a Direct Analysis in Real Time-Mass Spectral Database

Presenting Author: Rabi Ann Musah - Department of Chemistry, University at Albany

Non-Presenting Author: Samira Beyramysoltan, PhD - Department of Chemistry, University at Albany

Non-Presenting Author: Megan Chambers - Department of Chemistry

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Non-Presenting Author: Monica Ventura - Department of Chemistry

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Development of a psychoactive-plant database and user interface for plant identification from DART mass spectra

The widespread abuse of "legal high" psychoactive plants continues to be of global concern because of its negative impacts on public health and safety. A major challenge for law enforcement in controlling the use of these substances is the paucity of methods by which to identify them. In principle, knowledge of the species-specific chemical signatures of plants could enable their discrimination and identification. We used Direct Analysis in Real Time-High Resolution Mass Spectrometry (DART-HRMS) to generate a database of psychoactive plant chemical signatures. The rapid acquisition of mass spectra (i.e. a few seconds per analysis) and the ability to sample the materials in their native form without pre-treatment steps, enabled the generation of the vast amounts of spectral replicates required for database construction. A screening architecture with a graphical user interface (GUI) implemented in Python, was designed for the identification of plant unknowns, based on a machine learning approach. To create the database, 54 psychoactive plant species and plant-derived products spanning a range of sample types including flowers, stems, seeds, leaves, roots, extracts and brews, were analyzed by DART-MS in multiple replicates. A DART-SVP ion source (IonSense, USA) coupled with a JEOL AccuTOF high-resolution time-of-flight mass spectrometer (JEOL USA) operating in positive ion mode was used to collect soft-ionization mass spectra. The spectra were corrected for background and mass shifts, and aligned along common m/z values for multivariate analysis. A hierarchical classification tree was designed based on taxonomic relationships between plant species to reduce the 54 classes to a simplified multiclass problem. In each node of the tree, supervised and unsupervised classifiers were trained and their outputs were fused using the decision fusion approach for sample prediction. Performance analysis revealed the model to have 95% prediction accuracy for test samples. It enabled prediction of plant species identity from the raw DART mass spectra of unknowns, despite the complexity of their matrices. The user interface can be readily utilized by crime labs and forensic scientists, and does not require sample preparation steps or botanical knowledge.

Analysis of automotive paint and glass samples by combined LIBS and Raman spectroscopy

Presenting Author: Virginia Merk- LTB Lasertechnik Berlin

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Non-Presenting Author: Sven Merk- LTB Lasertechnik Berlin

Non-Presenting Author: Wolfgang Werncke- LTB Lasertechnik Berlin

Non-Presenting Author: Lutz Pfeifer- LTB Lasertechnik Berlin

Combined LIBS-Raman-spectroscopy with microscopic spatial resolution has the potential to improve forensic sample analysis significantly.

Two main goals in forensic analysis are the classification of samples present at the crime scene to obtain information on the origin and the discrimination from other samples found. The variety of possible samples is manifold and can range from organic and inorganic materials to samples of human origin. As diverse as the range of samples, the samples themselves contain much complexity. Another challenge in forensic analysis is that often only very small samples are available. Therefore, analytical techniques are required that exhibit high sensitivity and minimal sample destruction. Among other techniques like scanning electron microscopy-energy dispersive X-ray, X-ray fluorescence spectrometry and laser ablation ICP-MS, also Raman and Laser-induced Breakdown Spectroscopy (LIBS) have been established in forensic analysis. Here we will show with two different examples, namely the analysis of glass and automotive paint samples, the benefits of the combination of LIBS and Raman spectroscopy with multivariate analysis for forensic analysis. With the combination it is possible to obtain complementary (elemental as well as molecular) information of a sample and this can lead to an improved classification and discrimination of the samples. The data were obtained with a newly developed instrument that allows subsequent measurements with both techniques at the same microscopic sample spot by sharing one high-resolution, wide-range Echelle spectrometer. We measured sixteen different glass samples with LIBS and Raman. The results show that with LIBS and also the combination of LIBS and Raman fourteen of the sixteen samples could be differentiated. By using standardized integral values of selected LIBS lines instead of the whole spectrum we could significantly improve the differentiation. The combination with Raman leads to an improved stability of the differentiation due to the additional information. Automotive paint samples consist of different layers with varying composition. By measuring cross sections of eight different samples with LIBS and Raman we obtained layer and sublayer-specific chemical information with a spatial resolution of about 10 micrometer. The elemental cross section profiles together with the characteristic Raman and fluorescence signatures turned out to be very unique for macro- and microscopically similar samples.

Characterization and Dating of Archaeological Textile Fragments from the Seip Mound Complex, Ohio

Presenting Author: Ruth Ann Armitage - Department of Chemistry, Eastern Michigan University

Non-Presenting Author: Michaela Repaska - Department of Chemistry, Eastern Michigan University

Non-Presenting Author: Brenan Wilson - Department of Chemistry, Eastern Michigan University

Non-Presenting Author: Kathryn Jakes - Ohio State University

Understanding the composition of colored textile fragments aids in accurately measuring their radiocarbon ages.

Ohio Hopewell archaeological sites, and the Seip Mound complex in particular, are well known for their preserved textiles. Though what remains today is mostly fragmentary, degraded or charred, these precious remnants are indicative of the complex craftsmanship of the Hopewell. Modern analytical and dating techniques allow for characterization and chronological studies of these materials that have not been possible in the past. Segments of what was thought to be a fabric canopy with repeating patterns of green coloration were recovered during early 20th-century excavations at Seip-Pricer. Subsequent investigations of smaller fragments sharing the same physical characteristics as the canopy fabric indicate that it is made from plant fibers, though it remains unclear how the material was decorated. Minute samples were collected, taken from small bits of yarn that appear to have fallen away from the larger fabric fragments. These samples provided a unique opportunity for chemical characterization and radiocarbon dating of textile materials from the Seip Complex. A variety of analytical methods were used to characterize the green coloration and to determine if a binding medium was present, in an effort to better understand the process used to decorate the textile. Minimally-destructive sample preparation with pH 8 phosphate buffer and plasma oxidation was carried out on the minute samples which had been collected. Carbon dioxide produced by the plasma oxidation process was then subjected to graphitization and AMS radiocarbon analysis. An initial radiocarbon age for the green stained textile fragment was found to be consistent with previous dates for Seip, though at the earliest edge of the measured ranges. The copper carbonate pigment may decompose, causing a shift in the radiocarbon age compared to that of the plant fibers. The efficacy of removing the pigment with acid treatment is currently under evaluation. The results of these investigations demonstrate how such miniscule fragments of ancient textiles can be utilized to understand Hopewell technologies and place these human-made objects into the chronology of the Seip Mound complex.

Characterization of Beads from an Andean Inca Chullpa Funerary Assemblage

Presenting Author: Adelphine Bonneau, PhD - University of Oxford

Non-Presenting Author: Heather Walder - University of Wisconsin La Crosse

Non-Presenting Author: Ruth Ann Armitage - Department of Chemistry, Eastern Michigan University

Non-Presenting Author: William A. Lovis - Michigan State University

Characterization of small seed beads from an Andean mummy to investigate possible European post-contacts

In 1890 the U.S. Consul to Chile sent Michigan Agricultural College (now Michigan State University) the mummified remains of a young Andean girl interred in a chullpa tomb, reputedly located south of La

Paz, Bolivia. She was accompanied by a group of funerary objects, and the documentation indicated that she was 15th c CE Inca. She was repatriated to Bolivia in January 2019. During and following repatriation minimally destructive analyses were undertaken on the funerary objects. The estimated age of 1400-1500 CE was corroborated by a series of AMS ages on maize, leather, and gourd. However, a series of small 3 mm black and red beads gave the appearance of European manufactured glass "seed" beads common in the context of colonial exchange. If the beads were glass, and European, it would question the chronological homogeneity of the funerary assemblage since they would likely postdate ca. 1500 CE. Microscopic observations revealed structural characteristics consistent with fine sedimentary rock, or fine ceramic paste, but could not conclusively eliminate the possibility of weathered and eroded vitreous material such as glass. To explore the chemical composition of the beads one of each color was subjected to a series of analyses. The small sample was due to the sensitive nature of the assemblage. SEM-EDS (scanning electron microscopy coupled to X-ray energy dispersive spectroscopy), LA-ICP-MS (laser ablation - inductively coupled plasma – mass spectrometry), Raman spectroscopy, and DART-MS (direct analysis in real time – mass spectrometry) were deployed. Results of the analysis revealed that the beads were fine sedimentary stone or ceramic, and exhibited an organic coating potentially for coloration, or later museum curation. Therefore, glass was eliminated as the material of manufacture, but perhaps not ruling out the possibility of European manufacture, since small ceramic and stone beads were manufactured in Europe since at least the Middle Ages. However, very small, stone beads have been identified in Inka and other pre-Colombian contexts (e.g. Bernier, 2010, Davis, 2010, Carter and Helmer 2015, Lau, 2019). The most parsimonious interpretation of the beads analyzed seems to be indigenous Inka manufacture.

Characterization of Glue Recipes from Colonial America: Repairs on Repaired Ceramics from Ferry Farm

Presenting Author: Daniel Fraser, PhD - Lourdes University

Non-Presenting Author: Ruth Ann Armitage - Department of Chemistry, Eastern Michigan University

Non-Presenting Author: Mara Kaktins- George Washington Foundation

Non-Presenting Author: Melanie Marquis- George Washington Foundation

Understanding the composition of 18th-century glue residues may help elucidate why the ceramics were repaired.

During excavations at the 18th-century colonial American site of Ferry Farm, broken ceramics with traces of glue were discovered, indicating that the objects were repaired before ultimately being broken and discarded. This historic archaeological site is important as it was the boyhood home of George Washington. We present here our characterization of these 18th-century glues with Fourier transform infrared spectroscopy (FTIR), scanning electron microscopy-energy dispersive X-ray spectroscopy (SEM-EDS), and ambient ionization mass spectrometry. We compare the results from replica glues prepared from historic recipes based on cheese and milk, rendered animal collagen, and pine resins with those from the 18th-century glue samples. This reference collection of historic glue recipes allows us to test how these homemade glues function and change during burial, which aids in the interpretation of our analyses of the historic glue residues. While proteins are expected to break down rapidly when combined with calcium oxide lime, we were able to identify with paper spray ionization and time-of-flight mass spectrometry peptide markers in the replica glues of the milk protein casein several years after their preparation. Sample preparation to remove the mineral fraction plays an important role in

obtaining reliable ambient ionzation mass spectrometry results on the small molecule biomarkers like resin acids and beeswax esters. The carbonate mineral identified with FTIR, making up the bulk of the glue residues, was determined from the SEM-EDS to be consistent with lead white rather than calcium carbonate, which was expected based on the contemporary ceramic cement recipes that called for lime (calcium oxide). The results of our analyses of these glue residues will aid us in better understanding how and why the Washington family used household materials to repair their ceramic vessels.

Detection and Quantification of Fentanyl in Street Drugs by Portable Attenuated Total Reflection - Infrared Spectrometer

Presenting Author: Margo K. Ramsay- University of Victoria

Non-Presenting Author: Lea Gozdzialski- University of Victoria

Non-Presenting Author: Ashley Larnder - University of Victoria

Non-Presenting Author: Dennis Hore - University of Victoria

Non-Presenting Author: Bruce Wallace - University of Victoria

Fentanyl quantification in street drugs has the potential to reduce overdose deaths.

The opioid crisis has claimed over 9000 lives in Canada since 2016, with fentanyl and its analogues having been involved in the vast majority of these deaths. The detection and quantification of fentanyl in street drugs has the potential to reduce overdoses among people who use drugs. Using knowledge gained from drug checking services offered around Victoria, Canada a chemometric model for fentanyl quantification was created. The partial least squares regression model was built from ATR-IR spectra of lab-made opioid samples. This model was then used to analyze and quantify fentanyl in illicit opioid samples in Victoria, Canada.

Discrimination between human and animal blood by ATR FT-IR spectroscopy for forensic purposes

Presenting Author: Ewelina M. Mistek-Morabito- University at Albany, SUNY

Corresponding Author: Igor K. Lednev, PhD - University at Albany, SUNY

Nondestructive differentiation between human and animal blood for forensic purposes.

Forensic chemistry is an important area of analytical chemistry. This field has been rapidly growing over the last several decades. Confirmation of the human origins of bloodstains is important in practical forensics. Current serological blood tests are destructive and often provide false-positive results. Here, we report on the development of a nondestructive method that could potentially be applied at the scene for differentiation of human and animal blood using attenuated total reflection Fourier transform-infrared (ATR FT-IR) spectroscopy and statistical analysis. The following species were used to build statistical models for binary human–animal blood differentiation: cat, dog, rabbit, horse, cow, pig, opossum, and raccoon. Three other species (deer, elk, and ferret) were used for external validation. A partial least squares discriminant analysis (PLSDA) was used for classification purposes and showed excellent performance in internal cross-validation (CV). The method was externally validated first using blood samples from new donors of species used in the training data set, and second using donors of new

species that were not used to construct the model. Both validations showed excellent results demonstrating potential of the developed approach for nondestructive, rapid, and statistically confident discrimination between human and animal blood for forensic purposes.

Examining the Effects of Human Processing on Australian Archaeological Ochre Pigments

Presenting Author: Jolene M. Anthony, BSc - Flinders University
Non-Presenting Author: Claire Lenehan, PhD - Flinders University
Non-Presenting Author: Rachel Popelka-Filcoff, PhD - Melbourne University & Flinders Unversity
Non-Presenting Author: Shane Tobe, PhD - Murdoch University
Non-Presenting Author: Amy Roberts, PhD - Flinders Unversity
Non-Presenting Author: Claire Smith, PhD - Flinders University

Application of spectroscopy to understand the effects of human/cultural processes on archaeological ochre for provenancing.

Ochre is principally a group of Fe-based (oxide or hydroxide) mineral pigments which have been used worldwide by various cultures for tens of thousands of years. Aboriginal Australian people use ochre-based pigments for a variety of uses including on rock art and ochre-treated objects. Fundamentally, ochres occur naturally in various forms, with the main components composed of goethite (α-FeOOH, brown/yellow pigments) or hematite (Fe_2O_3, red pigments), which are typically accompanied by diverse mixtures of minerals such as aluminosilicates and quartz, as well as organic components from the original source. Characterisation of the original source materials provides a unique chemical signature that can be exploited to determine provenance of cultural ochre-based pigments. However, when traditionally used as pigments, ochre potentially undergoes various forms of human processing including heating, grinding and/or mixing with organic binder materials, which remains a complicating factor in provenance of pigments. The aim of this research is to examine the effects of cultural/ human processing as it relates to provenance determination of Australian cultural ochre. This area of research seeks to further characterise ochre via spectroscopy to understand Australian pigments and binders of cultural significance, providing an insight into past and present cultural practices and significant traditional processes.

Integrating Cavities for Raman Spectroscopy Trace Detection in Fibers Containing Dye Mixtures

Presenting Author: Benjamin R. Anderson, PhD - Washington State University
Non-Presenting Author: Natalie Gese - Washington State University
Non-Presenting Author: Hergen Eilers, PhD - Washington State University

We use a UV integrating cavity to measure minor components of dye mixtures.

Currently there is a strong desire in the forensics community to utilize Raman spectroscopy for trace detection of minor dye components in dyed cloth fibers. This is challenging, however, as minor dye components typically present small signals in the Raman spectra, which can have significant contributions from other dye components. Therefore it is necessary to use novel Raman spectroscopy techniques in order to obtain significant signal to identify minor components. To address this need we have developed a UV Raman spectroscopy technique involving a highly reflecting integrating cavity,

which enhances the Raman signal using multiple reflections and resonance enhancement. We demonstrate this capability by measuring the Raman spectra of fibers doped with mixtures of two acid dyes at different concentrations using both a standard Raman spectroscopy setup and our UV Integrating cavity.

Probing Menstrual Bloodstains Aging with Fluorescence Spectroscopy

Presenting Author: Anna Wójtowicz, MS - University at Albany, State University of New York
Non-Presenting Author: Alexis Weber, MS - University at Albany, State University of New York
Corresponding Author: Igor K. Lednev, PhD - University at Albany, SUNY

Schematic mechanism for changes occurring in menstrual blood's endogenous fluorophores over time is proposed.

Determination of the time since deposition (TSD) of body fluids can be the key to establishing the time of a crime, identifying biological stains which are relevant to the crime, and isolating individuals involved. Menstrual blood (MB) is a common and important type of forensic evidence, especially during the investigation of sexual assault cases. In forensic investigations, the differentiation of menstrual and peripheral blood is crucial because the differentiation can distinguish if the blood present is a result of tissue damage from an assault or a natural cause, like menstruation. Therefore, if crime scenes contain multiple sets of bloodstains, the crime-related stains can be established. The next step is to determine the TSD of a bloodstain as knowing when a stain was deposited would increase the probative value of the analysis results. Before developing a complete regression model for determining the TSD of menstrual blood, one must first understand the biochemical changes that are occurring within a bloodstain as it dries. MB is composed of peripheral blood and vaginal fluid, as well as other constituents such as epithelia, basal lamina, and biomaterial from the stroma and uterine glands. Fluorescence spectroscopy, which was previously applied to measure the changes to amino acids and proteins within peripheral blood, was used to capture changes that occurred in the menstrual blood's chemical composition over time. For this preliminary study, menstrual blood samples were deposited on aluminum covered glass slides and aged in ambient conditions. Fluorescence spectroscopy data was collected over nine hours post deposition for each of the samples. Changes in the relative concentrations of an amino acid - tryptophan, and two electron transporters: reduced nicotinamide adenine dinucleotide (NADH) and flavin adenine dinucleotide (FAD) were observed with samples aging. Knowing how the concentration of these species vary over time, provides a fundamental understating of what reaction mechanisms occurs within bloodstain as the samples aged. Moving forward, by measuring these changes as a factor of time, the ability to approximate the TSD of menstrual bloodstains will be possible. The observed spectral changes, their kinetics and inter-donor variations will be discussed.

Revisiting the Elemental Analysis of Bullet Lead: Laser-Induced Breakdown Spectroscopy (LIBS)

Presenting Author: Brooke W. Kammrath, PhD - University of New Haven
Non-Presenting Author: Lauren Vallee - University of New Haven
Non-Presenting Author: Charles Sisson - Applied Spectra, Inc.
Non-Presenting Author: Peter Valentin - University of New Haven
Non-Presenting Author: John Reffner- John Jay College of Criminal Justice

Non-Presenting Author: Peter De Forest - John Jay College of Criminal Justice

This presentation will demonstrate the merits of the elemental analysis of bullet lead by LIBS

Bullets are a frequently encountered component of physical evidence found at the scenes of shooting crimes. There are many situations in which comparison of striations and other surface markings on bullets are not feasible due to the severe fragmentation or deformation of a bullet, or a specific firearm is not recovered to be associated with the bullet or suspect in question. For these, analytical methods which determine and compare the elements present in the lead component of bullets can be valuable. Early in the 21st century, a considerable controversy arose concerning the evidentiary significance obtained with comparative bullet lead analysis. Although the NRC of the NAS determined that the analytical chemical approach had a firm scientific background, cited examples of poor testimony were criticized and questions arose about its interpretation. Unfortunately, instead of investing in additional research to investigate its evidential significance, the FBI announced in 2005 that they were no longer conducting comparative bullet lead analysis. Some scientists criticized this decision concluding that the FBI ultimately 'threw the baby out with the bathwater' because it effectively eliminated the ability for comparative bullet lead analysis to be used in all forensic casework despite its proven scientific merits and utility for resolving specific forensic questions. There is a need to revisit comparative bullet lead analysis, especially with the recent availability of new instrumental methods of elemental analysis which will enable more forensic laboratories to perform this type of scientific investigation. In this research, LIBS was evaluated for its ability to perform comparative bullet lead analysis. LIBS offers the advantages of a faster and more economical elemental analysis, thus enabling it to be more widely employed by a variety of resourced forensic laboratories. This could enable a large bullet lead database to be generated, shared and used to calculate robust statistics, and then potentially initiate a revival for comparative bullet lead analysis as part of a more readily available forensic laboratory procedure.

The Analysis and Characterization of Microplastics in Soil – Can Environmental Microplastics be Useful in Forensic Investigations?

Presenting Author: Michaela A. Sullivan- University of New Haven
Non-Presenting Author: Brooke W. Kammrath, PhD - University of New Haven

The study of microplastics may potentially have a place in the realm of forensic science.

Microplastics, which are particles that are defined as being smaller than 1 mm but are often in the range of several microns, have become a growing area of research because of their prevalence and permanence in the environment. Although the occurrence of microplastics have been thoroughly studied in marine environments and on beaches, there is a lack of literature on the occurrences of microplastic material in soil. In the field of forensic science, soil evidence may be frequently encountered in civil action or criminal cases. For the latter, soil analysis can provide information valuable to investigative efforts and crime scene reconstruction. In cases of violent crimes, soil samples recovered from a victim or from physical evidence can reveal information about where a crime took place and where items or people were located prior to, during, or after the commission of a crime. The complex nature of soil lends itself a high level of discrimination that may stand to be elevated to an even greater power of discrimination when also considering the anthropogenic material found in soil samples, such as microplastics. Should results obtained from the analysis of microplastics be found to be reliable and reproducible in replicate environmental samples, then the study of microplastics may potentially have a place in the realm of

forensic science, especially in the analysis of terrestrial samples recovered from crime scenes. This research will consist of a literature review of several studies involving environmental samples containing microplastics and will be supplemented by lab research that will employ microscopic examination and Raman spectroscopy, both via the Thermo Fisher Scientific DXR microRaman spectrometer and the HORIBA XPLORATM PLUS + Particle Finder, to analyze microplastics within collected soil and sediment samples. The main issue being addressed in this research is whether the results from the analysis of microplastics in terrestrial samples will be reproducible enough to be useful in forensic investigations. The secondary goal is to characterize microplastics found in terrestrial systems by their morphology and their molecular properties, and to determine if the levels of certain microplastics accumulated in soil are characteristic to specific classes of regions.

The Power of Combining X-Ray Fluorescence Spectroscopy with Other Analytical Techniques: Highlights from the Investigations of Cultural Heritage Materials in Rogaland, Norway

Presenting Author: Kidane Gebremariam, PhD - University of Stavanger, The Museum of Archaeology

Power of combining XRF with complementary techniques, for heritage materials' investigations in Rogaland, is demonstrated.

X-ray Fluorescence Spectroscopy (XRF) is increasingly employed for the investigation of archaeological and cultural heritage objects. This mainly originates from the possibility the technique offers to conduct analysis in a non-destructive and non-invasive manner. Maintaining the integrity, of the precious and unique cultural heritage objects, is always given the utmost priority. The advancements in the X-ray sources, optics, detection systems and software developments have made the XRF instruments, like the portable XRF, more powerful, moveable, affordable and, subsequently, accessible to wider users. Archaeometric and heritage science related investigations have gained from these developments and will do so in the future. The portable XRF has also drawbacks that can be addressed through the applications of complementary elemental, molecular, microscopic and imaging methods. This presentation highlights few case studies from the utilization of portable XRF, along with other methods, for the characterization of archaeological, historical, artistic and cultural heritage materials in Rogaland. The studied materials are diverse and include, among others, mortars, plasters, crucibles, metals, coins, lithic objects, gildings, glasses, enamels, gems, paintings, polychrome sculptures, rock paintings, slag and slag-like materials. The applications range from in-situ examinations for quick, on-site identifications in remote sites, to the use in museum and laboratory settings. The application area of the analytical methods demands highly multidisciplinary approaches starting from the formulation of the analytical problems to be tackled, to the interpretation of the experimental results. It involves working at the interface of humanities, art, technology and sciences with interactions of scientists, archaeologists, conservators, building experts, curators, historians, artists, etc. The results from the investigations help to gain better insight into the culture, materials and technologies of the human past and the interactions with nature. Characterization of materials, authentication, provenance, dating, classifications, corrosions and deteriorations can be addressed by such investigations. In addition to facilitating archaeological and art historical studies, they play crucial roles in the well-informed conservation of the unique cultural heritage objects. The dissemination of the investigations on the fascinating heritage objects could be utilized for inspiration of the youth in STEM and demonstration of the great importance of multi-disciplinary engagements for better understandings.

Atomic Spectroscopy

A Spatiotemporally Resolved Characterization of a Radiofrequency Pulsed Glow Discharge Fundamental Parameters Under Optical Emission Spectroscopy Elemental Mapping Operating Conditions

Presenting Author: Kevin Finch- Texas Tech University

Non-Presenting Author: Aldo Hernandez- Texas Tech University

Corresponding Author: Gerardo Gamez, PhD - Texas Tech University

First time insights into fundamental parameters of RF GDOES under elemental mapping conditions are elucidated.

Glow discharges (GD) have been widely used for the direct elemental analysis of solids, throughout a wide variety of applications. These plasmas offer many advantages including high-throughput (fast sputtering rates) simultaneous multi-elemental analysis, low operating costs, depth-profiling capabilities, and the ability to perform accurate analysis on low mass elements while the majority of other techniques fall short. While typical GD optical emission spectroscopy (OES) operating conditions result in poor lateral resolution (mm), due to the mixing of the atoms in the discharge, it has been shown that operating the GD under pulsed-power mode and higher-pressure allows improved elemental mapping. Furthermore, radiofrequency (RF) powered GD offers the advantage of being able to analyze nonconductive samples in comparison to traditional direct current GD. However, the underlying species behavior and mechanisms that govern the plasma-based chemical analysis are not fully understood, especially as a function of space and time. Therefore, it is imperative that systematic studies are carried out to monitor the fundamental parameters, including temporally resolved maps of electron/gas temperatures and densities. The methods of choice to probe these species are laser Thomson and Rayleigh scattering. Laser scattering diagnostic techniques have inherent spatial and temporal resolution, do not perturb the plasma (if the laser intensity is strictly controlled), and do not require the assumption of local thermodynamic equilibrium. Furthermore, Thomson scattering enables the simultaneous but independent measurement of electron temperature and density. Here, a newly constructed, novel transmission type triple grating spectrograph, will be utilized to obtain maps of electron/gas temperatures and densities, pertaining to RF-pulsed glow discharge under a variety of operating conditions used during GDOES elemental mapping. The effects of pressure, voltage, current, RF pulse width, and RF pulse frequency will be studied as a function of spatial position in the plasma and time along the RF pulse train to gain much needed insights into the governing mechanisms.

Characterization of Atmospheric Pressure Plasma Jets by Optical Emission Spectroscopy and by LIBS

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Applications of atmospheric pressure plasma jets require characterization of plasma species (type, density, energy etc).

Atmospheric pressure plasma (APP) jets are increasingly employed for industrial applications such as cleaning and modification of surfaces and deposition of coatings and for bio-medical application such as skin treatment and bacterial inactivation. We investigate cold and hot APP jets that are operated at low (300 W) and high (several kW) power of the plasma generator, respectively. The spatially resolved optical emission of the plasma jet and of the plasma induced by laser breakdown inside the APP jet is measured for different gaseous working media. For the cold atmospheric plasma device operated with air and N2 gas various emission bands of molecular nitrogen, oxygen, and nitrogen oxide are detected. The emission intensities are highest close to the device nozzle and decay rapidly with increasing distance (few mm). Intense atomic emission lines of neutral and ionized nitrogen, oxygen, and Ar in addition to the molecular emissions are detected by laser-induced breakdown spectroscopy (LIBS). Mixing of the N2 plasma jet with the surrounding air is observed by detection of oxygen lines with LIBS. The hot APP jet operated with Ar reveals many neutral and ionized Ar lines in the emission spectrum (industrial device Acerios by Fronius). The intensities of Ar I lines increase approx. linearly with the generator drive current whereas an exponential increase is observed for the Ar II lines. 2D mapping of Ar I and Ar II emissions reveals maximum intensities at the nozzle and different spatial ranges for the neutral and ionized species. Acknowledgements: Financial support by the Austrian Research Promotion Agency FFG is gratefully acknowledged (project CAPCOAT Plus 872846).

Chemometric Approaches to Direct Analysis of Lanthanides in REE-rich Ores by TXRF and WDXRF

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Improving the accuracy of direct simultaneous quantification of lanthanides by X-Ray fluorescence through chemometrics.

X-Ray fluorescence (XRF) is a useful tool for rapid multielemental analysis of geological samples that does not require acid digestion. One of the important tasks given to XRF is the simultaneous quantification of lanthanides in ores. However, strong line overlapping makes it challenging. Thus, high resolution wavelength-dispersive XRF (WDXRF) is used. However, the question still stands: can cheaper low-resolution energy-dispersive spectrometers accomplish this task? To answer this question we provided an assessment of total reflection XRF (TXRF) as a tool for direct analysis of REE-rich ores. In this work, we used partial least squares (PLS) and principal component regression (PCR) to

circumvent spectral interferences in both TXRF (S2 Picofox, Bruker GmbH) and WDXRF (S8 Tiger, Bruker AXS) spectra. To provide a uniform distribution of concentrations of lanthanides in a test set we implemented specialized design of experiment (DoE) based on Latin hypercube sampling (LHS) [1]. We obtained the matrix of 5 factors (elements) and 20 levels (concentrations as well as samples). The maximum correlation is 0.03, which is several times better than in [2]. In addition, this design has an advantage over DoE in [3] where the number of samples equals a square of the levels. Thereafter, our model was applied for the determination of Ce (460–39500 ppm), La (260–24100 ppm), Nd (150–11800 ppm) by both TXRF and WDXRF in standard reference materials of niobium and uranium ores used for validation. In addition, WDXRF provided quantitative determination of Pr (47–3800 ppm), Sm (24–1500 ppm) in these samples. We also present a comparison of PLS, PCR and integrated software results. Chemometrics provided twice the best average root mean square error of prediction (RMSEp) for Ce, La, and Nd for TXRF and thrice for WDXRF (average RMSEp for all elements) compared to the integrated software. 1. M. D. McKay, R. J. Beckman, W. J. Conover., Technometrics. 1979. 21. 239-245. 2. D. Kirsanov et al. Spectrochimica Acta Part B. 2015. 113. 126–131. 3. A. Maltsev, A. Shulyumova, N. Umarova. X-Ray Spectrometry. 2018. 5. 396–404.

Determination of ultratrace levels of boron in organic matrices

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A method to determine important elements in difficult matrices at ultra-trace levels.

Plasma source spectrometry is an indispensable analytical tool for the analysis of trace elements because of its ability to determine analytes simultaneously at the sub part-per-billion (ppt) level in various process chemicals. For the direct analysis of organic solvents, however, it is extremely important to address certain potentially problematic areas. Boron is a critical element in polymers because of its electrical properties in semiconductor materials application. Direct introduction of organic samples into ICP-MS presents challenges; volatility and viscosity effects can significantly affect the efficiency of the sample introduction system, plasma stability and data quality, among other factors. Traditional sample preparation approaches using digestion with mineral acids lead to analyte losses, memory effects and the increased risk of contamination for ultra-trace concentration level. In addition, ultra-trace determination of B by ICP-MS in high carbon matrices is exacerbated by the presence of the extremely high ^{12}C peak adjacent to the ^{11}B peak. Two simultaneous approaches using sample pretreatment and the introduction of reaction gas have been evaluated. In the first approach a sealed combustion tube is used as a sample preparation tool to burn and trap the analyte. The second approach involves the use of favorable gas phase ion-molecule reaction in the reaction cell, $C^+ + NH_3 = NH_3^+ + C$, as well as introducing oxygen to eliminate the effects of carbon interferences. Using these approaches the carbon interference has been shown to be minimized allowing the ultra-trace level determination of B in various matrices. The concept has been validated with direct introduction into ICP-OES which does not suffer this type of interference. M. Tichy, A. B. Raksit, D. G. Lister, N. D. Twiddy, N. G. Adams and D. Smith, "A study of the reactions of the ground and metastable states of C^+, N^+, S^+ and N_2^+ at 300 K", Int. J. Mass Spectrom. Ion Proc., 29, 231-247(1979).

Development of a Magnetic Confinement Attachment for Enhanced Signal in Handheld Laser Induced Breakdown Spectroscopy Soil Analysis

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The development and validation of a novel magnetic confinement fixture for handheld LIBS

A simple, low cost magnetic confinement attachment has been developed to enhance the ability of a handheld Laser Induced Breakdown Spectroscopy (LIBS) instrument to quantify low levels of heavy elements in soil samples. The identification of heavy elements in soil is important in several fields including geological mining and forensics, among others. This work focuses particularly on the identification of iron and uranium. Traditional handheld LIBS techniques can struggle to analyze heavy elements in soil because they typically have exceptionally low concentrations and thus weaker spectral peaks. LIBS spectra from soils are also notorious for their complexity and shot-to-shot variability due to their natural elemental heterogeneity and often-inconsistent moisture content. These factors combine to make low-level identification and quantification of heavy elements in soil via handheld LIBS challenging. This work partially alleviates the difficulties of handheld LIBS on soils by amplifying the peak intensities of all elements via magnetic confinement. Magnetic confinement is achieved via an external fixture designed specifically for the SciAps Z300 handheld LIBS, but easily adaptable to other models. The introduction of an external magnetic field accelerates the plasma's free electrons leading to an increased rate of electron collisions, increased electron density, and increased atomic emission. Initial results indicate the system can achieve signal enhancement factors of two to three in intensity, despite the presence of an increased continuum. The signal enhancement is most pronounced for elements with a small initial intensity which is ideal for this application. Potential future applications of this technique include identification of trace elements in metals, identification of trace heavy metals in biological samples and food testing among others. This paper will also discuss continuing research efforts to include evaluations of which spectral lines are most sensitive to magnetic confinement, as well as prototype development and testing.

Diffusive Gradients in Thin Films (DGT) for the Simultaneous Assessment of Plant-Available Strontium and Lead Isotope Ratios in Soil by Multi-Collector Inductively Coupled Plasma Mass Spectrometry

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Diffusion-based speciation, preconcentration and matrix separation for targeted Sr and Pb isotope ratio soil analysis

Stable isotope ratios of strontium (Sr) and lead (Pb) are widely applied to trace source-sink pathways in natural soil-plant systems. Yet, the assessment of the isotopic composition of labile (reversibly adsorbed) Sr and Pb species in soil available for plant uptake remains challenging. Analyte contents in soils in the low µg/g range and high matrix loads often require laborious sample preparation procedures towards the metrologically sound determination of Sr and Pb isotope ratios by mass spectrometry. In this study, we further develop and apply the diffusive gradients in thin films (DGT) technique for the targeted and rapid assessment of plant-available Sr and Pb stable isotope ratios in complex soil sample matrices. The approach favors the combination of in situ analyte speciation, preconcentration and matrix separation by DGT with accurate isotope ratio measurement by multi-collector inductively coupled plasma mass spectrometry (MC ICP-MS). Besides the application of the well-established Chelex® 100 DGT technique for sampling labile Pb, we report on the development of a novel DGT technique based on re-usable PTFE binding layers with a modified crown-ether functionality (TK100 DGT) showing high selectivity for both Sr and Pb under environmental conditions. The aim of this work is to establish a simultaneous Sr and Pb DGT MC ICP-MS technique to assess labile Sr and Pb isotopic signatures in topsoils as geo-reference in food provenancing. Results show that Chelex® 100 DGT enables the precise determination of labile $\delta(207/206)$Pb(SRM 981) patterns at ultra-trace levels (pg/g) with excellent measurement reproducibility (RSD ≤0.2%) and low combined measurement uncertainty (Urel = 0.025%, k = 2). The TK100 DGT shows high potential to assess the isotopic composition of both Sr and Pb with quantitative (≥99.9%) separation of matrix elements (Na, Mg, K, Ca, Rb), adequate recovery of Sr (>70%) and Pb (>80%) in DGT-purified fractions and low combined measurement uncertainty (Urel = 0.023%, k = 2). First analysis of $\delta(87/86)$Sr(SRM 987) sampled by TK100 DGT as relative to the $\delta(87/86)$Sr(SRM 987) of the corresponding Sr(NO3)2 standard solution in a synthetic soil solution matrix indicates no significant fractionation of Sr isotopes due to the diffusion-based sampling approach.

Elemental Mapping of Silver Nanoparticles on Microarrays using Glow Discharge Optical Emission Spectrometry

Presenting Author: Aldo Hernandez- Texas Tech University

Non-Presenting Author: Kevin Finch- Texas Tech University

Non-Presenting Author: Yue She- Texas Tech University

Corresponding Author: Gerardo Gamez, PhD - Texas Tech University

Glow discharge optical emission spectrometry allows sensitive high-throughput elemental mapping of nanoparticle microarrays.

Nanoparticles (NPs) have been an important tool in various applications, notably the medical and industrial fields; their unique properties allow capabilities from enhanced imaging, to catalysis, to drug delivery, among many others. This reveals a high importance on elucidating said properties of NPs. However, many of the current techniques used to image NPs have multiple downsides including large costs, the assumption of the shape of the analyzed NPs, or low information throughput. Thus, the need for more accessible, higher throughput techniques that yield complementary information becomes apparent. Glow discharge optical emission spectrometry (GDOES) is a technique that traditionally allows for quantitative and qualitative, multi-elemental and direct-solid analysis. Currently, GDOES

elemental mapping capabilities are being developed to take advantage of its ultra-high throughput analysis benefits. Here, the application of GDOES elemental mapping on silver nanoparticles in a microarray platform will be explored; these include observations of the effects of the size of the NPs, GDOES operating parameters, and substrate type. The quantitative figures-of-merit will also be discussed through limits of detection and sputtering rates.

Evaluation of a Commonly Used Calibration Scheme for the Measurement of Particle Size and Number Concentration by spICP-MS

Presenting Author: Karen E. Murphy - National Institute of Standards and Technology

Non-Presenting Author: Ingo H. Strenge - University of Siegen

Non-Presenting Author: Antonio R. Montoro Bustos- NIST

Non-Presenting Author: Monique E. Johnson - National Institute of Standards and Technology

A first, comprehensive, study of the accuracy of a common spICP-MS calibration scheme is presented.

Single-particle inductively coupled plasma mass spectrometry (spICP-MS) has emerged as a convenient method for characterizing particle size, size distribution, number and mass concentration of inorganic nanoparticles in liquid suspension. Our group has engaged in research to validate the accuracy, precision and robustness of spICP-MS [1, 2]. We have identified important differences in the mass versus particle transport of Ag and Au in select suspensions [2]. These differences impact the commonly adopted calibration scheme for spICP-MS where the analyte response is established with ionic standards and the transport efficiency is calibrated by either measuring the particle flux (frequency method) or particle response (size method) of a single, well-characterized monodisperse NP standard typically composed of AuNPs [3]. In this work we extend our validation studies to NPs of different composition (SiO_2 and Pt) and further expand our investigation of AuNPs and AgNPs of varying sizes and surface coatings. Utilizing high-resolution scanning electron microscopy (HR-SEM) to thoroughly characterize particle size distributions (typically > 1000 particles measured per material) and conventional ICP-MS to characterize mass fractions, an accurate measure of the median particle size, size distribution and derived number concentration is obtained. spICP-MS size results calibrated using the above-described size method scheme were within 20 % of known values for SiO_2 NPs ranging from 200 nm to 800 nm, Pt NPs (30 nm to 70 nm), Au NPs (30 nm to 200 nm) and Ag NPs (40 nm to 200 nm). Number concentration results by spICP-MS calibrated using the frequency method with a single AuNP reference material showed differences ranging from 2 % to 100 % indicating that the processes governing particle transport and calibration for the measurement of number concentration are not well understood. [1] Montoro Bustos, A. R., Kavuri, P. P., Possolo, A., Farkas, N., Vladar, A. E., Murphy, K. E., Winchester, M. R., Anal. Chem., 90, 14376-14386 (2018). [2] Liu, J. Y., Murphy, K. E., Winchester, M. R., and Hackley, V. A., Anal. Bioanal. Chem., 409, 6027-6039 (2017). [3] H. E. Pace, N. J. Rogers, C. Jarolimek, V. A. Coleman, C. P. Higgins, J. F. Ranville, Anal. Chem., 83, 9361–9369 (2011).

Evaluation of the Potential of Single Particle ICP-MS for the Accurate Measurement of Number Concentration of AuNPs of Different Sizes and Coatings

Presenting Author: Antonio R. Montoro Bustos- NIST

Non-Presenting Author: Karen E. Murphy - National Institute of Standards and Technology

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A rigorous assessment of spICP-MS capabilities for measuring PNC is presented for the first time

Single particle inductively coupled plasma-mass spectrometry (spICP-MS) is an emerging technique that is capable of simultaneous measurement of size and number concentration of metal-containing nanoparticles (NPs) at environmentally relevant levels. Although spICP-MS is widely applied to different fields, challenges remain in obtaining accurate and consistent particle number concentration (PNC) measurements. This communication presents for the first time, a rigorous assessment of spICP-MS capabilities for measuring PNC of gold (Au) NP suspensions. Calibration of spICP-MS is accomplished with the monodispersed NIST RM 8013, nominal 60 nm AuNPs, with well-defined mean size, size distribution and Au mass fraction. The comparability of both spICP-MS direct and derived determination of PNC and reference PNC derived based on the mean particle size or the particle size distribution obtained by different reference sizing techniques was first assessed using the monodispersed NIST AuNP RM 8012, nominal diameter 30 nm. To enable a proper assessment of the accuracy of the spICP-MS results, a comprehensive estimation of the expanded uncertainty for PNC determination by spICP-MS including the main sources of error is carried out. Following this, the influence of particle size, and surface coating on the quantification capabilities of spICP-MS is evaluated for different commercially available AuNP suspensions with three different sizes (30 nm, 60 nm, and 100 nm) and four different coatings: citrate, polyvinylpyrrolidone (PVP), polyethylene glycol (PEG), and branched polyethyleneime (bPEI). Regardless of NP size or surface coating, spICP-MS leads to quantitative PNC recoveries (90 % - 110%) only when reliable in-house Au mass concentrations and thorough mean particle size (central tendency) determinations are included in the calculation of the expected PNCs. The use of the full-size distribution over the mean size to derive PNC results in non-quantitative PNC recoveries for the materials with a low contribution (< 2 %) of smaller NPs (30 nm), materials with higher polydispersity (100 nm) or with two distinct sub-populations of particles (60 nm) regardless of NP coating.

GDOES Elemental Mapping And Quantification Via Single Pixel Compressed Sensing Imaging

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Compressed sensing allows GDOES-EM via cost-effective single-pixel imaging to have higher resolution and throughput.

Elemental mapping (EM) can help to understand the underlying mechanisms of materials' function. However, typical EM techniques are highly time consuming (several to tens of hours per sample). Glow discharge optical emission spectroscopy (GDOES) is known for its fast direct analysis of solid samples and yields quantitative, qualitative, and high depth-resolution information with minimal-to-no sample preparation requirements. When the GD is operated at pulsed power and higher pressures (10 –20 torr), laterally resolved information can be collected as well, thus enabling EM, as previously demonstrated with ICCD detectors. While ICCDs allow high throughput spectral imaging, their high cost restrict their availability. Conversely, single-pixel detectors are an order of magnitude more cost effective, but the typical pixel-by-pixel imaging requires significantly more time. Compressed sensing (CS), on the other hand, allows to perform compression during the acquisition process, so that the same resolution image

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can be reconstructed from far less measurements. Here, GDOES EM is coupled to a single-pixel imaging system based on the theory of CS. This results in significantly higher throughput and improved resolution compared to typical single-pixel imaging systems, as well as low cost advantages compared with 2D imaging systems. The effects of CS parameters (matrix density, compression factor, basis and reconstruction algorithm) on the fidelity, resolution, and GDOES quantitative figures-of-merit will be presented.

Geological Fingerprinting of Columbite-Tantalite

Presenting Author: Samuel E. Kessinger- South Dakota School of Mines and Technology

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The geo-fingerprinting of coltan ore allows for the strategic mineral's provenance to be verified.

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. LIBS, in conjunction with other spectroscopy methods, including XRF and Raman, is applied for the analysis of coltan (columbite-tantalite) samples. The resulting spectra, generated via these spectroscopic methods, enables the geological fingerprinting of samples. To facilitate the analysis, a significantly large number of samples, originating from numerous locations globally, were collected and tested. Due to the sizeable variety of individual coltan samples tested and the complexity of the resulting spectra, machine learning models and simulations are applied to the generated spectral results. When applied to a substantially diverse collection of spectral results, this advanced hybrid machine learning approach allows for sample identification, classification, and pattern recognition, resulting in the determination of individual coltan samples' provenances.

Improving analytical performance of a portable laser-induced breakdown spectroscopy device through use of a boosted regression ensemble

Presenting Author: Ashwin P. Rao - Air Force Institute of Technology

Corresponding Author: Michael B. Shattan - Air Force Institute of Technology

Novel analytical technique for rapid, accurate, in-situ chemical composition analysis of nuclear fuel material.

Boosted regression ensembles were used to analyze optical emission spectra of laser-induced plasma from pellets of cerium, a common chemical surrogate for plutonium, doped with silicon. A predictive model using the boosted ensemble method was built to determine Si content in the Ce matrix; this model was compared to commonly used chemometric regression tools such as PCA/PLS and artificial neural networks. The boosted regression ensemble outperformed the traditional regression techniques, yielding a higher R-squared value for the regression fit (0.982) and a lower mean-squared error (0.182) for Si content determination, providing the most accurate predictive model. While decision tree-based methods

have seldom been investigated for radiochemical analysis of optical emission spectra, the results of this study indicate that boosted ensemble methods could provide a promising new solution for these types of chemometric regression problems. Combined with a portable laser-induced breakdown spectroscopy (LIBS) device, these models can provide instantaneous, in-situ, accurate determination of elemental dopants in nuclear materials, yielding improved metallurgical quality assurance/quality control (QA/QC).

Investigating Complex LIBS Samples Through the Integration of Raman Spectroscopy and Advanced Machine Learning Methods

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Improving LIBS spectra data analysis through the integration of advanced machine learning methods.

Laser-induced breakdown spectroscopy (LIBS) which is an optical spectroscopy technique relies on data visualizations to interpret experimental results of spectra data. Dendrograms are most commonly used for this task, however, there are a number of algorithmic issues that arise with this method. Thus, to limit the practical issues that arise within hierarchical clustering methods, this paper explores additional advanced machine learning methods to more effectively visualize and analyze LIBS spectra data. These additional methods include refinements to the already commonly used clustering approach, as well as the addition of K-Means methods in conjunction with Raman spectroscopy data for faster and more reliable sample identification, classification, and pattern recognition. To best illustrate the proposed methodology, we used spectra data that was obtained from a laboratory LIBS set up as well as a handheld LIBS unit. The data constituted of spectra obtained from various complex samples consisting of Lead (Pb), Chromium (Cr), Tin (Sn), Gold (Au), Tantalum (Ta), Niobium (Nb). To perform the physical analysis, the open-source statistical programming language R was used in combination with various packages that will be described in detail in the presentation. In summary, by using this newly refined approach we aim to improve spectra visualization to allow for more efficient and effective interpretation.

Novel Use of Laser Induced Breakdown Spectroscopy on Lithium Hydride Samples to Determine Chemical Degradation and Chemical Reaction Rates

Presenting Author: James Stofel- Air Force Institute of Technology

Non-Presenting Author: Mark Gragston- UTSI

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Quantification of the extent and rate of oxidation, hydrolysis, and carbonation in lithium pressed pellets.

Lithium Hydride (LiH) is often used as a hydrogen storage material but chemical degradation in the purity of LiH can directly affect its effectiveness in this area. Common lithium compounds include LiH, LiOH, Li2O, and Li2CO3. The latter three compounds are products of reactions of LiH with H2O, O2, and CO2 in the air. Therefore, presence of these compounds represent degradation in a lithium hydride sample. This work will report on the initial success of an ongoing project to use Laser Induced Breakdown Spectroscopy (LIBS) to produce three-dimensional chemical maps of pressed lithium pellets. These maps will be used to determine the sample composition as a function of depth below the surface and to quantify the rate at which processes such as hydrolysis, oxidation, and carbonation permeate the pellet. Multiple multivariate statistical analysis techniques such as Principle Component Regression (PCR) and Partial Least Squares Regression (PLSR) are used to improve the precision of lithium compound calibrations. Further work will compare the environmental reaction rates of lithium pellets exposed to a variety of storage conditions.

On-line Species-specific Isotopic Analysis of Sulfur by Hyphenation of Capillary Electrophoresis with Multicollector-ICP-MS

Presenting Author: Sebastian Faßbender- Federal Institute for Materials Research and Testing (BAM)

Non-Presenting Author: Katerina Rodiouchkina- Universiteit Gent

Non-Presenting Author: Frank Vanhaecke - Universiteit Gent

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First hyphenation of CE with MC-ICP-MS for isotopic analysis of sulfur in different species

In many scientific fields, isotopic analysis can offer valuable information. Up to date, typically bulk analysis is aimed at measuring the isotopic composition of the entire elemental content of the sample. However, the analyte element is usually present under the form of different species. Thus, separating species of interest from one another and from matrix components prior to isotope ratio measurements can provide species-specific isotopic information, which could be used for tracing the origin of environmental pollutants and elucidation of (environmental) speciation. Using on-line hyphenations of separation techniques with multicollector-ICP-MS (MC-ICP-MS) can save time and effort and enables the analysis of different species during a single measurement. Whereas some works hyphenating GC and IC with MC-ICP-MS have already been reported, LC and CE hyphenations are still inadequately represented based on the capabilities of these separation techniques. In this work, we developed an on-line hyphenation of CE with multicollector-ICP-MS (CE/MC-ICP-MS) for isotopic analysis of sulfur species using a multiple-injection approach for instrumental mass bias correction by standard-sample bracketing [1]. With this method, the isotopic composition of sulfur in sulfate originating from river water could be analyzed without sample preparation. The results were compared to data from off-line

analysis of the same samples for validation. The repeatability of the results of the on-line measurements was promising regarding the differentiation of the river systems by the isotopic signature of river water sulfate. The great potential of this method is based on the versatility of the applied separation technique, not only in the environmental field but also for, e.g., biomolecules because sulfur is the only covalently bound constituent of proteins that can be analyzed by MC-ICP-MS. [1] Faßbender et al. (2020) Anal. Bioanal. Chem., DOI: 10.1007/s00216-020-02781-8.

Relating Physical and Chemical Development of Cerium Plasma Plumes using LIBS and Schlieren Imaging

Presenting Author: MICHAEL G. RYNDERS- Air Force

Non-Presenting Author: Mark Gragston- UTSI

Non-Presenting Author: Anil Patnaik - Air Force Institute of technology

Non-Presenting Author: Michael B. Shattan - Air Force Institute of Technology

Using Schlieren and dp-LIBS to understand physical and chemical evolution of cerium LPP plumes

Understanding plasma plumes and the distribution of species in Laser-Produced Plasmas (LPPs) microseconds after their formation has a variety of applications in fields such as hypersonics, explosion and combustion dynamics, and plasma chemistry studies; the behavior actinides in LPPs of is of particular interest to the nuclear community. This work studies cerium in LPPs created from CeO and CeBr6 targets. Chemical properties of cerium are similar to plutonium and hence, Ce is often used as a plutonium surrogate. Temporally and 1D-spatially-resolved chemical maps of the plume were produced using double pulsed Laser Induced Breakdown Spectroscopy (dp-LIBS) using 532nm light. Schlieren imaging at 40khz was used to relate the plume development to the plume chemical species information. Inclusion of calcium impurities in the CeBr6 sample showed how calcium species are located relative to cerium species in the plume. We present here our progress towards a quantitative measurement of the distribution of cerium within a plasma plume from zero to thirty microseconds after ablation.

Speciation analysis of arsenic in edible seaweeds sold in the United States

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The paper presents the determination of arsenic species in seaweeds sold for consumption using HPLC-ICP-MS.

Seaweeds are increasingly being cultivated for food as they are rich in nutrients including amino acids, Vitamin K, and iodine. On the other hand, seaweeds are known to accumulate arsenic in a range of chemical forms, some of which are of known toxicities and others which have yet to be fully elucidated for any potential effects. Most risk assessment practices associated with dietary arsenic are based on monitoring inorganic arsenic, which is a Class I carcinogen. This is generally adequate, as inorganic is

generally considered more toxic than organic arsenic and most products are known to accumulate arsenic in forms of defined properties. However, the approach may leave species of potential or unknown toxicities unidentified when applied to seaweeds, where arsenic has a complex and variable distribution of species. Comprehensive speciation analysis which aims at capturing a complete picture of the distribution of arsenicals is recommended. The poster presents the determination of arsenic species in edible seaweeds sold in the United States based on extraction of water-soluble and nonpolar arsenic. Fifty-six samples of brown, green and red algae that were purchased from local supermarkets and over the internet were analyzed by methods recently developed [1] and single-lab validated [2] at the FDA. The accuracy of the analytical results was evaluated by analyzing certified reference materials and using spike recovery tests. [1] M. M. Wolle, S. D. Conklin; Anal Bioanal Chem 410 (2018) 5675 [2] M. M. Wolle, S. D. Conklin; Anal Bioanal Chem 410 (2018) 5689

Synthesis, Characterization and Functionalization of Nanophosphors as a Light Source and Study of X-Ray Induced Behavior of Caenorhabditis elegans For Future X-Optogenetics.

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Synthesizing bright nano-scintillators and studying x-ray stimulated behavior of Caenorhabditis elegans for future noninvasive x-optogenetics.

Optogenetics uses visible light to modulate neural activities and allows us to understand the neural mechanism, to improve and fix when malfunctioning. However, this technique is limited by invasive nature and depth limitation. Therefore, we are investigating tools for non-invasive, in vivo optogenetic neuron stimulation by synthesizing and functionalizing nanophosphors that emit bright visible-light when irradiated with x-ray and studying x-ray stimulated behavior of Caenorhabditis elegans that we might bypass the need of visible-light in the future. We are synthesizing Eu- and Tb-doped $NaGdF_4$ nanoparticles using co-precipitate and hydrothermal processes and annealed at high temperature to increase the emission intensity. To prevent sintering during annealing and to facilitate biotin functionalization, nanoparticles are encapsulated in a silica shell. We study generating and collecting light through tissue by exciting nanophosphors using a focused X-ray source and ability to use as an MRI contrast agent. The nanoparticle size is ~100 nm. x-ray excited optical luminescence (XEOL) showed low emission intensity at low- and high-dopant levels. Hydrothermal treatment and annealing without a silica-shell increased the emission intensity. However, silica-shell decreased it. After annealing silica-coated $NaGdF_4$: Eu, there is no significant increase in intensity, the structure changed to Eu-doped sodium-gadolinium-silicate. We confirmed biotin-functionalization by attaching nanoparticles to streptavidin in vitro. Light-generating and collecting through tissue were confirmed using XEOL and x-ray excited luminescence chemical imaging. The particles served as MRI contrast agents. In the future, we plan to optimize synthesis and annealing protocols to obtain bright nanophosphors. To study C. elegans x-ray avoidance behavior, wild-type is exposed to 0-1Gy/s focused x-ray beam to determine

the dose-response relationship. Then, wild-type and dysfunctional Lite-1, Gur-3 and both Lite-1 and Gur-3 C. elegans are used to discover the photoreceptor that responsible for x-ray stimulation and pmyo-3::Lite-1 is used for x-ray stimulated egg ejection by exposing to 1Gy/s focused x-ray radiation. The above experiments proved that C. elegans x-ray avoidance behavior is proportional to x-ray dose and Lite-1 photoreceptor is responsible for this behavior. Further, 50% of pmyo-3::Lite-1 ejected eggs after x-ray on. In the future, we will reveal whether Lite-1 has the potential to control neurons in mammalian-cells.

The Effect of Laser Fluence on Surface Morphology During a (ns) Pulsed Laser Ablation Process of a Low Melting Point Alloy

Presenting Author: Tariq Alharby, MSc - University of Missouri-Kansas City

Non-Presenting Author: Omar Musaev, PhD - University of Missouri-Kansas City

Non-Presenting Author: Paul Rulis, PhD - University of Missouri-Kansas City

For first time, BiSn alloys ablated to observe surface morphology modifications as laser fluences differ.

The surface of multiple Bismuth-Tin (BiSn) alloys with different weight percents was irradiated with a (ns) pulsed excimer laser with a wavelength of 351 nm and a pulse duration of 20 ns. Each alloy was ablated with different laser fluences where the energy of the laser pulses ranged between 2 mJ to 100 mJ. Each location on the surface was ablated with only one laser shot with a specific laser fluence. The morphology of the ablated craters and their vicinities was analyzed using scanning electron microscopy (SEM) and energy dispersive spectroscopy (EDS) techniques. The aim of this research was to investigate the effect of different laser fluences on the morphology of the ablated low melting point surfaces with different weight percents including a eutectic composition. Due to the low melting points of the ablated targets, there were some manifest hydrodynamic effects in the ablated areas. This is due to the fact that the time interval that is required for the molten materials to solidify is large enough for this phenomenon to occur. Plus, the recoil pressure taking a place during the ablation process resulted in a radial liquid metal flow. Therefore, the solidification of the melt flow is also the reason for the formation of rims around the craters. Moreover, there was another solidification of capillary waves in the liquid film observed in the vicinity of the craters which is due to the mechanical shock from the recoil pressure. The SEM images showed a micro sized droplets in the vicinity of the craters. These could be ejected in the early stage of the melt flow when the viscosity is low. It was observed that as the weight percent of Bi increased in the alloy, the hydrodynamic effects; especially the solidified melt, became more pronounced in the vicinity of the craters. However, as the weight percent of Sn became the dominant in the composition of the alloy, higher rims around the craters were observed.

Trace Metal Analysis of Arabidopsis thaliana shoots by handheld XRF

Presenting Author: Eva-Maria Rudler- Saint Anselm College

Non-Presenting Author: Eva-Maria Rudler- Saint Anselm College

This research will allow for method development to be used further in research.

Toxic metals exist in excess in our soils today due to both natural sources and human activity. As plants grow on these contaminated soils, it is important to determine how much of these toxic metals they are taking into their systems as they may ultimately be incorporated into the shoots and seeds. Additionally,

it is important to observe how toxic metal exposure influences the concentrations of essential metals in these plants. The goal of this research project is to develop a method to determine metal concentrations in Arabidopsis thaliana shoots after growth on wild-type media and media containing cadmium using the handheld XRF Bruker Tracer III-V +. A standard addition protocol for solid powdered samples will be made for comparison and analysis in regards to iron, manganese, cadmium, and zinc. This method has been used before, for quantification at the ppm level. Data will be presented and results will be discussed.

Multi-sensor Imaging by LIBS

Presenting Author: Jhanis J. Gonzalez, PhD - Applied Spectra, Inc. / Lawrence Berkeley National Laboratory

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Over the last several decades, laser-induced breakdown spectroscopy (LIBS) has gained significant momentum in the scientific community, becoming an invaluable analytical spectroscopic technique for many applications. LIBS has several advantages over other analytical spectroscopic techniques. These include little to no sample preparation, rapid assessment and processing, and the ability to work at room temperature and under ambient pressure conditions. More importantly, LIBS can simultaneously detect organic and inorganic elements with either 2D (spatial mapping) or 3D (depth profiling) characterization. Another significant asset of this technique is the multisensory capability and its acquisition speed. Multiple sensors could be used to simultaneously collect information from the Laser-Induced Plasma, and the particles generated after the laser-material interaction. The speed of producing chemical images using LIBS is only limited by the laser repetition rate (up to kHz) and the detector speed. During this presentation, the imaging capability and the multisensory approach of LIBS are demonstrated with a demonstration of the powerful software for data and imaging processing called Clarity Image Plus by Applied Spectra, Inc.

Biomedical and Bioanalytical

A 3D Fluorescence Anisotropy Matrix Method for Characterization of Small Molecule Interaction with Macromolecules

Presenting Author: Karen E. Gall, PhD - HORIBA Scientific

Non-Presenting Author: Alex Siemiarczuk, Ph.D. - HORIBA Scientific

A fluorescence anisotropy matrix method differentiates small molecule local environments in polymers or protein solutions.

Fluorescence anisotropy is a method that has long been used for measuring the reorientation of fluorescent molecules and the physical properties that cause change to this type of molecular motion: size, binding/dissociation, local viscosity, encapsulation/release, and temperature. Fluorescence excitation emission matrices (EEMs) are used widely for detection and identification of components within mixtures of molecules. We present a method of using a fluorescence anisotropy excitation emission matrix with CCD-detection, for measuring mixtures of two fluorescent reporters and how they associate differently with macromolecules. In addition, the fluorescence signals that go into calculating the anisotropy ratios are measured simultaneously with absorbance/transmittance. From the absorbance data, the fluorescence signals are corrected for inner- filter effects to prevent non-linearity in measurements of high concentrations of fluorophore in some solutions. Fluorescence anisotropy absorbance-transmittance excitation emission matrices (FA-A-TEEM) is the resulting acquisition. Solutions of a hydrophobic dye and a hydrophilic dye are combined with an A-B-A triblock copolymer in micelle form and in unimer form of the polymer. The FA-A-TEEM matrices show the anisotropy at different wavelength profiles as a function of the two reporters and the local environment within different regions of the polymer solution. Using the same two dyes with a solution of bovine serum albumin protein, a biomolecule with known hydrophobic pockets, FA-A-TEEM can elucidate the local region of specificity for a hydrophobic small molecule and a hydrophilic small molecule in the same matrix profile. This method is presented as a potential use in the study of small molecule behavior within macromolecule and protein formulations.

Accessing the Metabolic Activity of Single Heterotrophic Bacterial Cells via Raman Microspectroscopy and Stable Isotope Labeling

Presenting Author: Georgette Azemtsop Matanfack - 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

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Deuterium uptake is strongly influenced by the organic carbon source and bacterial identity.

Understanding of metabolism is essential to unravel the basic mechanisms of physiological and pathological processes. Hence, the need for a rapid and reliable method to identify the metabolic activity state of bacteria in their natural habitat is of great concern in clinical diagnosis, pharmaceutical manufacturing, and environmental microbiology. However, in-situ metabolic tools are still lacking, due to the large diversity of bacteria, the complexity of interactions between community members and the influence of environmental factors [1]. Although Raman microspectroscopy has a high specificity to provide intrinsic fingerprint information about the molecular composition of bacterial cells, stable isotope labeling with deuterium improves the spectral sensitivity and specificity, due to the emergence of a C-D stretching vibration band in the Raman silent region, when hydrogen is exchanged by deuterium . By adding a deuterated substrate such as heavy water (D_2O) to cell culture medium, deuterium uptake can be traced [2]. Since hydrogen is one of the essential element of biomolecules, the

concentration of deuterium uptake can serve as robust indicator for substrate assimilation in bacteria; hence, this indicator can be used to characterize and identify microorganisms responsible for the metabolism of specific substrates [2, 3]. Here we combined Raman microspectroscopy with deuterium labeling to probe metabolic activity in bacterial cells under various carbon source conditions. We present two classification approaches to differentiate between deuterium labeled and non-labeled cells. Our technique is suited to single cell analysis; it underlines the influence of carbon source on deuterium uptake and promotes a better understanding of the heterogeneity and functions of heterotrophic bacteria.

 References [1] G. Azemtsop Matanfack, M. Taubert, S. Guo, R. Houhou, T. Bocklitz, K. Küsel, P. Rösch, J. Popp, Anal. Chem. 92, 11429−11437 (2020). [2] M. Taubert, S. Stöckel, P. Geesink, S. Girnus, N. Jehmlich, M. von Bergen, P. Rosch, J. Popp, K. Küsel, Environ. Microbiol. 20, 369-384 (2018). [3] K. Yang, H.-Z. Li, X. Zhu, J.-Q. Su, B. Ren, Y.-G. Zhu, L. Cui, Anal. Chem. 91, 6296-6303 (2019).

Advances in optical photothermal infrared (O-PTIR) spectroscopy and imaging to investigate cortical and trabecular human bone at submicron spatial resolution

Presenting Author: William Querido- Temple University
Non-Presenting Author: Nancy Pleshko, PhD - Temple University

Optical photothermal infrared (O-PTIR) spectroscopy and imaging reveals details of bone composition at submicron resolution

Bone strength depends on the quality and quantity of bone, factors that are linked to fracture risk. These include bone geometry, density and tissue-level composition. Over recent decades, advances in infrared spectroscopy and imaging have supported assessment of bone tissue composition. However, traditional Fourier transform infrared (FTIR) methodologies are limited to a spatial resolution of ~10 microns, limiting the detection of features of individual tissue structures. Here, we applied novel optical photothermal infrared (O-PTIR) methodology to evaluate human bone tissue composition at the submicron scale, focusing on structural units forming cortical and trabecular bone. Cadaveric femoral neck samples were analyzed, as quality changes in this region are linked to fracture risk. They were cut into 1 mm thick sections and cleaned to remove excess interstitial fat. The bone slices were analyzed using the mIRage O-PTIR microscope (Photothermal Systems). Visualization of bone tissue structures and infrared data were obtained with 200 nm point spacing from individual osteons and trabeculae. The spectra had high signal-to-noise quality and the primary components of bone tissue were identifiable. Absorbance peaks of amide I (1665 cm-1), amide II (1550 cm-1), collagen (1338 cm-1), phosphate (1040 cm-1) and carbonate (875 cm-1) were apparent in both cortical osteonal bone, and trabecular bone. Single wavenumber scans were used to obtain highly detailed images showing the distribution of each component within individual osteons and trabeculae. For example, it was possible to see the concentric distribution of mineral in the osteon lamellae, as well as mineral alignment parallel to the trabecula axis. Further analysis of the spectra allowed quantification of compositional properties, including degree of mineralization, collagen content, and carbonate substitution in mineral. Differences between osteons and trabecula were evident using this approach. This preliminary study highlights the straightforward application of O-PTIR spectroscopy and imaging to reveal compositional properties of cortical and trabecular bone with submicron spatial resolution. Further, in contrast to other spectral imaging methods, processing of bone tissue was not required for these data. Correlations of specific

metrics with bone strength may yield insight into aspects of molecular structure that relate to fracture risk.

Classification of cell types within ovarian tissue using optical-photothermal imaging

Presenting Author: Chalapathi Charan Gajjela - Research Assistant

Non-Presenting Author: Rohith K Reddy - Assistant Professor

Enhancement in spatial resolution for observing different cell types and structures using Mid-infrared spectroscopic imaging

A pathologist performs ovarian cancer diagnosis after histological staining of tissue and examination under a visible microscope. The identification of the morphology of epithelial and stromal cells using Hematoxylin and Eosin (H&E) stains is an important first step and constitutes the current standard of care. Cancer change both morphology and biochemical properties of different cell types in the tissue. Mid-infrared spectroscopic imaging (MIRSI) provides both bio-chemical and morphological information without stains and is a promising technique for ovarian cancer diagnosis. However, the major limitation of the previous generation MIRSI technologies such as Fourier Transform Infrared (FT-IR) imaging is its spatial resolution due to the relatively long wavelengths used. We overcome this limitation by vibrational excitation with a mid-infrared laser and probing the absorption with a visible laser through the thermal lensing effect. In our current work, we study ovarian cancer tissue using the aforementioned imaging technique, Optical Photothermal Infrared Imaging(O-PTIR)). We utilize the order of magnitude improvement in spatial resolution by using the new technique and combine it with spectral information. This enhancement in resolution paves the way in observing different cell types and certain subcellular structures like nuclei, which were not detected with previous MIRSI techniques. In our current work, we use different machine learning algorithms on the data obtained to perform classification of epithelial and stroma cell types within ovarian tissue.

Computational Modelling of Vibrational Spectroscopy in Bone and Connective Tissues

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Non-Presenting Author: Alexander Dumont

Non-Presenting Author: Shital Kandel- Temple University

Non-Presenting Author: Nancy Pleshko, PhD - Temple University

Monte Carlo (MC) modeling is a valuable tool for gaining a fundamental understanding of light-tissue interactions, guiding and evaluating the optical design of probes, preliminary simulation of translational studies, and informing analysis of empirically measured data. The use of MC simulations has accelerated the clinical development of diffuse reflectance-based techniques used to assess bulk tissues such as the breast and brain, however state-of-the-art approaches for computational modeling are not widely utilized in the development of vibrational spectroscopy of the bone and connective tissues. MC models capable of guiding translational development of vibrational spectroscopy should include the ability to simulate a wide spectral range, faithfully reproduce irregular tissue microstructures, incorporate background signals, which can often complicate in vivo measurements of bulk tissues, and finally meet some important practical considerations such as fast and flexible implementation. In order

to address these issues, an MC model for NIR vibrational spectroscopy and Raman spectroscopy has been developed atop the Monte Carlo eXtreme (MCX) framework. The MCX framework utilizes GPU-parallelization to dramatically increase computational throughput, which facilitates simulation across a multitude of biochemical vibrational modes, tissue architectures, background signals, and illumination/collection geometries. A novel MC model for Raman spectroscopy will be described, along with validation in mineralized and collagenous tissue phantom models. Moreover, the ability of GPU-accelerated MC modeling to inform the relative contribution of collagen in bulk tissues including both bone and cartilage will be discussed. Furthermore, the resulting implications for spectral analysis and comparison of probe configurations will be addressed. As advancements in musculoskeletal applications of vibrational spectroscopy continue to evolve, the concurrent development of computational modeling described here represents a valuable approach to further accelerate advancements in the field.

Confocal-Raman Microscopy Investigation of His-tagged Protein Capture by Nickel-Ligands on Hybrid Supported Lipid Bilayers

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Non-Presenting Author: Grant Myres - University of Utah

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This work demonstrates a unique method to detect and characterize protein accumulation at lipid bilayers.

One of the most vital interactions in protein purification is between the amino acid histidine and immobilized transition-metal ligands, which form weak coordinate bonds. Histidine residues can be easily added to either the C or N terminus of proteins prior to expression, creating a tag which allows modified proteins to be separated with high specificity. While traditionally used for protein separation schemes, the flexibility and specificity of this interaction lends itself to more complex protein binding assays. It is important to understand the dynamics of this binding scheme, and potential benefits and limitations as a basis for immunoassay or biosensing applications. Traditionally, protein purification is performed on agarose hydrogels. While this technology is sufficient for capture and release, the agarose support is not ideal for access to immobilized protein, a requirement for biosensing applications. An alternative support can be created by forming a lipid bilayer on the interior surface of highly porous silica chromatographic particles. Nickel-modified lipids are easily incorporated into a fluid-phase bilayer during preparation, creating a controllable amount of mobile binding sites. Raman microscopy can then be used to probe the interior surfaces of these particles. Because of the high specific surface area of these particles, the local concentration of lipid and protein in the confocal probe volume within the particle allows the weak Raman scattering from these interfacial molecules to be detected without the need for exogenous tags or plasmonic enhancement. Raman microscopy also gives important structural information about the lipid surface and bound protein, allowing structural changes upon binding to be observed. This work examines the behavior of 6-histidine tagged protein G at Ni-NTA decorated POPC supported bilayers within porous silica particles. This configuration enables in situ observation of protein binding kinetics in real time, without the need for removing samples for ex situ analysis. POPC lipid peaks act as an internal standard, allowing for quantitative determination of protein coverage at lipid surfaces. Through these methods binding valency, binding strength, and binding kinetics are examined.

Fast Infrared Imaging for the Early Detection of Cancer

Presenting Author: Thomas J. Tague, Jr., PhD - Bruker Scientific, LLC
Non-Presenting Author: Stephen Luettjohann - Bruker Scientific, LLC

Rapid Ultrahigh signal-to-noise IR imaging over the full region of the MIR

Early detection and accuracy are key elements to the successful characterization of cancer. The earlier the detection is conducted, the more successful the outcomes. Infrared (IR) spectroscopy is an emerging technique in the detection of cancer. Most importantly, changes in cell biochemistry precede any morphological manifestations allowing for earlier detection of disease. This would include not only the detection of significantly diseased tissue, but also early lesions and tissue dysplasia. Traditionally, light microscopy has been a key element of the analysis of tissue samples by pathologists. The interpretation of tissue and tumors containing borderline or indeterminant changes can lead to a false negative assessment. FTIR has been used successfully to assess the presence of cancer in the cervix, colon, pancreas, and other tissue. Full field IR imaging provides an alternative that can provide a non-subjective analysis in the early detection of cancer over a large area. No tissue fixation or staining is required and the cost per test can be inexpensive. For this investigation, a LumosII (Bruker Corporation) was utilized to conduct fast full-field FPA analysis of various relevant tissue samples over the full middle IR region. Data was collected over a 5.12mm2 area yielding 1.05 million spectra in total with a pixel resolution of 5 µm. A SiC IR source was utilized as well as a 32x32 pixel focal-plane array PV-MCT detector. The spectral range was 5000-750 cm-1 collected at a spectral resolution of 4 cm-1. The total acquisition time was 35 minutes. The benefits of utilizing the infrared spectrum are clear in that the presence and distribution of each component of the colon are illustrated to facilitate the assessment of the tissue. Each molecule has its own "fingerprint" signature in the middle infrared region of the spectrum (400-4,000 cm-1). Because of this high degree of specificity, small changes to tissue can be detected. Examples will also be shown of the potential for kidney and bone tissue analysis in the detection and characterization of cancer.

Glutathione detection in blood: a trial of strength between Mass Spectrometry (MS) and Surface Enhanced Raman Scattering (SERS)

Presenting Author: Isabel Ten-Doménech, PhD - Neonatal Research Unit, Health Research Institute La Fe

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Paper-based devices for glutathione monitoring: UPLC coupled to mass spectrometry vs SERS

Glutathione (GSH) is the most abundant non-enzymatic antioxidant in most cells and tissues, including whole blood, and its usefulness as a biomarker for oxidative stress monitoring has been known for

decades. However, its accurate quantification remains challenging due to the limited stability of GSH in biofluids. Here the strengths and weaknesses of two complementary approaches for the quantification of GSH in whole blood samples are compared: 1) ultraperformance liquid chromatography coupled to tandem MS (UPLC-MS/MS) from dried blood spot (DBS) samples after in-situ derivatization employing N-ethylmaleimide (NEM) and 2) the direct determination based on SERS employing a portable Raman probe. Our results show that DBS cards impregnated with NEM solution allow the simultaneous quantification of reduced and oxidized GSH. NEM-DBS cards are stable during at least one week and 10 µL blood samples can be stored after in-situ derivatization during at least 24h at 4 ºC with no significant changes in the GSH and GSSG concentrations. On the other hand, the use of an internal standard (GSH-13C15N) allows the accurate quantification of GSH in 5 µL whole blood samples by SERS within minutes with minimal sample manipulation, by using a paper-based device with deposited silver nanoparticles suitable for point-of-care testing. Both methods will be tested for GSH monitoring in newborns and their performance will be compared in the near future.

Large-Scale Photothermal Microscopy Using Smart Compressive Sampling

Presenting Author: Mahsa Lotfollahi, PhD - University of Houston

Non-Presenting Author: Nguyen Tran - Research Assistant

Non-Presenting Author: Sebastian Berisha - ASSISTANT PROFESSOR

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Non-Presenting Author: Zhu Han - Professor

Non-Presenting Author: Rohith K Reddy - Assistant Professor

Corresponding Author: Mayerich Mayerich - Assistant Professor

Propose an adaptive sampling method for HSI reconstruction. Leverage a pre-trained classifier as a metric.

Fourier transform infrared (FTIR) spectroscopy is extremely common for label-free molecular identification and quantification. However, long optical wavelengths (2.5-12.5 □m) used in FTIR imaging place a severe diffraction limit on resolution. This limit is particularly limiting in materials and biomedical imaging. Photothermal imaging overcomes this diffraction limit by using a multimodal pump/probe approach. The sample is excited using a mid-infrared quantum cascade laser source, while localized absorbance is measured using an atomic force microscope (PTIR) or secondary high-resolution beam (O-PTIR). These measurements require approximately 1s per spectrum, making them impractical for large samples. This paper introduces an adaptive compressive sampling technique to dramatically reduce image acquisition time by taking advantage of both spectral and spatial sparsity. This method identifies the most informative spatial and spectral features and integrates a fast tensor completion algorithm to reconstruct megapixel-scale images while competing with traditional FTIR speed.

Live-cell FTIR spectroscopy for pharmaceutical research

Presenting Author: K. L. Andrew Chan, PhD - King's College London

This presentation shows three novel applications of FTIR spectroscopy of living mammalian cells.

Analysing drug using cell-culture models has been an important tool for the assessment of the

effectiveness of drug. However, most biochemical assays are laborious, destructive and costly to run. A label-free screening tool that can directly measure the chemical compositional changes in cells as a function of treatment of drug may help to streamline the search of new drug candidates, hence reducing the time and cost for the overall drug development process. In this presentation, we will provide with three examples of live-cell FTIR applications for studying drug-cell interactions. We will first discuss how live-cell FTIR data, in combination with principle component analysis, can potentially be used to identify the mode of action of anti-cancer agents. We have shown that the technique can both identified the different responses from MDA cells to drugs of different modes of actions and the different responses from MDA and MCF-7 cells against the same drug. Second, we will show that live-cell FTIR measurements can detect the biochemical changes inside HepG2 cells when they become diabetic after exposing in high glucose medium for 24 hours. Finally, we will show that live-cell FTIR can quantify the amount of an inhaled drug in Calu-3 cells and study the effect of glycerol to the permeation of the drug.

Macrophage stimulation by Cold Atmospheric Plasma and Electroporation

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Non-Presenting Author: Jordan Hoops - South Dakota School of Mines & Technology

Non-Presenting Author: Timothy M. Brenza, PhD - South Dakota School of Mines & Technology

Non-Presenting Author: Rajesh Sani, Post Doc. - South Dakota School of Mines & Technology

Through CAP and EP therapy, a mechanism of inflammation can be established.

The molecular oxygen known for its exceptional electron acceptor abilities within the mitochondria can be infiltrated throughout its incomplete reduction. Certain cells are more susceptible to this potential for damage and diseases are constantly linked to this production. Type I Diabetes, which affects millions of individuals in the United States and around the world, is believed to stem from this attack, the overproduction of these species lead to a signal released by the mitochondria of β and it is followed by a cytotoxic attack of T-cells. Recent research studies have indicated that the increase of an immune response when faced with an attack by ROS generated by Cold Atmospheric Plasma (CAP) and Electroporation (EP). In the presentation an in vitro study of the stimulus of immune cells faced with ROS and the mechanism of their response is explored using experimental and stimulation approaches. THP-1 macrophages are used and differentiated by DMSO and PMA. This will lead to an idea of how pancreatic β cells are destroyed and what steps could be taken to mitigate the early damage. The stimulus of the immune responses has potential to be a positive process resulting in the protection and potential restoration of function in β cells. Modeling via Modeller v9.23 provide a bioinformatic perspective in silico. Experiments presented will establish a threshold reactivity of CAP and EP within a redox environment common to T1D.

Multimodal Fiber Spectroscopy in 0.3-16μm range for Industrial and Biomedical Applications

Presenting Author: Viacheslav Artyushenko - art photonics GmbH

Corresponding Author: Olga Bibikova, PhD - art photonics GmbH

Non-Presenting Author: Iskander Usenov - art photonics GmbH

Non-Presenting Author: Tatiana Sakharova - art photonics GmbH

Several examples of multimodal fiber optic spectroscopy approaches are presented for industrial and biomedical applications.

Fiber optics spectroscopy provides compact, flexible, and cost-effective solutions to match the fast grown demands of industrial process control, remote environment monitoring and biomedical diagnostics. Fiber probes enable industrial process analysis in-line in so harsh environment which is not possible by common lab analysis, as it does not need sampling at all and can be done at high or low temperatures, in vacuum or under high pressure and vibrations, robust fiber probes are resistant to aggressive or toxic media and cleanable with approved solvents, they can be easy installed and removed. Customized probe design and length can be adapted to various process installations and process-interfaces with optical coupling to different spectrometer types: FTIR, QCL, NIR, Raman, Fluorescent, etc. We present the latest results on the development and application of fiber optic probes designed for the key spectroscopy methods used in a 0.3-16µm range for the analysis of media ATR-absorption, transmission or transflection, Raman scattering, diffuse reflection, fluorescence, etc. Several examples of multimodal approaches are presented for industrial and biomedical applications. Multichannel NIRaman fiber optic probes have been developed by art photonics in cooperation with Company M.A.C. (Measure Analyse Control BV) to measure in-situ NIR diffuse reflectance and Raman scattering of solids, powders, or liquids. This combi simultaneous measurement enables the hybrid modelling opportunities from the data fusion of these methods to enhance media analysis accuracy - that was impossible for them used separately. The other innovative combi probe was designed for simultaneous analysis of various liquid and solid samples using ATR-absorption in Mid InfraRed in combination with fluorescence spectra collected from the same spot. Complimentary data from Mid IR-absorption bands can be fused with fluorescence spectra to provide much better media composition analysis. The latest innovation in Mid IR-fiber spectroscopy comes now from very effective optical coupling of fiber probes with Quantum Cascade Lasers (QCL) - in huge contrast with their poor optical coupling with FTIR-spectrometers. A high coupling efficiency of IR-fibers and Hollow Waveguides with QCL will open the new horizon of spectral fiber sensors in broad range of various applications in industry, medicine and environment monitoring.

New techniques for tissue subtype identification with mid-infrared spectroscopic imaging

Presenting Author: Rohith Reddy- University of Houston

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We present for identification of ovarian tissue subtypes using O-PTIR imaging

Mid-Infrared spectroscopic imaging (MIRSI) enables biochemical identification in tissue. MIRSI combines the molecular specificity of mid-infrared vibrational spectroscopy with the spatial detail afforded by microscopy to provide biochemical maps of samples. This allows us to analyze biochemically heterogeneous samples such as cancerous tissue. Previous work has shown that machine learning on biochemical spectra signatures at each pixel can be used to perform diagnosis in several cancers including prostate, breast and colon cancer. We will present data analysis methods including supervised machine learning techniques in the context of cancer diagnosis and tissue sub-type identification. The advent of Quantum Cascade Lasers (QCLs) has resulted in novel imaging modalities with important advantages over traditional Fourier Transform Infrared (FT-IR) imaging. Moreover, techniques such as nano-IR or AFM-IR and photothermal IR have made it possible to perform MIRSI on the nano-scale. Optical photothermal infrared (O-PTIR) imaging is capable of providing high resolution images of tissue in a non-contact manner and has shown promise in biomedical diagnostics. Here, we present results from applying O-PTIR to problems in ovarian cancer diagnosis. We utilize both the spatial and spectral dimension of MIRSI data to perform tissue subtype classification in samples from 100 ovarian cancer patients.

Novel Disposable Attenuated Total Reflection (ATR) Substrate for Rapid Throughput Screening of Malaria.

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Novel modality for rapidly detect and quantify the malaria parasitemia in combination with Chemometric tool.

In this study, a novel, cost-effective and disposable Attenuated Total Reflection (ATR) substrate for rapid throughput screening of malaria is reported. Malaria has been creating a havoc with an estimated 300 million cases, resulting in 0.5 - 1 million deaths annually. The fingerprinting capability of ATR spectroscopy has already demonstrated the prognostic potential and facilitated the translation of the technology as a point-of-care (POC) diagnostic tool in resource-limited areas. ATR spectroscopy is known to be a convenient alternative to transmission spectroscopy for fluid specimens, as it yields a constant and reproducible effective optical path length and hence good sample-to-sample precision. However, after each measurement the ATR crystal or internal reflection element (IRE) requires to be cleaned and continuous collection of data can be difficult. As an alternative, herein, a novel ATR substrate was implemented mainly to reduce the sample preparation time as multiple samples can be prepared at once and dried together. Hence, the need to wait for the drying of every sample on the IRE can be overcome. The proposed substrate's performance was compared and validated with the traditional 'direct IRE' sampling method, where samples are directly placed on IRE, using signal to noise ratio (SNR) as a determining factor. The performance was statistically investigated and compared using Bland-Altman analysis. The Bland-Altman plot demonstrates that the smaller confidence intervals of mean difference and Limit of Agreement (L.O.A) (between -7.0 and +0.7) substantiates that there is a certain agreement between substrate and direct IRE method without any unambiguity. The novel modality was able to rapidly detect and quantify the parasitemia using ATR in combination with partial

least-squares regression (PLS) and the value of Root Mean Square Error of Cross Validation (RMSECV) and R2 were found to be 0.00342 and 0.996. Hence, the substrate is eminently suitable for the rapid throughput screening of malaria.

Orientation Matters: Polarization Dependent Infrared Spectroscopy of Collagen from Intact Tendon to the Single Fibril

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First combined FTIR-FPA, Optical Photothermal IR (O-PTIR) and sSNOM IR of tendon and isolated fibrils

Infrared (IR) spectroscopy has been used for decades to study collagen in mammalian tissues. While the intensities of many bands change under polarized IR light, all the absorption bands are naturally broad because of tissue thickness and heterogeneity. We have acquired spectra of intact tendon under 0 and 90 degree polarized far field (FF) FTIR imaging with a Focal Plane Array detector, and with the relatively new method of FF Optical Photothermal IR (O-PTIR). Polarized IR spectra of isolated fibrils were obtained with O-PTIR and with IR scattering-type scanning near-field optical microscopy (s-SNOM). Only the latter method enables spectroscopy of fibrils < 100 nm diameter. The FF methods were applied to sections of intact tendon with fibers aligned parallel and perpendicular to the polarized infrared light. The O-PTIR and sSNOM methods were applied to individual fibrils of 100-500 nm diameter. Taken together, the fibril spectra show the first confirmatory results for polarization perpendicular to fibril axis. Further, we also obtain complementary results, in that O-PTIR also enables polarization parallel to fibril axis. The Amide I and II bands from fibrils were narrower than those from intact tendon, while relative intensities and band shapes were altered. Variations are considered with respect to sub-fibril molecular structure and organization. These spectra represent reliable profiles for collagen type I fibrils of this dimension, under polarized IR light, and can serve as a benchmark for the study of collagenous tissues in health and disease.

Polyacrylic acid-based hydrogel as an injectable biosensor to radiographically measure pH in tumor microenvironment

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Injectable hydrogel biosensor for noninvasive monitoring of pH in tumor tissues by X-ray radiography.

An injectable biosensor was developed to measure pH in tumors noninvasively using a polyacrylic acid-

based hydrogel and the sensor was imaged by X-ray imaging technique. Measurement of pH by the developed sensor would enable to monitor disease response to treatment and track tumor progression. The pH in tumor tissues is heterogeneous with acidic regions typically in the range of pH 6.5 to 6.9 due to increased anaerobic glycolysis and poor clearance of metabolic acids such as lactic acid. As a result of this acidity, tumor tissues show more resistance to chemo- and radiotherapy, contribute to metastasis, and may influence immune responses. Therefore, pH monitoring in tumor tissues by the developed sensor can be used to study cancer pathophysiology and treatment effects. The sensor consists of a pH-responsive polyacrylic acid hydrogel which decreases in length at low pHs. The hydrogel was embedded with a radiodense tantalum bead and inserted in a porous metal sleeve, and they were all fitted inside a breast cancer biopsy marker needle. A breast cancer biopsy marker needles are used in needle biopsy procedures and it can be used to inject the sensor into the tumor tissue. At low pH of tumor tissues, the hydrogel would contract, causing the tantalum bead to move within the metal sleeve. The extent of motion of the bead can be determined using X-ray radiography. The dynamic range of the 5 mm sensor was determined to be between pH $4 - 8$ with a precision of 0.07 pH units. It also showed a repeatable response to pH cycling between pH 6.5 to 7.5 in bovine serum, most relevant to tumor acidosis. In future, the developed hydrogel-based biosensor will be tested in an animal tumor model to noninvasively measure tumor pH radiographically, in vivo. Thus, the sensor can be used for monitoring and studying of tumor microenvironment.

Probing Molecular Structure Of Neurotoxic Amyloid β Oligomers

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This contribution provides nanoscopic insights into the surface conformation of neurotoxic amyloid β oligomers

Soluble small oligomers of Aβ (1-42) peptide are neurotoxic and represent early biomarkers of AD for

their potential role in diffusing toxic species before the late aggregation process occurs. Probing molecular structure of such toxic oligomers is of utmost importance to understand the mechanism by which the early events of neurodegeneration trigger the irreversible neuron death. So far, the disordered nature of Aβ peptide has made any proposal for a structural model for oligomers extremely difficult and many empirical experiments still result controversial. A synergistic combined approach of Raman spectroscopies and extended molecular models is herein adopted to inspect structures and sub-molecular features of the conformation of toxic globular oligomers differentiating them from the non-toxic species [1,2]. Specifically, UV and visible Raman provide the average structural characterization with extent of secondary motifs and surface localization of aromatic amino acids in the toxic oligomers. On parallel, Raman assisted by localized plasmon resonance at the hot spots of intertwined silver nanowires is exploited to analyse structural features present on the surface of Aβ oligomers that appear critical in driving molecular interactions for neurodegeneration. Our investigation enabled to discriminate among different species of toxic and non-toxic Aβ oligomers, providing characteristic motifs of the aberrant conformers that were unravelled and further confirmed by computational models, while specific amino acid side chains associated with the toxic Aβ species that have so far escaped detection with other techniques were unambiguously identified [3].

 REFERENCES [1] D'Andrea C. et al. Nanoscale discrimination between toxic and nontoxic protein misfolded oligomers with tip-enhanced Raman spectroscopy. Small (2018), 14, 36. [2] La Penna, G. et al. Computational models explain how copper binding to amyloid-peptide oligomers enhances oxidative pathways. Phys. Chem. Chem. Phys. (2019), 21, 8774-8784. [3] Banchelli M. et al. Nanoscopic insights into the surface conformation of neurotoxic amyloid β oligomers. RSC Advances (2020), 37, 21662-22290.

Radiographic measurement of synovial fluid pH using an implantable hydrogel sensor for early detection of prosthetic hip infections

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X-ray radiographIc detection of post-surgery hip infections by an implantable synovial fluid pH sensor

We describe an implantable sensor developed to measure synovial fluid pH for non-invasive early detection and monitoring of hip infections using plain radiography. While hip replacement surgeries are generally safe and effective, about 1% of prosthetic hips become infected. If infections are not detected and treated promptly, the implant must be removed followed by reinsertion of the medical device after the infection is eradicated. During infection, studies show that in a well-mixed synovial joint fluid, pH correlates with white blood cell count and pH decreases from 7.5 to around 6.7 with a threshold around 7. The sensor developed can be used to measure synovial fluid pH in order to detect and monitor hip infections using plain radiography (X-ray imaging) which is already routinely acquired during patient follow up visits. The sensor was made of a pH responsive polyacrylic acid-based hydrogel, which expands at high pH and contracts at low pH. A radiodense tantalum bead and a metal wire were embedded in the two ends of the hydrogel in order to monitor the change in length of the hydrogel sensor in response to pH via plain radiography. The pKa of the hydrogel-based pH sensor was 5.25 with

a sensitivity of 0.3 cm/pH unit. The sensor showed a linear response and reversibility in the physiologically relevant pH range of pH 6.5 and 7.5 in both buffer and bovine synovial fluid solutions. The sensor was attached to a hip prosthetic implant and the change in length in response to pH was determined from the X-ray images by measuring the length between the tantalum bead and the radiopaque wire. Therefore, the developed sensor would enable noninvasive detection and studying of implant hip infection using plain radiography.

Raman Spectroscopy Based Molecular Bar Coding: Realizing the Value of High Wavenumber Region in Breast Cancer Assessment

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Our HWN ratiometric approach is simple, interpretable and exhibits distinct barcodes for different tissue.

There are over 300,000 new cases of breast cancer per year in the United States and breast conservation surgery (BCS) is a standard of care for treatment of early-stage breast cancer. Even with advancements in the standard of care, repeat surgery is necessary in 20% to as high as 40% of BCS cases due to the histopathological determination of positive margins days or even weeks after surgery. Cytological analysis such as touch preparation and frozen section histopathologic assessment are practiced, but these approaches are inadequate owing to sensitivity and specificity concerns, sample preparation artifacts and sampling errors. Notably, these methods are time-consuming and not practical for intraoperative time scales. Consequently, there is an urgent need for an intraoperative tool that can quickly, accurately, and non-invasively evaluate the margins of resected breast tissue to allow complete removal of all cancer tissue during primary surgery, reducing and ultimately eliminating the need for secondary procedures. Raman spectroscopy has been successfully employed to provide biomolecular attributes of cells and breast tissue under various benign and pathophysiological conditions. While Raman spectroscopy has demonstrated potential in breast cancer detection, it usually utilizes the fingerprint region and use of the rich spectral content in the high wavenumber (HWN) region is largely ignored. The HWN spectral region typically exhibits lower intrinsic fluorescence from the tissue and is compatible with glass substrates, facilitating easy integration into the pathological workflow, as well as the potential for low instrument cost. The "underappreciated" broad HWN region contains important information of characteristic stretching vibrations of biomolecules. Here we present a ratiometric intensity analysis of the Raman spectrum in the HWN region of three breast tissue conditions, namely adipose (fat), fibrous and malignant in a broad sampling of lumpectomy specimens. We demonstrate that these intensity ratios can be used as a "molecular barcode" in breast cancer pathology. Our ratiometric-based approach in HWN is simple, interpretable and exhibits distinct barcodes for different tissue types.

Raman spectroscopy characterization of the major classes of plasma lipoproteins

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Raman spectroscopy proves to be a rapid tool for the characterization of human lipoproteins

Raman spectroscopy has been vastly employed for the characterization of different bio-molecular species spanning from single protein to the in-vivo analysis of tissues. However, despite the huge work done, a detailed description of the Raman spectra acquired from the main classes of plasma lipoproteins is still missing. In this work, we extracted, the major classes of lipoproteins: the triacylglycerol-rich very low-density lipoproteins (VLDL); the more cholesterol-rich low-density lipoproteins (LDL); and the high-density lipoproteins (HDL); from human plasma of six fasting healthy volunteers. The extracted lipoproteins were dried on CaF2 slides and analyzed using a 633 nm laser line non-in resonance with the carotenoids present in the sample. The obtained spectra showed peaks relative to the different biomolecules composing lipoproteins: cholesterol, triglycerides, membrane lipids, carotenoids, and apolipoproteins (proteins). The intensity of the peaks from lipids and proteins are well in accordance with the measured composition of lipoproteins, but the information is acquired in a much faster way by Raman spectroscopy. Besides, Raman spectroscopy provides easily information on the levels of carotenoids and unsaturated fatty acid present in the samples

Spectroscopic assessment of connective tissues: from in vitro to in vivo characterization

Presenting Author: Isaac O. Afara, PhD - University of Eastern Finland

Connective tissues, such as articular cartilage, are essential musculoskeletal tissues critical for mobility. They generally consist of a collagen framework with embedded proteoglycan macromolecules that swell in the presence of water. The interaction and balance (homeostasis) between these components govern the functional integrity of the tissue, and alteration in this balance from trauma-induced (post-traumatic) or age-related (idiopathic) degeneration often result in musculoskeletal diseases like osteoarthritis (OA). OA is a debilitating joint disorder characterized by pain, severe morphological alteration of joint tissues (e.g., erosion of articular cartilage matrix) and joint dysfunction, with significant global impact. Although there are non-surgical therapies for the management of joint disorders, surgical approaches are common means of treating degenerative joint conditions. Surgical intervention is conducted via arthroscopy — the gold standard method for point-of-surgery assessment of the severity and extent of connective tissue damage, as well as treatment planning. However, traditional arthroscopy relies on subjective visual evaluation, leading to poor treatment outcomes as a result of poor reliability in assessing injury severity and extent. Spectroscopic techniques, such as near-infrared (NIR) spectroscopy, present a promising option for accurate, rapid, and label-free arthroscopic assessment of

connective tissue integrity in surgery. This non-destructive optical technique is capable of supporting early-stage disease detection by revealing tissue alteration at the molecular level before the development of visually apparent structural damage. Thus, non-destructive spectroscopic assessment of connective tissues generally involves detecting and characterizing the state of tissue composition and structure (e.g., content, quality, etc) as proxy indicators of tissue health. This talk presents a logical timeline of the adaptation and application of NIR spectroscopy for diagnostic assessment of connective tissue integrity. From basic (in vitro) to translational (ex vivo and in vivo) research, this presentation will highlight key fundamental and technical developments of NIR spectroscopy as a tool for real-time diagnostic assessment of connective tissue integrity in arthroscopic surgery.

Submicron O-PTIR Microscopy for Life Science Applications - Breast Tissue Microcalcifications & Amyloid Aggregates in Neurons

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Submicron OPTIR Microscopy provides new insights in breast tissue microcalcifications and amyloid proteins within neurons

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] Two examples in life science applications, which are otherwise impossible with traditional FTIR/QCL microscopy, will be presented, 1. ultra-high resolution images of breast tissue calcifications and 2. amyloid aggregates in neurons (neurites and dendritic spines).

Towards a non-invasive diagnosis of Basal Cell Carcinoma using deep Raman Spectroscopy

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This study sets the basis for the efficiently diagnosing BCC using Raman spectroscopy.

Basal cell carcinoma (BCC) is the most common skin cancer worldwide and in about one third of patients it appears as multiple synchronous or metachronous tumors. BCC arises from pluripotential cells in the basal layer of the epidermis and guidelines suggest surgery as the primary form of treatment. Although it rarely metastasizes, if left untreated or relapses after surgery in can cause significant local destruction. The gold standard for the diagnosis of a BCC is biopsy, which however remains an invasive procedure and cannot be used for the spatial evaluation of tumor growth. Therefore, there is imminent need for a non-invasive approach able to effectively assess the tumour growth and spread. Raman spectroscopy is an emerging group of techniques taking advantage of light-matter interaction to provide information of the sample (i.e. skin cells) on a molecular level. Deep Raman and specifically SORS, is able to look deeper inside the skin tissue and collect Raman photons with a bias towards the bottom layer of the epidermis, where BCC initially spreads. Biopsies have been collected with patient consent at the University of Ioannina hospital as a part of the standard clinical procedure and were used to confirm the pathology of the tissue. OCT scans and Raman spectra in a back-scattering mode were collected from the BCC and healthy areas prior to the biopsy, whereas the removal of biopsy was followed by histopathological analysis (H&E staining). In this feasibility study, we assess the correlation between tissue pathology, structural tissue information (OCT) and spectral biomarkers on BCC and healthy tissue of the same patient. The results indicate a strong correlation between tissue optical properties extracted from OCT scans and Raman spectra. With this study, we demonstrate that Raman spectroscopy has potential of providing adequate information to efficiently diagnose BCC. In order to characterize the malignant tissue at the exact depth where BCC occurs, Spatially offset Raman spectroscopy will be employed in later stages of this project. The results of this proof-of-concept study, can establish prognostic factors for a more efficient diagnosis and treatment of BCC treatment.

Towards The Development Of A Non-Invasive Optical Lactate Sensor for Early Detection of Sepsis

Presenting Author: Nystha Baishya, MSc., BE - City, University of London
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Non invasive Near Infrared spectrometry for lactate measurements.

Sepsis is unobtrusive and one of the prominent causes of death around the world. The most recent spike is seen during the global pandemic happening right now because of COVID-19, where more than 770,000 deaths have been reported as on August, 17, 2020. There is a need for technology with the capability to continuously monitoring patients in intensive care and identify the onset of sepsis, which will enable clinicians to plan an optimum treatment strategy. According to the guidelines set by Surviving Sepsis Campaign in 2018, lactate levels in blood can serve as an early indication for the inception of sepsis, hence lactate can be considered as a primary biomarker for the diagnosis of Sepsis. Unfortunately, in current clinical practice, lactate can only be measured intermittently using invasive techniques. Therefore, there is an urgent need for a disruptive technology which can offer rapid, continuous and most importantly, non-invasive measurements of lactate. The motivation of this study is to develop a non-invasive technology based on optical sensing of lactate. The research so far has comprehensively investigated the prominent Near Infrared (NIR) absorption wavelengths for lactate

both, in-vitro and in-vivo. The studies were conducted, using state-of-the-art bench top spectrometers, for varying concentrations of lactate in different media (human serum and whole blood) for in-vitro measurements. These were further tested on healthy human volunteers, undertaking a high-intensity incremental cycling experiment, where NIR spectra were collected using a portable NIR spectrometer. The knowledge obtained from these spectroscopic investigations, together with custom built computational models based on Machine Learning approaches such as Partial Least Square, support the prediction of lactate concentrations in real-time. Results from these studies suggest that lactate in human serum and whole blood could be predicted with a coefficient of determination, $R2$ of 0.77 and 0.75 and Root mean square of Cross-validation, RMSECV of 1.75 mM and 1.23 mM respectively. The $R2$ and RMSECV obtained from the volunteer study were 0.95 and 3.22 mM respectively. These initial results suggest the feasibility of a non-invasive lactate sensor used for the detection of the onset of sepsis in a critical care environment.

Towards the Utilization of the Human Cerumen (Earwax) Matrix for Disease Diagnostics: Chemical Profiling Using Two-Dimensional Gas Chromatography – Mass Spectrometry

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GC×GC-MS analysis of human cerumen can potentially be used for disease diagnosis

Earwax is an underutilized matrix that is receiving increasing attention because of its potential to reveal underlying pathologies. In contrast to biological materials such as blood and tissue that are often used in disease diagnosis, the collection of earwax is less invasive, which confers a number of advantages both in terms of its collection in a clinical setting, and patient comfort. However, its chemical complexity and lipid-rich nature complicate its analysis. In order to investigate the potential utility of earwax as a suitable matrix for the diagnosis of disease, approaches must be developed that can reveal disease markers. An important first step in this regard is the establishment of the chemical profile of "normal" earwax so that it can be contrasted with that associated with various disease states. Successful accomplishment of this task will provide a foundation upon which systematic investigations of potential correlations between earwax chemical signatures and the presence of a given illness can be conducted. Reported here is a method utilizing two-dimensional gas chromatography-mass spectrometry (GC×GC-MS) to identify the chemical profile of earwax. Earwax from healthy donors was collected in a clinical setting and prepared for analysis by ethyl acetate extraction and subsequent filtration. Several compound classes were detected including alkanes, alkenes, fatty acids, esters, triglycerides and cholesterol esters, and 44 compounds were tentatively identified. The observed chemical profile was then compared to the chemical profiles of earwax provided by donors who had been diagnosed with Ménière's disease. Visual comparison of the GC×GC-MS contour plots of the two displayed patterns that were readily recognizable as representing either "normal" earwax or Ménière's disease earwax. Notable distinctions between the two included differences in triacylglycerol profiles, and levels of free fatty acids. The results show that GC×GC-MS contour plots could potentially be used in a clinical setting for medical

diagnostics purposes. The chemical profile established for "normal" earwax provides a foundation for future studies in which wax acquired from donors exhibiting various diseases can be interrogated to determine the presence of attributes that can be correlated to illnesses.

Understanding Prebiotic Oligomerization of RNA with Mineral and Cationic Catalysts through Mass Spectrometry

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Native nanoelectrospray ionization mass spectrometry for detection of prebiotic RNA polymers

The spontaneous, abiotic condensation of monomeric species into biologically relevant polymers is of great interest in astrobiology in trying to understand the origins of life. The reactions that led to the formation of biopolymers probably took place in the presence of water. However, under watery prebiotic conditions, condensation reactions are thermodynamically disfavored due to the release of water molecules and kinetically hampered by high activation energies. There are many theories and scenarios which present feasible means of overcoming these challenges. The prebiotic synthesis of biopolymers such as ribonucleic acid (RNA) may have played an important role because it possesses multiple biologically relevant characteristics that could have started the basis of life in a competitive prebiotic chemical system. With an appropriate length and structure, RNA can store information as well as catalyze chemical reactions, including its own replication. Prebiotic RNA polymerization with mineral catalysts has been heavily investigated for decades. A variety of minerals, differing in both structure and composition, have been reported to produce RNA oligomers up to 33-mers. However, there has been some speculation about the true length of these oligomers due to the analytical methods employed. Here, we employ apply state-of-the-art mass spectrometry and chromatography tools to better characterize oligomer chain lengths produced from mineral- or cation-catalyzed abiotic reactions. The synthesis of RNA proceeded through the reaction of activated nucleotide monomers in an aqueous solution containing sodium chloride in the presence of montmorillonite mineral catalyst. Montmorillonite clay, treated through the Banin process to make it catalytically active, was found to produce the longest chain lengths. Untreated montmorillonite clay also led to oligomerization, but with shorter chain lengths (i.e., pentamers). In our replication of these experiments, nonamers were the maximum oligomer length detected via nanoelectrospray ionization mass spectrometry. Additionally, mass spectrometry was also

used to monitor prebiotic RNA synthesis to determine reaction pathways and efficiencies. Results suggest that abiotic RNA oligomerization could have occurred in a variety of realistic environments on the early Earth (e.g., on minerals, in salty seawater, or near hydrothermal vents).

Understanding the Mechanism of Proton-coupled Electron Transfer in the Bioinspired Artificial Photosynthetic Mimic, Benzimidazole Phenol Porphyrin

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Successful synthesis, simulation, and analysis of molecule that mimics the PCET of Photosystem II

Light-driven water oxidation in algae, cyanobacteria and higher plants generates dioxygen that supports life on Earth. The water-oxidation reaction is catalyzed by the oxygen-evolving complex (OEC) in photosystem II (PSII) that is comprised of the tetranuclear manganese calcium-oxo (Mn4Ca-oxo) cluster, redox-active tyrosine residue (YZ) and hydrogen-bonded network of amino acids and water molecules. The redoxactive tyrosine residue, YZ, mediates successive proton-coupled electron transfer (PCET) reactions that are essential for the oxidation of water to dioxygen at the Mn4Ca-oxo cluster. It is proposed that the strong hydrogen bond between YZ and and its conjugate base, D1-His190, likely renders YZ kinetically competent leading to highly efficient water oxidation. However, a detailed understanding of PCET at YZ remains elusive due to the lack of high-resolution structural methods to directly probe the electron- and proton-transfer reactions. In this study, we utilize high-resolution two-dimensional (2D) 14N hyperfine sublevel correlation (HYSCORE) spectroscopy to investigate the electronic structure of the bioinspired artificial reaction center, benzimidazole–phenol porphyrin, that mimics PCET at the YZ residue of PSII. Additionally, we perform density functional theory (DFT) calculations to determine the electron spin density distribution and electron-nuclear hyperfine coupling parameters of the benzimidazole–phenol porphyrin radical for comparison with the corresponding parameters that are obtained from the 2D HYSCORE measurements.

Urinary Short Chain Fatty Acids And Branched Chain Amino Acids Analysis For The Indirect Monitoring Of The Activity Of Gut Microbiota In Newborns

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This workflow allows to monitor newborn's activity of gut microbiota measuring non-invasive urine samples.

Short chain fatty acids (SCFAs) and branched chain amino acids (BCAAs) play an important role in physiological and pathological processes. These compounds are essential for the metabolism of newborns, especially preterm infants, which are highly dependent on intra-and-extrauterine nutrition. Little is known about the levels of these metabolites found in newborn's urine and their biological implications. We hypothesize that the levels of SCFAs found in urine correlate with SCFAs concentrations in feces, and hence, urine samples can be used for monitoring of the activity of gut microbiota. This work presents a targeted GC-MS method as a workflow tailored to the determination of SCFAs and BCAAs in small volume (300 µL) urine samples and reports concentration ranges of these compounds in term and preterm infants as well as their mothers. Significant differences in SCFAs and BCAAs concentrations between mothers (N = 60) and infants (both preterm (N = 34) and term infants (N = 43)) were found. Furthermore, butyric acids levels where higher in preterm than in term infants. No differences were found regarding the type of nutrition (own mother's milk vs. pasteurized donor human milk). In addition, a contamination originating from cotton used during the collection of infant's urine samples that hampered the measurement of acetic acid was observed. As a conclusion, this workflow evidence the capability of measuring easily collectable urine samples to determine SCFAs and BCAAs concentration in order to monitor of the activity of gut microbiota.

Chemometrics

A New Supervised Learning-based Workflow for the Processing of Chemical Data—Efficient Data Reduction-Multivariate Curve Resolution (EDR-MCR)

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New technique developed to classify high-dimensional data that offers added advantages compared to available methods

A prevailing challenge in the ability to accurately classify and/or draw inferences from data that enable prediction of trends and outcomes, remains the determination of the most straightforward and accurate approach to accomplish the task. A hallmark of this well-established field is the immensity of the range of algorithms that have been developed for this purpose. This reflects the truth of Wolpert's "no free lunch theorem", in that there is no single approach that can be used to solve a broad range of classification problems. Data reduction methods and performance evaluation methods are two fundamental and discrete steps used in the process of establishing a multivariate data analysis workflow that will accomplish classification and/or prediction for a given type of data. Reported here is a supervised learning method, termed "efficient data reduction-multivariate curve resolution" (EDR-MCR), that simultaneously accomplishes the task of optimal data reduction, learning and performance evaluation. It combines the abilities of principal component analysis (PCA), the principles of convex geometry, and multivariate curve resolution (MCR) methods to detect the training/test samples for model evaluation, reveal the most informative variables, and generate a classification model using MCR. The performance of the approach was investigated by applying it to various benchmark datasets, and forensic datasets acquired using Direct Analysis in Real Time-High Resolution Mass Spectrometry (DART-HRMS). The results were compared with the outputs of the ensemble-learning frameworks: linear discriminant analysis (LDA); k-nearest neighbors (KNN); classification and regression trees (CART); and support vector machines (SVM) methods. This comparative analysis revealed that relative to the aforementioned alternative approaches to the analysis of the data, EDR-MCR confers: (1) speed; (2) reduced need for the tuning of multiple parameters; (3) flexibility in the analysis of data that is characterized by low sample numbers and class imbalances; (4) the ability to include in the model additional system attributes as numerical constraints; and (5) the ability to resolve the relative weights of the variables that are most impactful in enabling class differentiation to be accomplished.

All-at-once Nesterov-like Extrapolated PARAFAC2-ALS: A Fast And Robust Complex Tensor Decomposition Algorithm for Analyzing GC-MS Data

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Proposed All-at-once Nesterov-like extrapolation PARAFAC2 algorithm will greatly advance GC-MS data analysis in scientific areas.

In the past years, multi-way data analysis also called tensor analysis has gained widespread acceptance and attractive research interests in chemometrics owing to the rapid development of advanced analytical instruments. Tensor decomposition techniques such as PARAllel FACtor analysis (PARAFAC) and PARAllel FACtor analysis2 (PARAFAC2) can be regarded as multi-way generalizations of two-way component analysis. Comparing with PARAFAC, PARAFAC2 has the extra ability to deal with a specific type of data problems such as observations have different lengths or measured profiles that slightly change position in the multi-way data. For example, gas chromatography-mass spectrometry data (GC-MS) is one of such typical type of data, where elution profiles can vary and shift between experimental runs. The most commonly used algorithm for fitting PARAFAC2 model is the PARAFAC2-Alternating Least Squares (PARAFAC2-ALS) algorithm, since it is very simple to implement and represents a good trade-off between computational cost and quality of the solution. However, PARAFAC2-ALS algorithm is very slow, especially when facing with 'swamps' or 'bottlenecks' in the data. In this research, we propose novel implementations of extrapolation-based PARAFAC2 algorithms. Besides the standard PARAFAC2-ALS algorithm, the PARAFAC2-Hierarchical Alternating Least Squares algorithm (PARAFAC2-HALS) is, for the first time, established and investigated in this research. We focus on 12 PARAFAC2 algorithms in total, which are composed of two PARAFAC2 fitting methods, PARAFAC2-ALS and PARAFAC2-HALS, in combination with five different extrapolation acceleration schemes including Enhanced line search, PLS_Toolbox line search, N-way toolbox line search, Nesterov-like extrapolation and All-at-once Nesterov-like extrapolation. The strengths and weaknesses of 12 PARAFAC2 algorithms are quantified in terms of algorithm speed, number of local minima, convergence ability and fitting process using both simulated and real GC-MS datasets. The results show that newly proposed All-at-once Nesterov-like extrapolation PARAFAC2-ALS algorithm achieves the fastest convergence speed whilst maintaining a low fraction of local minima solutions. This algorithm is shown to significantly outperform the latest extrapolation accelerated PARAFAC2 algorithms available in literature, which is deemed to greatly advance GC-MS data analysis in various scientific areas.

Development of a spectroscopic method to analyze food components

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Contactless quality controls to protect consumers are urgently needed.

Quality control is becoming increasingly important in various fields. Especially in the food industry, inexpensive and non-destructive measurement methods are required. In order to produce in a resource-friendly process and to reduce the risks for the consumers on plagiarism and contamination, there is great potential for application of spectroscopic methods such as FTIR and Raman spectroscopy. The aim of this project is to develop a measurement concept for quick and contactless analysis that enables an application online or even inline. Selected food components were analyzed by vibrational spectroscopic methods for their molecular properties and stability. In a subsequent evaluation step, chemometric data analysis tools like principal component analysis (PCA), principal component regression (PCR), and partial least squares (PLSR) regression were used. The quantitative analysis of fatty acid samples enabled to identify certain areas for the determination of concentration ratios as well as to make a

statement about the degree of saturation of the molecules. Overall, the results show areas of application in which FTIR and Raman spectroscopy can already be used. However, it was also possible to identify the need for further research.

Exploratory Analysis of Zooplankton Spectra

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Different approaches of chemometrics are used to study Raman and LIBS spectra of zooplankton

According to recent research, copepod crustaceans belonging to the Calanoida taxon accumulate lithium. The relation between atomic composition and molecular structure of tissues can reveal the mechanism of this behaviour. We carried out combined studies of zooplankton organisms by Raman spectroscopy and LIBS. Raman spectra were recorded with a 780 nm red laser, and a 20 mJ 266 nm Nd:YAG laser was used in LIBS. Signals were collected separately from dark, light, and medium-coloured spots on the surface of pelletized samples (29 samples of zooplankton, 14 of them from the Calanus taxon). The obtained data were subjected to different matrix decomposition techniques, viz., common principal component analysis (PCA), non-negative matrix factorization (NMF), and common components and specific weights analysis (ComDim) in its PCA-based realization. Bulk composition, obtained by ICP-AES and ICP-MS after digestion, was also available. For PCA and NMF, the influence of data fusion on the results of decomposition was studied. When applied either to Raman or LIBS data separately, the both techniques allowed classification of samples in terms of Li enrichment. Lithium in Calanus appears to be associated with carotenoid compounds and amino acids. Both datasets and the result of their concatenation are described with 3 PCs. The most easy-to-interpret results were obtained using ComDim which weights data of different origin to provide meaningful contribution of all sources. ComDim suggests the existence of just 2 common components. Besides the interrelation of Li with carotenoids and amino acids, ComDim revealed its link to potassium. Interestingly, this holds true only for dark and medium-coloured spots, but not for light spots. There is always a part of Li signal not separated from other elements. This might indicate the existence of different mechanisms of Li uptake by crustaceans. ICP-AES and ICP-MS elemental analyses of zooplankton samples were funded by the Russian Science Foundation (project No. 18-77-00064). The MATLAB code for performing ComDim was kindly provided by Prof. D.N. Rutledge (AgroParisTech, France). The authors are grateful to Á. Bélteki, A. Kéri, P. Janovszky, D. Palásti, Dr. K. Fintor (University of Szeged) and Dr. R. Rajkó (Universtity of Pécs, Hungary) for their valuable assistance.

Extra Virgin Argan oils' shelf-life monitoring and prediction based on chemical properties or FTIR fingerprints and chemometrics

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The potential of FTIR fingerprints with chemometrics to monitor the Argan oils' shelf-life were established

Argan oil has gained worldwide acceptance in edible and cosmetic applications, because of its nutritional composition, therapeutic and dermatologic benefits. Argan oil production is considered as pivotal leverage for social, economic and human development for the southwestern Moroccan populations. The determination of the shelf life and optimal storage conditions of a food product represents a way of safety, quality and health protection. In order to achieve a better understanding of the shelf-life behavior of extra virgin Argan oils (EVAO) during storage, the influences of storage periods, roasting process and packaging materials were studied. Oils were extracted from roasted or unroasted kernels. The EVAO shelf-life assessment was made by determining chemical properties (acidity, peroxide value, specific absorbances K232 and K270, tocopherol content, fatty-acids and sterol composition, and oxidative stability index) and FTIR spectra. Sixty EVAO samples (30 from roasted and 30 from unroasted kernels) were evaluated after production and being packed in two glass-bottle types (dark and clear), which resulted in 120 samples. They were stored under realistic storage conditions (ambient temperature) for two successive years and analyzed 6-monthly. Chemometric data analysis was applied to study the influence on shelf life. PCA and PLS-DA, on either the chemical data or the FTIR spectra, allowed the discrimination (100% classification rate) between fresh and oxidized oils. The oil shelf life was predicted by means of PLS regression ($R^2 > 90\%$). Thus, the time of storage after which the oil loses its extra virgin quality could be predicted. Moreover, the potential of FTIR fingerprinting to quantify four physicochemical properties (i.e. acidity, PV, K232 and K270) during EVAO storage was established using PLS regression. Good results were obtained which justify the applicability of FTIR. Proper packaging and storage conditions may conserve the quality of EVAO. However, conventional methods, determining the chemical properties, as well as sensorial characteristics, are classically applied to inspect their shelf life quality. Those procedures are high cost, and time-consuming. The results evidenced that FTIR and chemometrics form an interesting, cheap and fast approach to survey the shelf-life of EVAO.

Extracting Matrix Matched Samples from Large Spectral Libraries for Local Multivariate Calibration

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Novel local modeling method able to harness non-global analyte matching by comparing many calibration sets.

As spectral datasets continue to grow and their contents span greater information variance and spectral matrix effects (such as measurements on new instruments and/or novel sample compositions), it is becoming increasingly difficult to extract useful and specific information for an effective analyte specific multivariate calibration by, for example, partial least squares (PLS). The accuracy of prediction for novel (target) samples is dependent on the overall similarity between each novel sample undergoing prediction and the calibration sample domain used to form the prediction model. A higher degree of similarity on average indicates a more accurate prediction. Local modeling is concerned with selecting a subset of calibration samples from a large library tailored to each novel sample such that the two have similar chemical and spectral matrix effects, i.e., samples are matrix matched. Presented in this work is a method termed local adaptive fusion regression (LAFR) that uses an extensive process to determine the strongest local calibration set. For each sample to be predicted, LAFR first decimates the reference library by using spectral similarity measures. Then using the selected reference samples, LAFR forms many calibration sets by a window clustering approach of analyte values. Finally, the best calibration set matrix matched to each target sample is ultimately selected to form the PLS model and subsequent analyte prediction value. This local modeling protocol accrues sample-wise adaptability because the selected calibration set is identified as the best set by simultaneously matching by both spectra and analyte concentration relative to each target sample. A consensus approach is used for calibration set selection by utilizing data fusion to combine a multitude of similarity scores in order to robustly identify the strongest calibration set. Results are presented across multiple near-infrared datasets showing successful generation and selection of densely matched calibration sets for local regressions with higher accuracy than global regressions.

Generative Adversarial Linear Discriminant Analysis

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An algorithm is developed to out-perform PCA with high dimensional datasets.

A method is introduced to enable linear discriminant analysis with small numbers of high dimensional data sets and few classes, for which conventional LDA typically exhibits numerical instability preventing meaningful use. Linear generative adversarial strategies were devised to address "overfitting" limitations intrinsic to conventional LDA performed in such underdetermined conditions. The generative adversarial linear discriminant analysis (GALDA) algorithm is analogous to nonlinear generative adversarial strategies routinely used to minimize overfitting in artificial neural networks. By retention of class information, GALDA is shown to provide improvements in resolution better than principal component analysis (PCA), even in cases for which PCA normally would be expected to greatly outperform conventional LDA (i.e., a small number of high-dimensional training data). In the analysis of Raman spectra of different crystal forms of the blood thinner clopidogrel bisulphate, GALDA was shown to support discrimination between just three different spectral classes of testing data with as few

as three training spectra in each class. This case is far outside the regime in which conventional LDA is generally applicable, suggesting GALDA may expand the use of LDA into regimes it has hitherto been wholly inapplicable.

Iterative Target Detection for Detection and Classification with an Example Application in Hyperspectral Imaging.

Presenting Author: Neal B. Gallagher, PhD - Eigenvector Research, Inc.

mproving detection sensitivity using extended and generalized least squares with an application in hyperspectral imaging

Classical least squares (CLS) is the tool of choice for detection and classification in hyperspectral images because often target spectra are known but reference values for each pixel are rarely available. Generalized least squares (GLS) is a weighted CLS model used to suppress clutter signal (interferences and noise) while enhancing minor target signal. (GLS is also known as the matched-filter and the Aitken estimator.) An iterative target detection approach exhibits synergy between GLS and the extended mixture model (extended least squares, ELS) to further improve discrimination. To enhance visualization of the methodology an example is shown for a Landsat 8 image of Lake Chelan, WA USA. However, the concepts demonstrated are applicable to a wide range of applications including fault detection and classification in the process environment. The distinct advantages over approaches like principal components analysis and partial least squares include interpretability and ease of model updating (adaptability) relevant for time-series application. The example shown utilizes GLS iteratively in a hierarchical approach to classification, followed by a combined GLS / ELS model was used to further split a single class that was otherwise difficult to classify. Both objectives were complicated by the presence of significant interference signal but showed good results verified using ground truth.

Quality assessment of Cabernet Sauvignon from Chile based on simultaneous Absorbance-Transmission and Fluorescence Excitation-Emission Matrix analysis

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This paper evaluates a new combination of instrument methodology and data analysis for wine quality.

Cabernet Sauvignon (CS) wine is of high economic impact in Chile, with an approximate yearly volume of 358 million liters, corresponding to 35% of the total wine. CS is produced in a wide range of styles, thus, quality assessment of the grapes and wines is a key industrial goal. Grape and wine quality highly depends on chemical composition. Thus the objective of this work is to study the A-TEEM technique to predict the chemical composition of CS wines. Importantly, polyphenols exhibit high chromophoric and fluorescence properties, making them especially suitable for A-TEEM analysis. The method's automatic real-time Inner Filter Effect (IFE) correction furthermore allows the quantification of other minor compounds. The EEM and absorbance variable were combined using a multi-block tool, and regressed

against concentrations of phenolic and anthocyanin compounds measured independently by HPLC-DAD. The study focused on comparing Partial Least Squares Regression (PLSR) and Extreme Gradient Boost Regression (XGBR) for the single- (EEM) and multi-block data using the Solo toolbox (Eigenvector Inc.). As a general trend, validation of the multi-block data models with independent data using XGBR, compared to PLSR, yielded higher prediction correlation coefficients (R2) and lower Root Mean Square Errors for Prediction (RMSEP). A set of 138 files, and applying 80:20 random split, resulted in precise prediction fits with R2 between 0.95 and 0.985 for compounds > 10 mg/L. As an example, catechin in a range of 7.2 - 22.9 mg/L resulted in R2 = 0.982, and RMSEP of 0.56 by multi-block XGBR, while by using PLSR lower fits of R2 = 0.91, and RMSEP 1.4 for the same compound were obtained. These multi-block data sets were also associated with significantly higher R2 (and lower RMSEP) compared to a single block evaluation of the fluorescence EEMs. By using mean-centering and an Extended Mixture Model filter the multi-block data sets fit robustly using both XGBR and PLSR without the need to apply secondary variable selection algorithms. We conclude that analyzing the A-TEEM data using the multi-block organization and the XGBR algorithm facilitates a robust prediction of the key phenolic and anthocyanin compounds that influence CS wine quality.

The application of neural networks for single-step preprocessing of Raman spectra

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Computer driven methods for analyzing Raman spectrographic data require preprocessing for direct comparisons between observations. Preprocessing of Raman data should not be trivialized, as errors are likely to result in false conclusions. Raman preprocessing generally include signal correction for; a) local variations from measurement noise and the stochastic nature of Raman scattering, b) high energy spikes from cosmic radiation, c) baseline from external sources or fluorescence. There are many methods that can handle each of these preprocessing tasks separately. One disadvantage with a sequential approach it that dividing the processing into different tasks is likely to produce errors that propagate throughout the processing and contaminate the results. The objective of this investigation is to explore the possibility to perform the preprocessing in one step. Machine learning and artificial neural network ANNs have become popular tools for many data driven problems. ANNs are particularly useful, as they can be trained to make predictions on any digital dataset. Therefore, it comes naturally to hypothesize that ANNs can improve preprocessing of Raman data. In this investigation an ANN was implemented and trained to preprocess Raman data in a single step. To circumvent the need to acquire a database of measurement data, the ANN was trained solely on synthetic data. It would be difficult or even impossible to acquire a sufficiently large training sets from measurements – especially for samples with complicated molecular structure, where measurements are difficult and high-quality preprocessing is required. Synthetic data can be designed to contain the desired complexity to be suitable for any measurement situation. Compared to a reference prediction based on a Whittaker smoother, second difference and polynomial fitting on a set of 105 synthetic spectra, the ANN showed to be a more accurate predictor. 91.4% of all predictions had smaller absolute error (RMSE), 90.3% had improved quality (SSIM), and 94.5% had better signal-to-noise ratio (SNR). The same result was achieved on experimental Raman spectra from polyethylene, paraffin, and ethanol – all with background contamination from polystyrene. The results are a proof of concept for fast high-quality preprocessing of Raman spectra.

Two-Trace Two-Dimensional Correlation Spectroscopy for the Resolution of Multi-Component Drug Mixtures

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Initial demonstration of drug mixture analysis using two-trace two-dimensional (2T2D) resolution technique.

Infrared spectroscopy is a popular portable drug checking technology widely used in harm reduction applications to navigate an increasingly adulterated drug supply. Identity of common adulterants in drugs are generally known; however their presence is not always clear. The success of accurate identification relies on the availability of extensive libraries and searching algorithms. Complex mixtures (of 3 or more substances) continue to pose a challenge for accurate drug identification with infrared technology and the traditional approach of sequential spectral subtraction of pure component spectra is not often adequate to resolve the third or minor component due to propagation of error through over- or under- subtraction. As an initial demonstration for this application, this work uses synthetic spectra of relevant drug mixtures to minimize complication from sampling, such as noise and reproducibility. Difficulty of analyzing a ternary mixture using spectra of two known components and traditional subtraction methods is demonstrated. The potential of asynchronous and synchronous two-trace two-dimensional (2T2D) resolution analysis as a technique to capture the spectral features of the unknown third component, using the combination of two known spectra as the reference, is explored in the context of drug checking.

Use of Machine Learning to Further the Scope of LIBS

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LIBS is paired with machine learning to simplify classifications and identification of complex samples.

Laser-Induced Breakdown Spectroscopy (LIBS) is an analytical method for quantitative analysis of samples in gases, liquids, solids, and aerosols. This method uses a pulse laser to generate a plasma that results in ablation of miniscule amounts of the sample, which then leads to the atomization, excitation, and ionization of particles. The transition from high energy state to a low energy state results in an elemental spectra. LIBS method is portable and easy to use, however when used on complex samples, the spectra analysis becomes intricate and clouded. In this study, LIBS is paired with machine learning to simplify classification and identification of complex samples. Various samples were tested such as geological, soil, and metal samples. Future direction of data analysis will be presented.

Variable Selection Methods for the Improvement of PLS Calibration Models

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A comparison of variable selection methods for PLS calibrations when the variables are highly correlated.

With the quick and easy measurement of thousands of variables in FTIR spectra as wavenumber channels, the selection of significant and relevant variables to include for the creation of partial least squares (PLS) models is a non-intuitive problem, and often one that is overlooked. Here, we present a study of several variable selection methods used to remove wavenumber channels from a data set of FTIR spectra of ammonia gas collected for an ammonia concentration calibration. Results highlighting the differences between a filter approach (determining which variables to remove from the full data set) vs a wrapper approach (iteratively removing variables based on data refitted after prior variable removal steps) are presented. The effectiveness of each method, and the interpretation of the wavenumber channel removal process is discussed.

Electric-Field Driven Phenomena

A Tunable Insulator-Based Dielectrophoresis System for the Separation of Biomolecules

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Fine-tuning electric field gradients by actuation of a normally-closed valve

Insulator-based dielectrophoresis (iDEP) has been exploited for the manipulation of particles, organelles, and nucleic acids. Typical iDEP devices integrate insulating post arrays with different geometries to induce a nonuniform electric field. The dielectrophoretic (DEP) force scales with the magnitude of the squared electric field gradient. To maximize DEP forces, large potentials are applied across the fluidic channel; however, analytes can deteriorate and degrade when exposed to such conditions. To overcome these issues, we developed a microfluidic device that integrates a normally-open valve that serves as a dynamic insulating constriction when an electric potential is applied while the valve is deflected into the fluidic channel.1 In this device, a thin membrane made of polydimethylsiloxane (PDMS) was integrated by soft lithography. The device demonstrated the preconcentration of liposomes and partial trapping of DNA by fine-tuning the electric field to induce high dielectrophoretic forces. Here, we further optimized the valve by designing a normally-closed valve to fine-tune the electric field. The device is composed of three different fluidic channels in which each channel integrates four stem valves with varying dimensions: width 10-100 µm, length: 250-400 µm, and height 20-30 µm. The stem valve acts as a dynamic insulator constriction in the fluidic channel when it deflects into the control chamber while an electric potential is applied across the fluidic channel. The negative pressure required to fine-tune the actuation of the valve was characterized by comparing the different dimensions of the stem valve and position in the fluidic channel. Furthermore, a numerical study was developed to understand the relationship between the electric field gradient and the stem valve to substrate distance. We further suggest that serially aligning multiple dynamic constrictions will allow the design of a separation system for heterogeneous analyte mixtures and reaching high electric fields with reduced applied potentials. This novel method will be a crucial turnover for iDEP applications such as the separation of biomolecules, and further understanding of biomolecule manipulation with DEP. 1. Ros, A.; Kim, D.; Luo, Jinghui, Yang, M. Tunable Insulator-Based Dielectrophoresis (iDEP) with Membrane Valves. U.S. Patent 2019 / 0224689 A1. July 25, 2017.

Characterizing Unusual Particle Electromigration Effects in a Straight Microchannel

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First experimental assessment of the second kind electrophoretic mobility of cells and particles.

Electrokinetic (EK) microfluidic systems have proven to be a strong technique for manipulation and separation of microparticles and microorganisms. Recent studies presented a novel parameter: the electrokinetic equilibrium condition (EEEC), finding that has revolutionized the analysis of microparticles in microfluidic devices by considering a nonlinear electrophoretic (EP(3)) behavior. This study presents experimental characterization of linear and non-linear electrophoretic mobility (µEP(1) and µEP(3), as well as the EEEC of four polystyrene microparticles and microorganisms in EK-based devices with straight microchannels. The particles used in this study ranged in sizes from 2 µm to 6.8 µm, and the microorganisms studied were three bacteria cell strains (B. cereus, E. coli, S. enterica) and one yeast cell (S. cerevisiae). The EEEC and the nonlinear electrophoretic mobility parameters from this study will be useful for future design of insulator-based electrokinetic devices, challenging separations of multiple kinds of bioparticles and for the development of computational models closer to reality.

Customizing Nanopore Sensors with Synthetic Chemistry.

Presenting Author: James T. Hagan- The University of Rhode Island

Non-Presenting Author: Brian S. Sheetz - The University of Rhode Island

Corresponding Author: Jason R. Dwyer, PhD - The University of Rhode Island

Fabrication and application of chemically tuned silicon nitride (SiNx) nanopores for polysaccharide sensing.

The development of solid state nanopores as single molecule sensors has focused on optimization for direct analysis of biologically relevant species, particularly DNA. Over the short lifetime of this analytical technique researchers have been working to both improve the materials and conditions used for nanopore fabrication and sensing, as well as broadening the analyte scope for this technique. We have been working on both chemically tuning the surface of nanopores formed in commercially available silicon nitride (SiNx) membranes by controlled di-electric breakdown (CDB) and solidifying saccharides as an accessible analyte for nanopore sensing. Chemical modification can occur both during fabrication with the addition of sodium hypochlorite to the electrolyte solution used for CDB and after pore fabrication by photochemical reactions resulting in direct covalent attachment to the surface of the pore. Using alkene terminated small molecules, a broad spectrum of functional groups have been covalently attached to the surface of the pore (e.g. carboxylic acids, boronic acids, hydroxyl groups) which can provide increased pore selectivity and allow for analyte-specific interactions resulting in unique signals measured. Further exploration and development of these capabilities has the potential to benefit the whole nanopore community by increasing the customizability of nanopore sensing conditions.

Detection of biofouling on gold-coated MF membranes by in-situ electrical impedance spectroscopy

Presenting Author: Nan Zhang- McMaster

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A novel technique - EIS - to track biofouling on electrically conductive membranes

Detection of biofouling in microfiltration processes enables early and effective strategies for fouling prevention. This work investigates the use of electrical impedance spectroscopy (EIS) is used for online monitoring the biofilm development on the surface of electrically conductive membranes (ECMs). A simple technology to fabricate ECMs is introduced through sputter deposition of the ultrathin gold layer (30 nm) on microfiltration polyethersulfone (PES) membranes. Gold-coated membranes exhibit extremely high electrical conductivity ($\sim 5 \times 105$ S/m) and high water permeability. SEM and AFM images of modified membranes show a homogeneous and flat coating surface. To monitor the onset and development of biofouling in situ, a two-electrode crossflow filtration cell is customized in which a gold-coated membrane acted as the working electrode. Fouling experiments with a bacterial culture harvested from tap water were conducted over a duration of 12 h. Biofilm-induced permeate flux decline showed two stages of biofouling development. The initial stage was related to attachment and deposition of bacteria on the membrane surface. The second stage was associated with the accumulation of extracellular polymeric substances (EPS). Correspondingly, impedance spectra indicated the impedance at low frequency region (< 10 Hz) sharply decreased with fouling early on, and gradually decreased to the end of the experiments. Further, the measured impedance was modeled by an equivalent circuit from which a EIS-derived parameter, the normalized impedance related to diffusion was achieved. It is observed that the impedance-based detection is more sensitive to changes as compared to the decline of permeate flux during the early stage of biofouling. Hence EIS applied to ECMs has the potential to be used as canary cells installed on a side-stream of commercial membrane modules for fouling detection as well as fouling control in industrial applications.

Development and Characterization of a Lateral Flow Assay for Myoglobin Detection

Presenting Author: Dinah Pimentel- Maryville University of St. Louis

Non-Presenting Author: Annika Fahs - Maryville University

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The lateral flow assay provides rapid access to myoglobin testing results in a non-clinical setting.

Lateral flow assays (LFAs) are widely known to be an affordable and user-friendly point-of-care testing method in medical diagnoses. The objective of this project is to create an LFA strip using the sandwich technique for the detection of myoglobin in human blood, which indicates muscle injuries in patients. In developing this technique, cost-effective and accessible test strips would be available for indication of muscle injury in patients.

Electrokinetic separation of cells and polystyrene particles in an insulator-based device

Presenting Author: Abbi Miller- Rochester Institute of Technology

Non-Presenting Author: Nicole S. Hill- Rochester Institute of Technology

Non-Presenting Author: Adriana Coll De Peña - Rochester Institute of Technology

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Separation of polystyrene and biological samples by size and shape using insulator based electrokinetic chromatography.

In the field of biomedical analysis, there is currently a need for the development of fast, portable, and inexpensive devices for separation and analytical applications. Past research has proven that microfluidic electric field driven techniques are effective in separating cells and micron sized particles. Insulator-based electrokinetic (EK) techniques employ non-uniform electric fields to enhance the effect of nonlinear EK effects on particle migration. EK-based devices have the potential to become an inexpensive, easily fabricated, and disposable alternative method to separate and concentrate microparticles of interest, such as biological cells, from a given sample. The present work focuses on the use of insulator-based EK devices applied to yeast cells, fluorescent polystyrene (PS) particles, and non-fluorescent peanut shaped PS particles of similar size (5 µm). Different samples of these particles were prepared in an aqueous solution and injected as a mixture into two different EK channel designs. Each device was then utilized to separate the various particles into distinguishable fractions using an array of insulating posts and electrokinetic sample injection. The data collected from these experiments was plotted as a signal captured at an observation window located at the end of the post array. The efficiency obtained in our EK devices was evaluated in terms of retention time and separation resolution (Rs). The final results demonstrate the potential of insulator-based devices, which enhance with nonlinear EK phenomena, for carrying out separations of micron-sized particles and cells, by exploiting differences in particle shape, particle size and surface charge.

How Adsorption Isotherm Models Describe Ion Behavior at Air-Aqueous Interfaces: Observations with Ionizing Method Surface Potential

Presenting Author: Tehseen Adel- The Ohio State University

Corresponding Author: Heather C. Allen, Ph.D. - The Ohio State University

Surface potentials of electrolyte solution surfaces are measured and analyzed with adsorption isotherms.

Surface potential is a measure of the electric charge at any given interface. Using an instrument based on the electrochemical ionizing method surface potential, the surface electric charge of several sodium halide solutions is determined and compared to well-known surfactants. By relating these measurements to adsorption isotherm models, the intrinsic behavior of ions adsorbing to the air/aqueous interface can be quantified thermodynamically. For a series of sodium halide solutions, anions are found to be key contributors to the surface charge in an order similar to their Hofmeister arrangement: chloride < bromide < iodide. At -3.3 kcal/mol, iodide has the most negative free energy of adsorption compared to other halide anions.

Lesion Induced DNA Amplification (LIDA) Conducted In A Microfluidic Chip To Differentiate Between Different Ginseng Species

Presenting Author: Christopher A. Oberc- Simon Fraser University

Non-Presenting Author: Paul Li - Simon Fraser University

A microfluid chip was used to conduct solid-phase isothermal amplification to identify ginseng species.

Panax ginseng and Panax quinquefolius have different medical properties and market values; however, they can be difficult to differentiate from one another based on physical appearances. A molecular test is thus needed to overcome this difficulty. The single nucleotide polymorphism (SNP) site on the Panax genome that differs between. P. ginseng and P. quinquefolius has been selected. An isothermal DNA amplification technique known as LIDA has been developed for use in a microfluidic chip; this nucleic acid amplification test not only amplifies the extracted plant genomic samples but also allows for detection of specific SNPs better than conventional nucleic acid tests.

Microfluidic Pressure in Paper (µPiP) for Electrokinetically Assisted Ultra-Low Cost Diagnostics

Presenting Author: Md Nazibul Islam - Texas A&M University

Non-Presenting Author: Jarad W. Yost- Texas A&M University

Corresponding Author: Zachary Gagnon - Texas A&M University

An ultra-low cost pressurized paper method for continuous electrokinetic bio-molecular concentration and separation

Paper-based microfluidics has gained widespread attention as a novel platform for diagnostic devices in low-resource settings. However, variability in fluid transport due to evaporation and lack of fluidic reproducibility in real-world samples limits the commercial potential and widespread adoption of this method. To address these limitations, we have developed a novel fabrication technique called "Microfluidic Pressure in Paper" (µPiP). This approach combines thin laminating PDMS membranes and precision laser-cut paper microfluidic structures to produce paper microfluidic devices that are low-cost, capable of being manufactured at commercial scale and exhibit controllable and reproducible fluid flow dynamics similar to that of conventional microfluidic devices. Here we present a new µPiP DNA sample preparation and processing device that reduces the number of sample preparation steps and improves sensitivity of the quantitative polymerase chain reaction (qPCR) by electrophoretically separating and concentrating nucleic acids (NA's) continuously on paper. Device performance was characterized using different microfluidic paper channels with a larger pore (25 microns) size for bulk fluid transport and a smaller pore size (11 microns) for sample concentration. These two paper types were aligned and laminated within PDMS sheets, and integrated with electrodes comprised of adhesive copper tape. A solution containing a custom DNA sequence (88 bp) was introduced into the large pore size paper channel using a low-cost pressure system. A DC voltage was applied to the copper tape to then electrokinetically deflect the solution containing NA's into the paper channel with the smaller pore size. Samples were collected from both DNA enriched and depleted channels and analyzed using quantitative PCR (qPCR). Our results demonstrate the ability to use these paper devices to process and enrich the concentration of nucleic acids. Currently, qPCR sample preparation requires multiple steps to isolate and concentrate NA's from lysates and other biofluids containing PCR inhibitors. Our concentration device has the potential to reduce the number of sample preparation steps and to improve

qPCR sensitivity, which has immediate applications in disease diagnostics, microbial contamination, and public health monitoring.

Microfluidic Sample Handling to Broaden the Reach of Nanopore Single-Molecule Sensing

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Non-Presenting Author: James T. Hagan- The University of Rhode Island

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A method to characterize the surface chemistry of custom surfaces on solid state SiNx nanopores.

Nanopore single molecule sensing is a fast-growing field. The uses have grown to encompass research areas such as genomics, glycomics, proteomics, and much more. Controlling the surface chemistry of the SiNx pore opens a large opportunity to tune the pore's selectivity and flow characteristics at different experimental conditions and in response to different analyte classes. To broaden the reach of this platform, a microfluidic sample delivery system has been prototyped and implemented using a stereolithographic 3D printer and a poly(methyl methacrylate) resin. We created a closed low-volume system to streamline sample handling and to reduce conventional nanopore surface chemistry characterization times from hours to minutes. To probe different surface chemistries, our microfluidic flow system was used to sweep the pH of the pore's environment. As the pH is swept, the Debye layer charge changes depending on the pKa of the specific surface chemistry. This change contributes to the current flow through the nanopore in a way that is characteristic of the particular nanopore surface coating. This technique will help in the characterization of nanopores, while the microfluidic system will broaden the reach of nanopore experimentation.

Microparticle filtration and separation using cascade devices

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Microparticle filtration and separation using cascade devices

Particle samples manipulated in microfluidic devices might be highly complex and have several components that hold no interest to the user, components that might hinder the separation of analytes of interest. The ability to develop multipart microparticle separations without requiring additional processing could help mitigate sample loss caused by the need to repeatedly withdraw and transport samples to different devices or processing tools. Presented here is a multipart electrokinetic device that is able to trap particles on two different levels, facilitating the modification of electric field application to suit the current needs and allowing for multiple geometries, helpful when trying to selectively sort particles without having to apply a higher electric field. The multipart device works well for helping to selectively sort different particles and prevents the potential issue of clogging that some larger particles or debris might cause when trapping or flowing through the electrokinetic device. A multipart electrokinetic device allows for effective multi-stage particle separation without needing to manually transfer particles from one device to another, risking increased loss of sample.

Nucleic Acid Amplification Using Radio Frequency Electrokinetic Heating

Presenting Author: Jarad W. Yost- Texas A&M University
Corresponding Author: Zachary Gagnon - Texas A&M University

Radio frequency fields directly heat PCR samples without Faradaic reactions or biomolecular damage

Microfluidic PCR is one of the most common and widely used sample preparation techniques. Most heating methods for microfluidic PCR are heated in one of two ways: (1) the reaction is pumped between different boundary-heating zones, or (2) stationary reaction chamber boundaries are heated by resistive heating elements. Both heating methods are subject to limitations of boundary-driven heating, where an inherent thermal gradient exists between heater and reaction. Therefore, there is a need to develop a simple and rapid PCR heating mechanism that is not subject to limitations of boundary-driven heating. Here we show a novel nucleic acid amplification platform that drives amplification reactions using high-frequency (radio frequency) AC electrokinetic heating. Heating occurs by applying a current across the reaction, with electrodes in direct contact with the reaction mixture, a system we call E-NAAMP (Electrokinetic Nucleic Acid Amplification). We demonstrate that the heating system can be used in isothermal amplification reactions (LAMP) and non-isothermal amplification reactions (PCR), with amplification efficiencies rivaling those of traditional thermal cyclers. Additionally, we show that heating produced by the applied current is electrokinetic in nature due to the temperature and voltage relationship that arises seen in the mathematics of AC electrokinetic phenomenon. Our results demonstrate that direct heating of amplification reactions by Joule heating at radio frequencies can be achieved with minimal electrode damage or significant enzyme activity loss over the course of a reaction, both of which can occur at lower frequencies. We anticipate this heating method to be able to ramp heating of PCR reactions at rates that can accomplish amplification in under a few minutes.

Utilization of Nanodisc Affinity Capillary Electrophoresis to Study the Interaction of Gialpha Proteins with Cell Membranes

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Affinity capillary electrophoresis and lipid nanodiscs are employed to characterize protein binding to cell membranes.

Guanine nucleotide binding protein subunit alpha (Gi☐) is a signaling protein that interacts with membrane-bound receptors and effectors to transduce signals across cell membranes. Gi☐ lacks a hydrophobic region to stabilize its binding to the cell membrane, but during expression of the protein, myristic acid attaches to the N-terminal residue. The attachment of this saturated fatty acid stabilizes this interaction allowing for strong binding to occur to the membrane. Interactions between proteins and biological membranes are difficult to study due to the complex structure of the membranes and the often-limited quantities of available protein. Such studies can be simplified and facilitated, however, using a cell membrane mimic such as lipid bilayer nanodiscs combined with affinity capillary electrophoresis. In this study, nanodisc affinity capillary electrophoresis (NACE) has been applied to the

study of myristoylated and native Gi□ with polymer-belted lipid bilayer nanodics. The nanodiscs employed were comprised of 1-palmitoyl-2-oleoyl-glycero-3-phosphocholine (POPC) lipid stabilized with a styrene-maleic acid co-polymer belt.

Vacuum-actuated Integrated Nanowell Microfluidic Devices for Membrane Protein Crystallization and Structure Determination

Presenting Author: Abhik Manna- Arizona State University

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Membrane protein structure determination will be possible using minimal amount of valuable protein sample.

Membrane proteins are major targets for drug delivery as they play an important role in transport across the cell membrane. Drug molecules can be specifically targeted to the membrane protein of interest if the binding site structure and conformation is known at atomic resolution. The major bottleneck of finding a natural membrane protein structure is the difficulty of stabilizing these proteins outside the cell membrane. X-ray crystallography along with microfluidics facilitates structure determination of membrane proteins. To conserve precious proteins, microfluidic devices requiring minimal protein sample can be designed to screen for protein crystals and further deliver protein crystals to state-of-the-art X-ray sources for structure determination. We have designed a microfluidic device (1) with 204 nanowells separated from one another by valves which preserve unique crystallization conditions in each well. Devices were previously fabricated with polydimethylsiloxane (PDMS) requiring only 5µL of protein for screening crystallization conditions. Here, we report on devices fabricated with thermoplastic materials like cyclic olefin copolymer (COC) or cyclic olefin polymer (COP) with the same design and functionality facilitated through flexible PDMS membranes. These new devices can be used as fixed-targets to deliver crystals to X-ray light sources for structure determination of proteins in addition to screening for crystallization conditions. The different layers of the device have been imprinted successfully in the thermoplastic materials, but challenges remain on homogeneous bonding. To improve on device bonding, surfaces were modified chemically through plasma treatment and covalent functionalization with various agents, while chemical characteristics are studied with infrared spectroscopy and contact angle measurements. Water contact angle measurement revealed a hydrophilicity increases to below 30˚ after 5 seconds of oxygen plasma exposure, which only marginally changed over the course of several days in 100% humidity. With improved fabrication, membrane protein crystallization will be performed. To avoid the extremely high viscosity of the traditional lipidic cubic phase (LCP), sponge phase with larger aqueous pore sizes will be employed to promote crystallization. The device will be used as fixed-target in the newly developed Compact X-ray Light Source at Arizona State University. Reference: 1. Abdallah, et al., Cryst. Growth Des. 2016, 16, 2074–2082

Molecular Spectroscopy

A Second Derivative Method for Raman Peak Recognition and Range Independent background Subtraction Algorithm

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Helpful for full automated background removal implementation in Raman spectroscopy applications

We report a novel computational technique that recovers Raman peaks embedded in highly fluorescent contaminated spectra. Our technique uses a Second derivative method to identify the most intense Raman peak after which a modified Savisty Golay algorithm is used to iteratively filter and recover the hidden Raman peaks. This technique is an improvement on existing background removal algorithms in both performance and user objectivity

ATR-FTIR spectroscopy and spectroscopic imaging to investigate behaviour of proteins under freeze thaw cycling stress conditions

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Application of high-throughput ATR-FTIR spectroscopic imaging to monitor effect of freeze thaw cycles on proteins

In recent years biopharmaceutical production has grown significantly due to the ever-increasing applications of biologic drugs. These can be used to treat a wide range of illnesses from dementia to cancers, and can be more effective than small molecule drugs due to their specific binding capabilities. However, despite their obvious benefits, there are challenges to producing biopharmaceuticals. For example, they are commonly less stable than small molecule drugs, and have to be produced, transported, and stored in very specific conditions to ensure they retain structural and functional integrity on delivery to the patient. During these processes, biopharmaceuticals can go through multiple freeze thaw cycles (FTCs), these FTCs place stress on the protein, and can cause partial or full, reversible or irreversible, unfolding of the protein secondary structure, which can ultimately lead to aggregation. This aggregation of biopharmaceuticals can render them ineffective to the patient, or even lead to anaphylaxis and possibly death. In this research, both FTIR spectroscopy and FTIR spectroscopic imaging were used to investigate the impact of FTCs on protein behaviour. This employed single droplets for conventional FTIR spectroscopy, and attenuated total reflection (ATR) with a high throughput PDMS well device attached to the ZnSe crystal surface for FTIR spectroscopic imaging. These methods allow investigation of protein aggregation close to the crystal surface, and, using post spectral collection analysis methods we can elucidate the secondary structure of the protein in each imaged well. Using this approach, we found that 30 mg/ml lysozyme, used here as a model protein, followed a trend of increasing protein deposition and thus aggregation with increasing number of FTCs.

In contrast 30 mg/ml IgG monoclonal antibodies in phosphate buffer exhibited some increased protein deposition but this plateaued over the FTCs, indicating the protein was more stable in the buffer solution. This is the first time the effect of FTC on protein secondary structure in the context of biopharmaceuticals has been investigated using ATR-FTIR spectroscopic imaging, and will prompt more research into how this method can be used to fully understand how protein structure changes in production, transportation, and storage.

Attenuated Total Reflection - Fourier Transform Infrared Spectroscopy for Early Detection of Pancreatic Cancer in Human Serum

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Earlier diagnosis of pancreatic cancer to enable curative surgery or earlier treatment for patient management.

Pancreatic cancer is the 7th cause of cancer death worldwide, accounting for 5% of the total cancer deaths with over 400,000 victims per year. At present, the radioimmunoassay to individuate circulating levels of Cancer Antigen (CA) 19-9 is the first mean of operation in detecting pancreatic cancer; although, it can only give an approximate indication of inflammation in the pancreatic area. The serum CA 19-9 test is not able to provide certain information neither regarding the presence of a pancreatic tumour nor of other surrounding tumours, which can also affect the test results. In the last decade, vibrational spectroscopy has shown outstanding achievements in the clinical field, and attenuated total reflection – Fourier transform infrared spectroscopy (ATR-FTIR) has demonstrated exceptional potential in serum analysis for cancer diagnostics. Early detection is not only important for the improvement of quality of life and survival rates, but also for a better and worthier application of cancer treatments. The implementation of ATR-FTIR serum analysis in the clinical environment could potentially represent a significant step towards the early detection of many cancers. PANSPEC project originated in 2017 as a multicentre UK recruitment programme to collect serum samples of patients with suspected pancreatic cancer. It aims to investigate the use of ATR-FTIR spectroscopy on dried serum deposited onto a silicon internal reflection element (SIRE) as novel approach to the early diagnosis of pancreatic cancer. Recent results were achieved on a cohort of 100 cancer samples versus 100 healthy controls with the aid of machine learning algorithms. The random forest (RF) model outcomes of sensitivity and specificity were of $91.3 \pm 5.9\%$ and $84.7 \pm 7.0\%$ respectively; whilst, partial least squares – discriminant analysis (PLS-DA) model results amounted to $91.5 \pm 5.5\%$ of sensitivity and $87.8 \pm 5.4\%$ of specificity. Both models achieved κ values over 0.75. We herein present promising results in discriminating pancreatic cancer and healthy control samples; and, hence, demonstrate that ATR-FTIR serum analysis on SIREs aims to become a rapid, sensitive, specific, reliable, minimally invasive and cost-effective diagnostic test for the early detection of pancreatic cancer to break the barriers towards clinical translation.

Balanced Detection Enables Highly Sensitive External Cavity-Quantum Cascade Laser Based Mid-Infrared Transmission Spectroscopy of Proteins

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A new milestone in sensitivity for mid-IR transmission spectroscopy of proteins outperforms established FTIR approaches.

We present an external cavity-quantum cascade laser (EC-QCL) based double-beam mid-infrared (IR) transmission setup for qualitative and quantitative analysis of the protein amide I and amide II band. In conventional Fourier transform (FT)IR transmission spectroscopy of aqueous solutions, the applicable optical path length for the amide I region is restricted to <10 µm in order to avoid total IR absorption through the HOH-bending band of water. The herein applied high intensity laser light source allowed application of larger optical path lengths (26 µm) to ensure robust sample handling. The laser light was divided into two beams of equal intensity which were detected by a thermoelectrically cooled mercury cadmium telluride (MCT) balanced detection module. In balanced detection, one beam passes through the sample, while the other serves as a reference for beam intensity fluctuations. In this way, limits of detection (LODs) approx. 8 times lower than those achieved using high-end FTIR spectrometer at comparable acquisition time and spectral resolution. An acquisition time of 150 s can be used to achieve maximum sensitivity (LOD 0.0025 mg/mL). By using an acquisition time of 45 s per full spectrum, secondary structural features can still be identified at protein concentrations as low as 0.1 mg/mL. Thus, the presented setup combines high sensitivity, large optical path lengths as well as short measurement times and outperforms previous research type EC-QCL setups as well as commercially available instruments and constitutes a milestone in mid-IR protein sensing.

Characterization of Single Extraterrestrial Dust Particles Using Optical Trapping-Cavity Ringdown and Raman Spectroscopy

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Optical trapping was combined with spectroscopic techniques to obtain the physicochemical properties of single dusts.

Earth's atmosphere consists of interplanetary dust particles (IDPs), which are a key component to understand planetary phenomena, until now the study of their physicochemical properties without external interferences at the single-particle level is limited. A single dust particle with the optical-trapping (OT) technique can be stably trapped in air for subsequent characterization. We present on measurements of the single-particle extinction of trapped particles employing cavity ringdown spectroscopy at ultraviolet wavelength about 308 nm. Also, we report Raman spectral features of chemical groups in individually trapped particles. Two simulants of extraterrestrial materials (Martian and lunar analogs) and two from terrestrial materials (carbon spheres and volcanic ashes) were trapped and spectroscopically characterized. In addition to the on-trap measurements, the particles' mineralogical and morphological information was obtained from off- trap measurements using energy dispersive spectroscopy and scanning electron microscopy. This study explains that the integration of OT with cavity ringdown and Raman spectroscopy gives a new tool to gain multimodal information on the physicochemical properties of single IDPs with minimum to no external interferences.

Coherent Raman Correlation Spectroscopy

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The significance of this work is 2DCOS application in conjunction with coherent Raman techniques

Coherent Raman spectroscopy is a high throughput technique; however, interpretation of data received by this technique is frequently made complex by nonlinear optical mixing processes. An analytical tool, two-dimensional correlation spectroscopy (2DCOS) method, can be adopted to overcome this barrier. Despite being a well-known technique in spontaneous Raman spectroscopy, 2DCOS application in conjunction with coherent Raman techniques is relatively new. We use a 2DCOS spectroscopy method with the first-hand definition of one-dimensional second-order correlation function (in the frequency domain, which replicates the results of the diagonal projection of synchronous 2DCOS data), hence, reducing the two-dimensional analytical tool to a single dimension. The results were obtained for chemicals such as pyridine, water, and their mixtures in varying concentrations, where we observed butterfly shapes in the asynchronous 2DCOS data representing auto- and cross-correlated red and blue shifts of peaks attributed to hydrogen bonding. Furthermore, this analytic tool is generalized to the correlation analysis based on the probe pulse delay variable, with a change in the probe widths. This research is demonstrated as a simple analytic tool for data interpretation for coherent Raman spectroscopy and has a potential for multiple practical applications. In particular, these findings will help to explore in-depth the ongoing progressions in coherent optical processes.

Confocal Raman Microscopy Studies of the Shape Selectivity in Aromatic-Hydrocarbon Partitioning on Modified Reversed-Phase Chromatographic Surfaces

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Non-Presenting Author: Emily Heider - Professor
Corresponding Author: Joel M. Harris, PhD - University of Utah

The results provide support for n-alkyl chain ordering governs the greater retention of planar PAHs.

In chromatographic studies of polycyclic aromatic hydrocarbons (PAHs), shape selectivity of separation media plays an important role. Numerous liquid chromatographic studies have been carried out using reversed-phase C18-modified columns to understand how the shape of a molecule impacts its retention. Sander and Wise (Anal. Chem. 1999, 71, 4821-4830) noted that for PAH geometric isomers, those with higher planarity are more strongly retained, especially on surfaces with a higher density of n-alkyl chains, with longer alkyl-chain lengths, and at lower temperatures. They speculated that the reason of this higher selectivity for planar PAH compounds could be due to greater ordering of the n-alkyl chains. In this work, we investigate this hypothesis using hybrid-supported bilayers (HSBs) as stationary-phase models having more controllable alkyl-chain ordering. These are prepared by the self-assembly of a monolayer of phospholipid or mixed cationic-anionic surfactants on the hydrophobic surfaces of C18-modified porous silica particles, providing unique opportunity to control n-alkyl chain ordering. Confocal Raman microscopy was used to track the carbon chains order and corresponding partitioning of a planar versus a non-planar PAH. Depending on acyl chain length of phospholipid or surfactants and their degree of saturation, the hydrocarbon chains of the hybrid-supported bilayer can be either in the gel-phase (highly ordered C-C trans-to-gauche ratios in their Raman spectra) or in the liquid-crystalline phase (disordered with low trans-to-gauche intensity ratios). The quantification of partitioned PAH molecules is made relative the CH2-twisting peak of the hydrocarbon stationary phase as an internal standard. The spectroscopic results provide clear support for the concept that n-alkyl chain ordering governs the greater retention of planar versus non-planar PAH compounds in reversed-phase separations.

Confocal-Raman Microscopy of DNA Immobilized at Porous Silica Surfaces

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Non-Presenting Author: Eric Peterson, Ph.D - University of Utah

Quantitative and structural analysis of DNA immobilized at an interface with Raman spectroscopy.

Detection of specific sequences of DNA is crucial for diagnosis of genetic diseases. Although techniques like SPR and quartz microbalance can detect DNA hybridization at surfaces, these methods are insensitive to DNA structure and base content. The inability to detect base content makes distinguishing between single-nucleotide polymorphisms (SNP) and partially bound fragments a challenge. Although Raman spectroscopy is sensitive to both DNA conformation and base content, detection of DNA immobilized at planar surfaces is challenging due to small scattering cross sections. In this work, we overcome these sensitivity limitations by immobilizing DNA at surfaces of porous silica particles and characterizing their interior composition with confocal-Raman microscopy. The structurally insensitive 1094-cm-1 phosphate mode was used to quantify interfacial DNA hybridization, where unit capture of a 16-mer compliment to an immobilized 19-mer single-strand was observed. Two strands of differing lengths, 9-mer and 16-mer, complimentary to the same 19-mer immobilized strand were demonstrated to be spectroscopically distinguishable through the use of the phosphate (1094-cm-1), guanine (681 cm-1), cytosine (1530 cm-1), thymine (749 cm-1) and adenine (729 cm-1) vibrational modes. Sensitivity to a single-nucleotide polymorphism was demonstrated by distinguishing between a fully complementary 16-mer and a 16-mer SNP sequence containing a single thymine-base mismatch.

Disruption of Watson-Crick base-pairing was accompanied by the red-shifting of the 790-cm-1 C2'-endo/anti thymine mode to 777-cm-1 indicative of thymine in the unbound C3'-endo/anti conformation. In addition to detecting interfacial DNA hybridization, structural conformation changes of interfacial oligonucleotides can also be observed. To demonstrate this capability, an interfacial 15-mer thrombin-binding aptamer capable of forming a potassium-ion stabilized g-quadruplex was investigated. Introduction of potassium-ion in solution to the immobilized interfacial strand was accompanied by changes in the vibrational spectrum consistent with formation of a g-quadruplex. A 17-cm-1 red-shift in the 1498-cm-1 guanine C8=N7-H2 deformation mode characteristic of tetrad formation was observed, along with an increase in the 685-cm-1 guanine mode indicative of the C2'-endo/anti conformation, consistent with the antiparallel conformation of the immobilized g-quadruplex. The methodology represents a major breakthrough in spectroscopic characterization of interfacial DNA composition, reactivity, and structure.

Confocal-Raman Microscopy of Protein Ion-Exchange Interactions on Hybrid-Supported Lipid Bilayers

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Confocal-Raman microscopy can detect and quantify protein ion-exchange interactions with hybrid-lipid bilayers in porous silica.

Protein purification, often accomplished via ion-exchange chromatography, is a necessary precursor for characterizing the structure and activity of proteins, as well as developing quality research and clinical products. Though there is a need for increased understanding of electrostatic interactions of proteins with charged surfaces, driven by the growing application of ion-exchange separations to biopharmaceutical purifications, few methods currently exist that allow for molecular-scale quantification of molecules interacting with a charged interface. In this work, we investigate protein ion-exchange interactions with a novel interface formed through engineering positively-charged sites into hybrid-supported lipid bilayers within reversed-phase chromatographic silica particles. We utilize confocal-Raman microscopy to detect the interaction of proteins with charged sites within the particles. The absence of nonspecific protein adsorption to the interior surfaces of the particles is ensured through the self-assembly of the lipid 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) to the C18-modified silica surface, which forms a hybrid-bilayer that is highly ordered and protein-repellent based on its negligible interactions with bovine serum albumin. This property ensures that the observation of protein association to a small fraction of a cationic lipid, such as 1,2-dipalmitoyl-3-trimethylammonium-propane (16:0 TAP), into a DPPC bilayer is due to specific ion-exchange interactions. The positively-charged bilayers readily exhibit protein retention, detected through Raman scattering from the phenylalanine ring-breathing mode of the protein. Quantitative determinations of protein coverage on the bilayer surface are achievable through use of the C-N headgroup stretch of the lipids as an internal standard. The dependence of the surface-adsorbed protein on the pH and ionic strength of the surrounding buffer solution is investigated. The reversibility of protein adsorption, shown by monitoring the loss of protein from the interface in a wash-off experiment and supported by a good fit of the protein surface coverage isotherms with a Langmuir model, suggests the potential application of this material to ion-exchange separations.

Coupling of Raman Imaging and Machine Learning Approaches for the Assessment of Different Myeloid and Lymphoid Leukemia Subtypes in Patient Samples

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Raman imaging can distinguish leukemia subtypes usually difficult to diagnose by visual inspection

Background: Leukemia is the result of the uncontrolled expansion and differentiation of hematopoietic cells in bone marrow (BM), producing proliferation of abnormal cells in BM and peripheral blood (PB). According to specific clinical, morphological, immunological, and genetic features, more than forty leukemia subtypes are reported, and they should be correctly recognized to define diagnosis and treatment. Nowadays, the morphological evaluation is still the first fundamental diagnostic step and includes the recognition and counting of different cellular subtypes by examining manually hundreds of cells on stained smears. This step is highly subjective, scarcely reproducible, and error prone. The introduction of subjective, reproducible, and automatable approaches for the morphological assessment of leukemia subtypes would greatly improve the diagnosis of leukemia. Method: BM samples from 19 patients affected by 9 different leukemias, including 6 acute myeloid leukemia (AML) subtypes (0,1,2,3,5a and 6) and 3 acute lymphoid leukemia (ALL) subtypes (BPh+,BPh-,T) were selected. A total of 321 cells were studied by high-resolution Raman imaging using a home-built confocal Raman microspectrometer equipped with a 647nm laser and a 63xW objective. Each cell was scanned by 64x64 (4096) Raman spectra over the entire cell area using an average step-size of 180nm and an acquisition time of 100 ms per spectrum. After pre-processing, the dataset of >1.3M spectra was used; 1) to produce false color cell images by clustering and univariate approaches; 2) to determine the spatial distribution of cellular components related to each leukemia subtype by integration and fitting approaches and 3) to automatically identify different leukemia subtypes by multivariate and machine-learning approaches (i.e. different random forest variants). Results: Raman images reveal that AML subtypes show higher biochemical and morphological heterogeneity if compared with ALL ones. A few specific components (i.e. DNA, organic matrix, myeloperoxidase, esterase, hemoglobin) are sufficient to discriminate different AML subtypes ($p < 0.001$), and AML vs ALL, but not to discern ALL subtypes. Conversely

multivariate analysis and machine learning approaches permitted to both extract some new discriminant features, including those associate to ALL subtypes, and to automatically classify all leukemias included in the study with an average accuracy >90%, after validation.

Development of a Dielectric-Barrier Discharge Hydrogen Lyman-Alpha Source: Towards Raman Spectroscopy in the Vacuum Ultraviolet

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A new HLA source is described that allows for exploration of vacuum ultraviolet Raman spectroscopy.

Raman spectroscopy provides abundant information about molecular structure, crystal phases, and local chemical environment of analytes. Unfortunately, sensitivity is limited by the inherently low Raman scattering cross section for analytes, which leads to approximately one scattered photon per 10,000,000 photons incident on a sample. Therefore, Raman spectroscopy requires high intensity, coherent excitation sources (e.g., lasers). One approach to improve the cross section is to decrease excitation wavelength, which increases the intensity of Raman scattering by the fourth power. Recently, excitation wavelengths as low as 193 nm, in the deep ultraviolet (UV), have been successfully used for Raman spectroscopy. Shorter wavelengths would produce greater Raman scattering but powerful monochromatic sources below 193 nm are often large, complex, and costly. Photons in this wavelength range are also strongly attenuated by most optical materials and molecular species in air. However, there is little attenuation around the hydrogen Lyman-alpha (HLA) line (121.56 nm) by atmospheric gases, providing a suitable window for Raman spectroscopy. In addition, HLA lines are narrow and relatively simple to produce, as HLA is the most abundant emission line in the known universe. Here, an inexpensive, (trans)portable, and powerful HLA source for vacuum-UV Raman spectroscopy is described. The source is based on a dielectric-barrier discharge (DBD) at atmospheric pressure. In the DBD, a high-voltage, alternating-current waveform is applied to at least one electrode on the outside of a quartz tube, that is purged with a plasma gas. The discharge is sustained in Ne or He with a small amount of H2 or H2O as a source of hydrogen. Ultimately, the DBD requires minimal supply gas (< 1 L/min) and low powers (<5 W). Despite the simplicity and low average power, DBDs produce high instantaneous photon fluxes due to the formation of plasma bullets. To obtain the highest instantaneous power of HLA photons, a testbed DBD source has been constructed that allows rapid interchange of plasma gases, electrode configurations, and powering schemes. Emission characteristics were measured

under various parameters with a vacuum UV spectrometer. Preliminary results show that the DBD yields significantly more HLA photons than a commercial electron-beam-based HLA source.

Discrimination between MSSA and MRSA strains using UV-Resonance Raman spectroscopy

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Detection of methicillin-resistance in S.aureus strains using UV-Resonance Raman spectroscopy without previous exposure to antibiotics

Over the past decades, increasing antimicrobial resistance has developed into a major health issue. S. aureus is one of the most common causative agents of bacteraemia and sepsis. The prevalence of Methicillin-resistant Staphylococcus aureus (MRSA) strains is increasing; and its presence limits therapeutic options, especially in nosocomial infections. Routine microbiology techniques require several days and there is a need for the development of novel, highly sensitive and inexpensive laboratory techniques for the detection of resistant pathogens in limited time. Raman spectroscopy is a fast, label-free analytical technique which can provide information of the pathogen's chemical composition. With UV-Resonance Raman (UVRR) spectroscopy typing information including the DNA/RNA content, G/C ratio and aromatic amino acids content from the bacterial cells can be obtained. Previously, it has been shown that this method can facilitate an identification of the pathogen species as well as the detection of resistance determinants [1, 2]. In the present study we investigate the detection of bacterial resistance determinants using UVRR in pre-cultured, heat-inactivated MSSA and MRSA

strains. UVRR was applied on four MRSA and MSSA pairs that differentiated only the presence/absence of the mobile genetic SCC/mec element that harbors the resistance gene, mecA, in question. For each strain, three batches of 25x10 spectra were measured on different days. Statistical analysis was performed using chemometrics and machine learning methods to investigate whether it is possible to differentiate between MRSA and MSSA. The accuracy for differentiation in the pairs ranged between 50-86%. It was observed that the band at 780/786 cm-1 (C, U) was present in all MSSA but absent from all MRSA, indicating differences in RNA composition. Tryptophan ring breathing vibration 756/849cm-1, Phenylalanine band at 1002cm-1 and Tyrosin and at 1203cm-1 were only present in one pair showing differences in protein composition in this pair compared to the others. UVRR shows potential for further more detailed analyses with a larger set of defined strains. Financial support of the MCSA-COFUND Multiply Project (H2020 GA 713694) and the research campus InfectoGnostics (FKZ 13GW0096F) is gratefully acknowledged. References [1.] K.Gaus, et al. Biopolymers, 2006;82:286-290 [2.] A. Walter, . Anal Bioanal Chem. 2011;400:2769-2773

Experimental Study of the Kinetics of Particle Formation

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Investigation of particle formation from gaseous mixtures of water vapor, formic acid, and trimethylamine.

Fine particulates in air have significant health consequences, especially those classified as PM2.5. These particles are small enough to stay suspended in the air and penetrate far into the lungs. The principle source of PM2.5 is gaseous compounds reacting in the atmosphere. An understanding of the kinetics of PM2.5 formation is important to inform models that predict the concentration of particles in the atmosphere from the amount of gaseous pollutants released. Particles have been observed to form from clusters composed of short-chained carboxylic acids and water in the presence of trimethylamine. Our goal is to determine the kinetic rate constant associated with this process, using formic acid as the short-chained carboxylic acid. To measure the kinetics of new particle formation, a slow-flow reaction cell was constructed with a constant flow of water vapor and formic acid passing through it. Small amounts of trimethylamine are introduced at different points in the reaction vessel to vary the amount of time for the reaction to proceed before the end of the vessel. At the end of the flow cell, two scanning mobility particle sizers (SMPS) measure the concentration of particles along with their size distribution. Wavelength-modulated IR diode laser spectroscopy is used to determine the water vapor concentration in the cell. UV-vis spectroscopy is used to determine the formic acid and trimethylamine concentrations. The results of this research will allow for atmospheric models to more accurately predict the amount of PM2.5 in the atmosphere, leading to better regulation policies.

Fabricating Highly Reproducible Nanofibers Covered with Au Nanoparticles for SERS Optophysiology

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A facile strategy to fabricate nanofibers covered by AuNPs with tunable morphology and adjacent spacing.

Nanofibers as biosensors have attracted great attention due to its facile removal from the biosystem after a period of intra- or extracellular measurements and site-specific measurement among others. The application of nanofibers covered with Au nanoparticles as SERS sensor circumvents the aggregation and accumulation of Au nanoparticles in vitro or in vivo, in addition to the greater Raman enhancement of signal than on planar surface. We report here on a strategy using block copolymer brush-layer templating and ligand exchange for fabricating highly reproducible and stable SERS-active nanofibers with tip diameters down to 60 nm and covered with well-dispersed and uniformly distributed branched AuNPs, which have intrinsic hotspots favoring inherently high plasmonic sensitivity. In addition, AuNPs with tunable morphology and adjacent spacing on the nanofibers can be adjusted using an in situ growth technique, thereby enhanced SERS sensitivity was obtained due to the asymmetric structure and coupling between the adjacent AuNPs. Tunable AuNP morphologies and hence the optical characteristics of the AuNPs on the nanofibers can be easily controlled by choice of experimental parameters, particularly the growth time. Besides, finite difference time domain (FDTD) simulations were performed to gain more insight into the electric-field enhancement of AuNPs on the high-curvature substrates. Furthermore, SERS application of these nanosensors in pH sensing is demonstrated here, offering appealing and promising candidates for real time monitoring of extra/intra-cellular species in vitro or in vivo. In addition to SERS sensing, these highly uniform nanosensors have other far-reaching implications, including medical diagnostics, therapeutics and so on.

Handheld near infrared spectrometer applied to detect DNA conformation changes in simple Eukaryotic cells.

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Non-Presenting Author: Phil Heraud, PhD - Adjunct Senior Research Fellow

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We detected DNA B to A conformation changes in DNA using a Portable NIR device.

Real-time monitoring of DNA conformation within living cells is pivotal in prognosis of a disease progression and response to treatment. Largely, spectroscopy has been the "method of choice" for studying different conformations. However, most spectroscopic approaches exploited so far are high-priced and requires intricate procedures. Near infrared spectroscopy on the other hand is a less expensive alternative which recently gained popularity in the context of different biomedical and biophysical applications. The potential of this technique to become a leading biomedical tool in the next few decades was extensively reviewed. While majority of NIR spectroscopic studies focused on exploring the overtones of X-H stretching vibrations, only few interrogated other important overtones and combinations bands like the C-C combination, P-H stretching mode and asymmetric C-N-C stretch which are essential for probing biomolecules like DNAs Here we demonstrate for the first time a reversible B to A- like transition in DNA using an ultra-portable near infrared instrument, for synthetic

and extracted DNA bands emerging from functional groups peculiar to different DNA structures such as such as nucleobases, phosphate backbone and aromatic moieties showed distinct shifts or intensities variation upon exposure to hydration with water and D2O. Further to this, we showed that the same conformational effect can take place in intact chicken erythrocyte consequent to hydration and dehydration. Finally, using partial least square regression (PLS-R), we demonstrate the potential of the device to unequivocally predict different DNA concentration in a multianalyte system

Identifying Raw Materials Directly Through Paper SacksUsing a Hand-Held SORS Raman Spectrometer

Presenting Author: Dean H. Brown, MS - Agilent Technologies

Expanded capabilities of materials identification using SORS Raman spectroscopy for FDA compliance

Papers sacks are often used as primary or secondary containers for raw materials employed in the manufacturing of pharmaceutical products. Excipients like lactose monohydrate, mannitol, microcrystalline cellulose, and sucrose are often supplied to pharmaceutical manufacturers in multilayer paper sacks. On arrival, the excipients in paper sacks are unloaded from the truck and moved to a quarantine area in the warehouse. Up to 100% of the received paper sacks are moved to a sampling booth where they are opened and sampled. Next, the samples are analyzed either directly in the booth with a handheld Raman or NIR system, or they are sent to a QC lab for analysis with FTIR or wet chemistry methods. Conventional Raman works well for raw materials verification, but it needs line-of-sight of the contents. That means one must be able to see clearly through the container. With most packaging, conventional handheld Raman instruments don't work. Most containers arriving in a pharmaceutical plant are nontransparent, for example, sacks, tubs, bottles, and barrels. These packaging materials are incompatible with conventional. Once sampled/analyzed, the paper sacks are sealed and moved back to the quarantine area to await approval for release to production stock. This process is time and resource intensive and can take days to complete. The Agilent Vaya SORS Raman system is a handheld spectrometer capable of identifying raw materials through transparent and opaque containers to simplify and accelerate the receipt of raw materials in GMP environments.

Infrared Spectroscopic Study of Methanol and 1-Propanol Contaminats in Ethanol-Glycerol based Hand Sanitizers

Presenting Author: Aminur Rashid Chowdhury- The University of Texas at Austin
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This study is mainly focused on the detection of poisoning chemicals in hand sanitizers

Alcohol-glycerol mixtures are ubiquitously crucial to the food, beverage and pharmaceutical industry. While the demand for the alcohol-glycerol mixture as hand sanitizer is an all-time high, the Food and Drug Administration (FDA) has recommended avoiding hand sanitizers with methanol content in it. The effects of poisoning by methanol in hand sanitizers include nausea, vomiting, dizziness, headaches, weakness, visual disturbances and loss of consciousness. The recent pandemic situation has raised many unanswered questions regarding toxicity and the threshold limit of methanol in hand sanitizers. Also FDA has recently identified some brands of hand sanitizers with 1-propanol contamination which can

result in central nervous system depression that may cause death. These 1-propanol and methanol contaminants pose a serious threat to human health. Everyday availability of these life-threatening chemicals in hand sanitizers can be avoided by development of cost-effective and robust detection methods. Unfortunately, there have not been many studies addressing the detection of methanol and 1-propanol in the hand sanitizers. In this study, we use infrared spectrocsopy to study 1-propanol and methanol contaminats in ethanol and glycerol mixtures. The results of this study will aid in identifying the spectral frequiency ranges and the detection limits of those contaminats, and provide the first step towards developing a method for hand sanitizer contaminants.

Inkjet printed SERS sensors for opioid detection

Presenting Author: Li-Lin Tay, PHD - National Research Council Canada

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Non-Presenting Author: John Hulse- National Research Council Canada

Using total diffuse reflectance, we correlates the NP loading to the performance of SERS sensors.

Fentanyl is used as prescription drug for pain relief and surgical anesthesia and is 30 ~ 100 times more powerful than morphine. Fentanyl and many of its analogues have made it into the illicit drug trade. From January to September of 2017, 72% of apparent accidental opioid-related deaths involved fentanyl or fentanyl analogues. People who use drugs often are not aware whether or not fentanyl is present. Drug checking technologies provides a harm reduction measure by informing users of the composition of the drug. There are a number of drug checking technologies that are offered at safe injection sites to help users make an informed choice. Among the different technology offered are Raman, FTIR, mass-spectrometry and colourimetric test strips. In this presentation, we will discuss surface enhanced Raman scattering (SERS) based sensor for opioid detection. We have fabricated inkjet-printed SERS sensors and tested it against fentanyl molecules. The inkjet-printed SERS sensors are particularly suitable to be used with handheld Raman analyzer. 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. We will demonstrate that well designed paper-based SERS can have a detection limit comparable to the colorimetric test strip. We will also show that surface functionalization schemes can further improve the detection sensitivity reaching detection limit of 10 ng/mL for fentanyl. Lastly, we will also introduce the concept of analyte-loaded SERS witness sample, which can serve as reference standard for comparison and validation purposes.

Interfacial Response of Iron (III) Chloride Probed by Second Harmonic Generation Spectroscopy

Presenting Author: Ka Chon Ng- The Ohio State University

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Use nonlinear technique (SHG) to probe the iron(III) chloride species presents on surface

The interfacial dipole of Fe (III) species is probed by SHG spectroscopy to investigate the structure of Fe species on the top-most layers, and the response of $FeCl_3$ solution is concentration dependent. In higher concentration of $FeCl_3$, increasing surface dipole can be assumed the increment of surface

propensity of Fe species can be obtained by a Langmuir model. The signal in lower concentration is proposed that is dominated by the change in refractive index. Electrical surface potential experiment is employed for measuring the interfacial dipole of all the species and the data support the similar results of SHG by a gradually increased of the surface potential. Surface tension experiment shows higher difference of FeCl3 solution in higher concentration which indicates the depletion of cation on the surface in high concentration. Bulk Raman results shows more Fe-Cl bonds form in higher FeCl3 concentration with less O-H bonds, suggests that the proposed dominated species on the surface is FeCl2+.

Investigating Extracellular Vesicles Derived from Mesenchymal Stromal Cells by Surface-Enhanced Raman Spectroscopy

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Trapping single nanoscale biological samples in nanohole arrays and characterising their Raman fingerprints

Plasmon-enhanced spectroscopies are a promising field of techniques for the detection and characterisation of nanoscale biological samples. Surface-enhanced Raman spectroscopy (SERS) is a plasmon-based, non-destructive technique capable of single-molecule detection. Extracellular vesicles (EVs) are a biological area of research that could greatly benefit from this technology. EVs play an important role in intercellular signalling and communication, and so there is an interest in their role as biomarkers for disease detection, diagnosis, and prognosis. Many therapeutic claims have also been proposed, as EVs released from mesenchymal stromal cells (MSCs) are thought to be potent cell-free regenerative agents effective in bone and tissue repair as well as curative agents in certain life-threatening conditions. Exosomes, a subtype of EVs, are difficult to characterise due to their nanoscale size (30 – 150 nm) and the compositional heterogeneity that exists within each population. In this work, we have designed and fabricated a SERS substrate capable of trapping single exosomes and enhancing signals of specific protein markers. Gold nanohole arrays of varying shapes (circles, squares, and triangles) and sizes (100 – 1000 nm) have been fabricated by electron-beam lithography. MSC-generated EVs derived from bone marrow and pancreatic tissues have been trapped by these nanohole arrays and irradiated with a 632.8 nm laser. The Raman fingerprint information obtained from these experiments has been subsequently analysed for the characterisation of these individual vesicles. This is a great step forward in the development of point-of-care techniques for disease detection and diagnosis.

Investigation of structural stability for methyl cyclohexane in the viewpoint of electronic states

Presenting Author: Yugo Higaki- Kindai University

Non-Presenting Author: Yusuke Morisawa, PhD - School of Science and Engneering, Kindai University

Instability for axial conformation methyl cyclohexane is accounted for electron distribution changes in HOMO-2 orbital.

It is believed that axial conformation for methyl cyclohexane is more unstable than equatorial one because of repulsive 1,3-diaxial interaction for methyl substituent. According to quantum theory of atoms in molecules, QTAIM, conformational selectivity should due not to 1,3-diaxial repulsion but to changes in electronic density of cyclohexane. Specifically, it has reported that charge transfers from ring to substituent make substituent stable but ring of cyclohexane unstable due to reduction for electron density around substituted carbon atom. Then, substituent in axial position gets greater unstable than that in equatorial position. There is no experimental evidence of changes in electronic states by substitute in cyclohexane. Systematic study of electronic transition for monosubstituted cyclohexane derivative will give information of electronic states of those molecules. If change in σ-electronic transition between these molecules could be observed experimentally, it would become parameter for cycloalkane stability with substituent. We measured FUV spectra for a neat liquid of cyclohexane and methyl cyclohexane by attenuated total reflection (ATR) technique, and assigned their transition using TD-DFT calculation. Moreover, we also investigated methyl cyclohexane to discuss the structural stability of methyl-substitution in the viewpoint of electronic states. ATR-FUV spectra of cyclohexane and methyl cyclohexane show an absorption band which involves at least two bands. Using Gaussian fitting, peaks of the bands are 147.7 and 148.3, and 159.2 and 160.5 nm for cyclohexane and methyl cyclohexane, respectively. TD-DFT calculations with GAUSSIAN 09 at CAM-B3LYP /aug-cc-pVDZ level were used to explore red shift by methyl substitution. According to these calculations, the most contributed transition of the band is second highest occupied molecular orbital (HOMO-2) to Rydberg 3p for both molecules, and the orbital energy of HOMO-2 of methyl cyclohexane is higher than that of cyclohexane.

Isolation and Characterization of Microplastics in Water

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This work describes a rapid method for microplastics detection in water samples.

Microplastics are a growing thread to safe drinking water, our food supply, and the environment due to the degradation of plastic waste in our oceans and waterways. This portends a need to characterize microplastic content in a rapid, simple, and cost-effective fashion. Water samples with microplastics of various sizes and chemical compositions were evaluated for microplastic content. Microplastics were size separated by passing through size-separating sieves and filters. Larger (generally larger than 20 microns) microplastics were isolated with a benchtop micromanipulator. Raman spectra were obtained a confocal Raman microscope and a Raman spectral library of common plastics. The system was equipped for single-point measurements, a mapping stage, and a configuration for rapid laser scanning of a 100 x 100 micron area on the surface. Combination of the laser scanning and mapping functionalities allows for rapid characterization of a full filter for microplastic quantification and identification. Results will be presented on the samples to illustrate the quantity of microplastics found as well as a discussion on the speed and cost of microplastic characterization in water samples.

Label-free Imaging Of Hepatocyte Maturation By Raman Microscopy

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Raman Microscopy can realize non-invasive evaluation of cell state and quality for regenerative medicine.

Along with the development of regenerative medicine, which is aiming at fabricating artificial tissues and organs for transplantation, there is an increasing demand for non-invasive methods to monitor the growing process of different cell populations and evaluate the quality of the final tissue constructs. However, conventional methods generally require disruption of the tissue transplants, such as tissue sectioning and immunostaining. A method which can discriminate and access different cell population in a label-free and non-destructive manner is necessary for this field. Raman microscopy has emerged as a powerful tool in label-free observation and characterization of biological samples since it can detect vibrational frequencies given by the chemical structure of the molecules. After simply shining the laser light onto the living specimens, specific biochemical information of cells and tissues can be collected and analyzed without additional treatment, enabling non-destructive quality control of cells and tissue constructs for the subsequent transplantation. We reported several Raman peaks which can be assigned for the differentiation and maturation markers of the hepatocytes. Reconstructed Raman images depicted the distribution of different cellular components, showing the variance of Raman intensity at the single-cell level. To quantify this variance, the averaged spectrum of the individual cells was extracted and Raman intensity at each peak was calculated. At the end of culture, cell population with different Raman spectra was identified which differ from the cells at the beginning of the culture. Raman peaks related to hepatocyte maturation were identified. In this research, cell maturation process was monitored, and different cell population was identified by Raman Microscopy. It suggests that Raman microscopy has great potential in evaluating the quality of cell products for regenerative medicine.

Label-Free Raman Microspectroscopy For Studying Infections With Bacteriophage

Presenting Author: Indra Monsees- Universität Duisburg-Essen

Non-Presenting Author: Alexander Probst - Universität Duisburg-Essen

Shifts in Raman spectra can be used to differentiate bacteriophage-infected Pseudomonas cells from non-infected cells.

Raman microspectroscopy allows differentiation of bacterial cells different growth phases [1] as well as the characterisation of viral particles [2]. Therefore, we propose that Raman microspectroscopy is sensitive enough to differentiate phage-infected cells from non-infected cells. Hence, the aim of this work was to identify Raman marker shifts of viral infections in bacterial pure cultures using multivariate data analysis and to define a spectral marker in univariate statistics. To achieve this goal, Pseudomonas sp. was infected with phage phi 6, and samples for Raman spectroscopy were fixed and measured using

a Renishaw inVia™ confocal Raman Microscope before and after infection. The acquired spectra were analysed using the R package Micro Raman [3] using ordination analyses, hierarchical clustering and Monte Carlo-based permutation procedures (multi response permutation procedure). Spectral differences were analysed using contrast plots and univariate markers (intensity ratios) for identifying infected cells were based off Raman shifts with the highest values in the contrast plot. Before infection, no significant difference was observed between the individual cultures (chance corrected within-group agreement A = 0.0093 p = 0.002), whereas a strong divergence of certain cells was observed after infection (A = 0.06202 p = 0.001). In detail, these cells showed a greater abundance in nucleic acids and lower abundance in proteins, respectively. We conclude that a shift within the nucleic acid:protein ratio can be used as discriminator for non-infected and infected cells. We assume that in heavily infected cells viral gene expression and reproduction of the viral genome cause shifts in the nucleic acid abundance. We propose that Raman spectroscopy in combination with other microscopy techniques has a high potential for providing key information for detecting viral infections 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. 3. García-Timermans, C., et al. (2018) Journal of Microbiological Methods 151: p. 69-75.

Mid-IR laser-based dispersion spectroscopy in liquid

Presenting Author: Alicja Dabrowska- Technische Universität Wien

Non-Presenting Author: Andreas Schwaighofer - Technische Universität Wien

Corresponding Author: Bernhard Lendl, Prof. Dr. - Technische Universität Wien

Dispersion spectroscopy is a novel alternative approach for qualitative and quantitative analysis of liquid-phase samples.

While mid-infrared (mid-IR) spectroscopy has been an important analytical technique widely used in a variety of scientific fields for its capability of molecule specific detection. The development of the quantum cascade laser (QCL), a powerful and coherent source of mid-IR radiation, opened new possibilities and approaches for spectroscopic sensing, triggering advancements in mid-IR spectroscopy. External cavity QCLs (EC-QCL) has been shown to outperform established FTIR spectrometers in terms of sensitivity and throughput. However, inherent properties of these sources, their coherence nature in particular, enable new spectroscopy schemes that go beyond classical absorption spectroscopy. Measurements of the changes in refractive index (dispersion) induced by a molecule rather than absorption is one alternative approach to classical laser-based spectroscopic techniques. Dispersion sensing offers quantitative and qualitative information equivalent to absorption spectroscopy, but is experimentally more challenging than conventional absorption measurements. Nevertheless it is increasingly implemented because of multiple advantages, i.e. it is immune to source intensity fluctuations and offers high dynamic range for chemical detection. While a number of methods for dispersion sensing of gaseous samples has been developed based on QCLs, QCL based dispersion spectroscopy of liquid-phase samples so far has found little interest. We present a novel sensing concept based on an EC-QCL and a Mach-Zehnder interferometer setup for dispersion and absorption spectroscopy (i.e. real and imaginary part of the refractive index) of liquid-phase samples - information which is not accessible with conventional or commercial instrumentation and therefore had to be calculated using the Kramers-Kronig transform. The method is independent of the sampling technique, allowing to use transmission absorption measurements for highest sensitivity or attenuated total

reflection for robustness. Our example applications demonstrate the power of our technique for quantitative and qualitative analysis of solutes in water (e.g. proteins, carbohydrates, …). Using electromagnetic theory, we are also able to devise a Beer's law analogue for quantitation using the real part of the refractive index.

Monitoring Curing Adhesives: A Diamond FTIR-ATR Comparison

Presenting Author: Bisirat Araya- Harrick Scientific Products
Non-Presenting Author: Susan Berets, PhD - Harrick Scientific Products
Non-Presenting Author: James Delaney - Harrick Scientific Products
Non-Presenting Author: Jeff Christenson - Harrick Scientific Products

Analysis of diamond ATR accessories in 2600-1500 cm-1 region using samples with isocyanate functional group.

Adhesives are frequently analyzed for quality control purposes and in order to develop improved formulations. Diamond FTIR-ATR spectroscopy can be useful in this endeavor. The low coefficient of friction of diamond makes permanent adhesion to the ATR crystal unlikely, and its hardness makes it possible to scrape off residual hardened adhesive using metal blades should it be needed. Thus many types of durable and difficult-to-remove residues from processes in which liquids harden into solid form, such as curing adhesives and epoxies, can be examined easily using diamond ATR spectroscopy. The major hindrance for this type of study is the diamond lattice absorbances in the 2600-1500 cm-1 region and their potential to interfere with absorbance bands characteristic of triple-bonded species such as nitrile, cyanate and azide functional groups. This work compares the performance of several diamond ATR accessories during the curing process. Several adhesives are examined, including one with an isocyanate functional group.

Multimodal Spectroscopic Characterization Of Buried Electrochemical Interfaces

Presenting Author: Sasha Moonitz- University of Utah
Non-Presenting Author: Noah Shephard- University of Utah
Non-Presenting Author: Rodrigo Noriega, PhD - University of Utah

Probe interactions between biomolecules, interfacial solvent environment, and ionic species under applied electric fields

Electrified interfaces are a complex environment where electrode surfaces, solvent molecules, ions, and free and surface-bound species interact on multiple time scales. Probing electrochemically-active buried interfaces is a challenge that requires the combination of a wide variety of experimental tools. To enable the spectroscopic and electrochemical characterization of electrified interfaces, mid-infrared surface plasmon resonances and time-resolved fluorescence are employed to probe the interface between a liquid electrolyte and the working electrode of an electrochemical cell. With these complementary tools it is possible to detect the effects of interfacial electric fields on the conformation of macromolecules and the concentration of ions within the interfacial layer, as well as how these changes in the microenvironment affect the time scale and extent of local molecular motions.

Near-Infrared Imaging for Quantitative Determination in Pharmaceutical Tablets

Presenting Author: Shigeaki Morita, Ph.D. - Osaka Electro-Communication University

Two independent values were quantitatively determined during a manufacturing process of pharmaceutical tablet.

A quantitative determination of physicochemical information for pharmaceutical tablets by using non-contacting and non-destructive method during a manufacturing process is strongly demanded. Near-infrared spectroscopy can obtain both physical and chemical information from an opaque sample such as pharmaceutical tablets. In the present study, near-infrared hyperspectral imaging was applied for quantitative determination of two independent values of a content of sarpogrelate hydrochloride in a tablet and a thickness of film coating on a surface of the same tablet. Near-infrared hyperspectral images of tables on a moving conveyor-belt were sequentially obtained. For one tablet having a diameter of approximately 7 mm, a total of 121 spectra were measured. By using spectral data from 121 tablets, i.e., 14,641 spectra, two independent calibration models were constructed by means of partial least squares (PLS) regression, respectively. Results of validation for the two independent models and potential usefulness of near-infrared hyperspectral imaging for pharmaceutical process control will be discussed in detail.

NIR spectral analysis of natural medicines supported by quantum chemical calculation

Presenting Author: Justyna Grabska, PhD - Leopold-Franzens-Universität
Non-Presenting Author: Krzysztof B. Bec - Leopold-Franzens-Universität
Non-Presenting Author: Christian W. Huck, Mag.Dr. - Leopold-Franzens-Universität

Near-infrared spectroscopy is a potent tool and is used for analysis of natural products

Natural medicines processed from plants are of great importance as pure compounds as well as standardized extracts. A variety of medicinal plants have traditionally been used as a source of therapeutic agents and are presently rapidly re-gaining their worldwide importance, following the society's preference for using natural drugs. Due to the increasing demand from the global market for the top performing screening programs for seeking therapeutic drugs from natural products, keen interest is placed in efficient analytical approaches. Near-infrared (NIR) spectroscopy is a potent tool and is often used for analysis of natural products. The spectrum of the applications of spectroscopic methods include: direct quality control of natural medicines; determination of the product's chemical composition and quantification of the content of bioactive compound; control/optimization of the cultivation parameters of medicinal plants, e.g. location, conditions, harvest time. These methods are applicable in laboratory and directly in field when using novel NIR miniaturized spectrometers. Difficult interpretability of the spectra hampers the evolution of NIR spectroscopy in certain areas, e.g. structural discrimination and identification. Further, portable spectrometers often operate only in narrow wavenumber regions. This influences their performance of analysis, depending on the NIR absorption lineshape bands of the analysed compounds. Quantum chemical simulation of NIR spectra provides new insights and helpful assistance to such analyses. We present our achievements in developing new approaches capable of providing practical benefits, e.g. understanding of the meaningful wavenumber regions in calibration models and prediction of the analytical performance of handheld NIR

spectrometers for classes of compounds with no need to perform tedious measurements of analytical standards.

Non-Contact Electrostatic Sampling of Powders Enables Solvent Free Mass Spectral Analysis in Seconds-per-Sample

Presenting Author: Brian D. Musselman, PhD - IonSense, Inc.

Non-Presenting Author: Brittany D. Laramee - IonSense, Inc.

Non-contact collection of particles facilitates rapid chemical analysis without use of solvents.

Considerable time can be spent preparing solid samples for chemical analysis. Typically, solids are pulverized into powder form then dissolved using solvents to facilitate sample introduction via syringe into the injector of either a gas chromatograph or Liquid Chromatograph/Mass Spectrometer. A knowledge of the solubility of the chemical and its contaminants is often necessary to complete successful analysis. Insoluble materials, and mixtures containing soluble and insoluble materials present problems for the analyst that must be solved to permit successful analysis. Considerable volumes of solvent are thus used for sample preparation and in the case of LC/MS for the analysis. Solvent waste are generated in copious quantities in many laboratories as analytical methods are used over and over for raw material and product quality assessment. We describe a method using a low-cost handheld Van de Graaf (VDG) Generator to generate a high voltage, low current electrical field sufficient for non-contact sampling of particles from surfaces and TLC plates. Particles are collected from those surfaces by positioning a metal pin, or metal mesh sampler within 1mm of the sample, activating the VDG, and touching its output to a conducting holder to which the metal is affixed. Post-collection the metal sampler position in the desorption ionization region of a direct analysis in real time (DART) equipped mass detector to facilitate analysis. The DART source directs heated ionized inert gas at the sample laden metal resulting in thermal desorption and subsequent ionization resulting in formation of either intact protonated molecules or intact deprotonated molecules for accurate MS or MS/MS analysis.. DART-MS results from the analysis of fine chemicals, trace contaminants in finished products and natural products demonstrate the range of this method. Non-contact sampling from thin layer chromatography (TLC) plates is utilized to demonstrate utility for identification of multiple chemicals in comlpex samples with limited solvent consumption. The method is rapid and reduces the use of solvents for sample prep, except when completing TLC separation prior to sampling. The method reduces the need for lab glass, caps, septa, and reduces the volume of solvent thus mitigating waste generation.

Optical Sensor for Monitoring Quality of 3D Metal Printing

Presenting Author: Giuseppe Pignatelli- BAM - Bundesanstalt für Materialforschung und -prüfung

Corresponding Author: Igor Gornushkin, PhD - BAM - Federal Institute for Material Research and Testing

Non-Presenting Author: Anne Strasse - BAM - Federal Institute for Material Research and Testing

Non-Presenting Author: Andrey Gumenyuk - BAM - Federal Institute for Material Research and Testing

Improving online monitoring for additive manufacturing

There is wide interest regarding Additive Manufacturing (AM) techniques, which allow building items

made of different materials based on a computer-aided design (CAD). Among them there is Laser Metal Deposition (LMD), a technique that uses laser and metal powder to manufacture on, cover or repair a metal substrate. While it is of high interest for different industrial sectors, its deployment is slowed down by a lack of on-line monitoring devices that would detect production flaws during the process. Up to now, the most reliable way to test a workpiece is after its completion with invasive and time-consuming techniques, while several attempts to acquire information online in different ways have met only a limited success. Here we show how a portable spectroscopic set-up that uses the light emitted during the laser-matter interaction process can helps in identification of defects online while printing. We acquire the light emitted while printing straight stripes of steel on metal substrate, where several holes-like defects have been made on purpose. The light emitted from the molten steel during printing does not show characteristic emission lines, as is common in similar set-ups, but only a featureless broadband emission in the analysed range of 200-1650 nm. Thus, the sources of information are not peaks of atoms or ions or molecular bands, but the whole spectrum. Every collected spectrum is compared with reference spectra recorded while printing on a regular substrate in standard condition. This comparison is performed calculating Pearson or rank correlation coefficients, a metric that measures the similarity between two datasets. By calculating this coefficient for every spectrum acquired online, we measure a noticeable change in its value when the laser prints over the artificial defects; thus proving this being a suitable technique for online monitoring of defects. Moreover, since there is no plasma breakdown involved, the emitted light is related only to blackbody radiation emitted by molten steel. It is possible to fit the recorded spectra with the Plank equation(1) and estimate the liquid steel temperature, thus improving the monitoring capabilities. With these preliminary results, the accuracy and limitations of this method are evaluated.

Paving the way for real-time monitoring of nitrite via SERS through signal and substrate processing techniques and low-cost SERS substrates

Presenting Author: Robert B. Chevalier, N/A- University of Rhode Island

Non-Presenting Author: Brian S. Sheetz - The University of Rhode Island

Non-Presenting Author: James T. Hagan- The University of Rhode Island

Corresponding Author: Jason R. Dwyer, PhD - The University of Rhode Island

Development of SERS processing techniques and low-cost SERS substrates for real-time nitrite monitoring in seawater

Nitrite is a prevalent contaminant found in most wastewater treatments, industrial processes, and agricultural runoffs. Nitrite levels in seawater from natural and anthropogenic activities provide important information when assessing coastal ecosystems. The detection and monitoring of nitrites is thus crucial. At the moment, nitrite test kits using the colorimetric Griess reaction are available. We are exploring SERS sensing as a buoy-deployable method for real-time nitrite monitoring in marine environments. Along with high sensitivity, technological advances have made portable spectrometers possible, which allow for SERS to be used in the field versus in a lab. We will present several advances from our work focusing on developing signal-processing, SERS substrate processing, and creation of low-cost SERS sensors in order to support this drive for the deployment of real-time SERS sensing platforms.

Performing SERS Measurements in Complex Tissues with Plasmonic Nanofibers

Presenting Author: Gregory Q. Wallace - Université de Montréal

Non-Presenting Author: Benoit Delignat-Lavaud - Université de Montréal

Non-Presenting Author: Xingjuan Zhao- University of Montreal

Non-Presenting Author: Louis-Eric Trudeau - Université de Montréal

Corresponding Author: Jean-Francois Masson- Université de Montréal

Development of a blueprint for performing SERS measurements in thin ex vivo brain slices.

Neurological and psychiatric diseases are often associated with changes in brain neurochemistry. It is therefore necessary to better understand the composition of secreted neurotransmitters across various domains of healthy and disease-state brain tissue. Unfortunately, many of the currently used methods suffer from drawbacks, most notably poor temporal resolution or a lack of multiplexing capability. Without both criteria, it is nearly impossible to critically evaluate the near-real-time composition of secreted neurotransmitters. Inspired by traditional patch clamp experiments, the nanosensors used are composed of a pulled glass rod decorated with gold nanoparticles arranged by nanotemplating. This allows for the near-real-time detection of neurotransmitters using a label-free SERS approach. To date, our group has emphasized evaluating these types of processes using neuronal cultures. This work will cover our group's more recent efforts in the development of a blueprint for performing SERS measurements in thin ex vivo brain slices. Given the small diameter of the nanosensor, observing the sensor once embedded into the brain tissue becomes rather challenging. As a result, considerable time has been spent evaluating different nanosensor designs and detection schemes used to focus the incident excitation light onto the embedded sensor. We then demonstrate the SERS sensing capabilities of these nanosensors once embedded into the brain tissue. Potassium depolarization and optogenetics are subsequently used to trigger the secretion of the neurotransmitters. By adding pharmacological agents, the rates of secretion and/or re-uptake of the neurotransmitters can be altered to further improve and validate our detection scheme. By performing these measurements across different portions of the brain tissue, and under basal and stimulated conditions, we can achieve a better understanding of neurochemistry in near-real-time.

Plasmon-Mediated Reduction of Diazonium Salts Using Gold Nanostructures.

Presenting Author: Denis A. Therien- University of Western Ontario

Non-Presenting Author: Danielle McRae- Western University

Corresponding Author: François Lagugné-Labarthet, Ph.D. - Western University

Gold Nanostructures with three exploitable plasmon resonances in the visible for surface-specific chemistry

Metastructures with plasmonic 'hot-spots' can be further utilized to pattern chemical reactions at a nanoscale level using the hot electrons emitted in these specific areas. In the present work, we have a developed a 'cross-hair' gold nanostructure organized in arrays that has 3 resonances in the visible region and that can be addressed independently and with specific polarizations. The crosshair-like structures, designed using finite domain time domain calculation, display three distinct plasmon resonances at 532, 633, and 800 nm that can be addressed with common laser wavelengths. The

structures were then fabricated by electron beam lithography. This work focuses on the tuning, the fabrication, and the pattering of these designed nanostructures with diazonium salts. The 4-branched crosshair structures were irradiated at the three different wavelengths and further imaged to estimate the efficiency of the chemical reactions and confirm the plasmonic origin of the reaction. Such structures can be exploited for multiplexed functionalization using distinct guest molecules to be patterned over the structure.

Polarimetric Balanced Detection: Enhanced Attenuated Total Reflection Mid-Infrared Laser Spectroscopy for Sensing in Liquids.

Presenting Author: Stephan Freitag- Technische Universität Wien

Non-Presenting Author: Matthias Baer - Friedrich-Alexander-University Erlangen-Nuremberg

Non-Presenting Author: Laura Buntzoll - Friedrich-Alexander-University Erlangen-Nuremberg

Non-Presenting Author: Andreas Schwaighofer - Technische Universität Wien

Non-Presenting Author: Georg Ramer, MSc PhD - Technische Universität Wien

Non-Presenting Author: Bernhard Schmauss - Friedrich-Alexander-University Erlangen-Nuremberg

Corresponding Author: Bernhard Lendl, Prof. Dr. - Technische Universität Wien

Polarimetric balanced detection enables long-term stable and background free evanescent field mid infrared laser spectroscopy.

Classic attenuated total reflection (ATR) infrared (IR) absorption spectroscopy approaches e.g. in process monitoring, utilize a background spectrum usually recorded prior to sample measurements, hence introducing a temporal mismatch between background and sample spectra. As environmental and instrument parameters drift over time (especially in process analytical settings) this difference in time often leads to artifacts in the resulting absorbance spectra and impair successful analysis. Polarimetric balanced detection is a new ATR mid-IR laser based sensing scheme that exploits unequal effective thicknesses achieved with laser light of different polarization, hence allowing for simultaneous recording of background and sample spectra and eliminating artifacts due to instrument or environmental drifts. A widely tunable monolithic Vernier quantum cascade laser (QCL-XT), a multi-bounce zinc sulfide ATR element and a thermoelectrically cooled balanced detection module consisting of two mercury cadmium telluride elements were combined for analysis of liquids. Fully-automated sample injection into a custom built ATR flow-cell was performed via sequential injection analysis. The device performance was evaluated by on-site measurements of ethanol in water inside of a water treatment plant (i.e. a process analytical setting). Polarimetric balanced detection improved the root mean square noise by a factor of 10 over classic absorption measurements. In addition the noise suppression capabilities of polarimetric balanced detection improved the limit of detection for ethanol in water by a factor of 2. This demonstrates the potential of our new polarimetric laser-based ATR mid IR sensing scheme for process monitoring or in-field applications prone to a multitude of interference. This work also illustrates the application of QCL-XT technology, a broadly tunable mid-IR laser source as a monolithic alternative to the more commonly used external cavity QCLs used for liquid phase investigations.

Polymer Characterization via Laser-Assisted Pyrolysis Program Flowing Atmospheric Pressure Afterglow Ambient Mass Spectrometry

Presenting Author: Dong Zhang- Texas Tech University

Non-Presenting Author: Mohsen Latif- Texas Tech University

Non-Presenting Author: Maureen Oliva- Texas Tech University

Corresponding Author: Gerardo Gamez, PhD - Texas Tech University

A novel laser-assisted pyrolysis program technique is demonstrated for profiling polymers by FAPA AMS

Ambient mass spectrometry (AMS) allows direct sample desorption and ionization with minimal-to-no sample preparation, when combined with pyrolysis techniques, is a powerful tool for polymer analysis. However, the mass spectra are usually complicated without thermal separation since all pyrolysis products and additives present in the same spectrum, thus makes it difficult to interpret especially for copolymers. Gradually elevating the pyrolysis temperature allows thermal separation of the additives and different pyrolysis products. In the present study, a novel laser-assisted pyrolysis program (LAPP) technique is demonstrated and combined with flowing atmospheric pressure afterglow (FAPA) AMS for instantaneous of profiling polymers and their additives. The FAPA source design has a pin-to-capillary configuration. A negative DC voltage is applied to the pin cathode through a ballast resistor of 4.5 kΩ while the inner capillary electrode is grounded, leading to a glow discharge generated in the discharge chamber between the two electrodes with voltage of ~450 V and current ~30 mA. The inter-electrode distance is set to 6 mm and helium is used as discharge gas. The duty cycle of a 405 nm diode laser (J Tech Photonics) is controlled by an Arduino board. Mass spectra are collected by Thermo LTQ XL orbitrap mass spectrometer (Thermo Scientific). Samples including pressure sensitive tapes, nitrile gloves, and plastic bags are analyzed. The optimized laser power is 0.8 W with frequency at 100 Hz. The duty cycle of laser is set to 0%, 10%, 20%, 50%, and 100% with 25 s for every duty cycle, which makes the analysis ~2 min/sample. For the duct tape samples, the peaks of phthalate additives already present when the laser is off, but the peak patterns of pyrolysis products are evident until the laser duty cycle reaches 50% or 100%. Further mass spectra elucidation is performed by plotting the Kendrick mass plot, which shows the CH2 repeating unit of polyethylene. The peaks of C5H8 repeating unit are also evident for duct tapes, which comes from the adhesive rubber polyisoprene. More comprehensive peak identification will be presented for different laser duty cycles which result in varying pyrolysis temperatures.

Quantum Chemical Simulation of Near-Infrared Spectra - Current Development Towards Applications in Analytical Chemistry

Presenting Author: Krzysztof B. Bec - Leopold-Franzens-Universität

Non-Presenting Author: Justyna Grabska, PhD - Leopold-Franzens-Universität

Non-Presenting Author: Christian W. Huck, Mag.Dr. - Leopold-Franzens-Universität

This is quite unique and novel direction of research, yet attracting high interest (citation count)

The spectra in near-infrared (near-IR, NIR) region in their complexity extend far beyond those in infrared (IR; or mid-infrared, mid-IR; 4000–400 cm-1) and Raman. This fact imposes difficulties in the interpretation of the observed NIR bands. The intrinsic convolution of NIR spectra resulting from band overlapping also the source of its strength as rich information about the sample properties is brought within it for chemometric analysis. The advances made in the area of theoretical NIR spectroscopy over

the last few years, have offered an ability to interpret NIR spectra in detail. Quantum chemical methods yield an independent insight here and offer new opportunities in analytical applications of NIR spectroscopy. These methods shed light on the physicochemical background which determines the spectral variability that is keen interest of analytical applications of this technique. This presentation overviews the latest accomplishments in obtaining a better comprehension of this underdeveloped area with special attention paid to the innovative role and considerable potential attained by theoretical simulation of NIR spectra [1]. Ref. [1]. Beć, K.B.; Huck, C.W. Breakthrough potential in near-infrared spectroscopy: spectra simulation. A review of recent developments. Front. Chem. 2019, 7, 48.

Raman Hyperspectral Imaging with Multivariate Analysis for Investigating Enzyme Immobilization

Presenting Author: Joseph P. Smith, Ph.D. - Merck

Non-Presenting Author: Melinda Liu - UCLA

Non-Presenting Author: Mackenzie Lauro, PhD - Merck

Non-Presenting Author: Madhu Balasubramanian - Merck

Non-Presenting Author: Jacob Forstater - Merck

Non-Presenting Author: Shane Grosser - Merck

Non-Presenting Author: Zachary Dance - Merck

Non-Presenting Author: Timothy Rhodes - Merck

Non-Presenting Author: Xiaodong Bu - Merck

Non-Presenting Author: Karl S. Booksh, PhD - University of Delaware

For the first time, Raman hyperspectral imaging with chemometrics investigates immobilized enzymes for biocatalysis

Directed enzyme evolution has led to significant application of biocatalysis for improved chemical transformations throughout the scientific and industrial communities. Biocatalytic reactions utilizing evolved enzymes immobilized within microporous supports have realized unique advantages, including notably higher enzyme stability, higher enzyme load, enzyme reusability, and efficient product-enzyme separation. To date, limited analytical methodology is available to discern the spatial and chemical distribution of immobilized enzymes, in which techniques for surface visualization, enzyme stability, or activity are instead employed. New analytical tools to investigate enzyme immobilization are therefore needed. In this work, Raman hyperspectral imaging with principal component analysis, a multivariate method, is demonstrated for the first time to investigate evolved enzymes immobilized to microporous supports for biocatalysis. Herein we demonstrate the ability to spatially and spectrally resolve evolved pantothenate kinase (PanK) immobilized onto two commercially-available, chemically-diverse porous resins. This analytical methodology is able to chemically distinguish evolved enzyme, resin, and chemical species pertinent to immobilization. As such, a new analytical approach to study immobilized biocatalysts is demonstrated, offering wide application for development of protein or biomolecule immobilization.

Raman Microscopy Investigation of Antibody-Antigen Interactions at Hybrid Supported Phospholipid Bilayers in Porous Silica Supports

Presenting Author: Jay P. Kitt, PhD, MS - University of Utah

Non-Presenting Author: Joel M. Harris, PhD - University of Utah

Absolute quantitation (surface coverage) of an antibody assay with structural investigation of antibody-antigen interactions

Antibody-antigen interactions are important in disease pathology, disease detection, and as promising novel drug candidates. Despite this, quantitative methods for measuring antibody-antigen interactions capable of reporting binding stoichiometry are limited; those few methods which can provide quantitative results rarely yield structural insights into the mechanism. These mechanisms can drive differences in binding strength for varying epitopes on an antigen or variation in binding domains across antibodies to the same antigen. In this work, we report a method for immobilization of antibody proteins at a hybrid supported phospholipid bilayer within porous silica and demonstrate quantitative and structurally informative measurement of antibody-antigen interactions using confocal Raman microscopy. Biotin-modified lipid incorporated into the within-particle hybrid lipid bilayer was used to capture streptavidin, a multivalent protein with high (near-covalent) affinity for biotin, immobilizing streptavidin to the lipid membrane. The bilayer was then exposed to either biotinylated antibody or biotinylated protein G to attach either to the bilayer interface. Protein G is an antibody-binding protein derived from bacterial membranes that binds the constant region of an antibody thereby orienting the antibody to the interface. It is commonly incorporated into antibody assays and thought to increase efficiency by orienting the Fab-fragments toward the solution. In the case of protein G, the bilayer is then exposed to unlabeled antibody, which is captured by protein G. By detecting scattering from the phenylalanine ring stretch relative to scattering from the phospholipid headgroup C-N stretch as an internal standard, protein accumulation was quantitatively measured at each step, allowing assessment of the efficiency of each binding interaction in the immobilization scheme. The antibody-modified particles were then exposed to model antigen in solution and binding efficiency of the immobilized antibody measured. The impact of protein G on binding efficiency was determined by comparing protein G immobilized antibody to biotin immobilized. Comparing antibodies with different Fab (binding) regions was carried out to investigate both quantitative binding and structure to examine the underlying mechanism of biorecognition.

Raman Spectroscopy Of The Family Of Two-Dimensional MXenes

Presenting Author: Asia Sarycheva- A.J. Drexel Nanomaterials Institute

Corresponding Author: Yury Gogotsi - Department of Materials Science and Engineering, and A.J. Drexel Nanomaterials Institute, Drexel University

The first study on the essential characterization for the emerging class of 2D materials MXenes

In the recent years, Raman spectroscopy was widely used in the field of 2D materials: graphene, transition metal dichalcogenides, boron nitride and others. It has been the tool to evaluate the composition and defects in the structure of those materials. Moreover, Raman spectrum could be collected even from a monolayer of 2D material. Apart from structural properties, Raman spectroscopy has been used to count the number of layers, mechanical properties and thermal conductivity of 2D

materials and its composites. This big impact on the field shows that Raman spectroscopy can give a lot of information about 2D materials, therefore is an essential tool for study the new families of 2D materials. One of those families is MXenes, 2-dimensional transition metal carbides and nitrides, discovered at Drexel University at 2011, which gained already a lot of interest in a variety of applications: from a promising electrode material for supercapacitors to transparent flexible radio-frequency antennas To date, there is a lack of detailed systematic experimental Raman spectroscopy study on MXenes, therefore a database for quick assessment of a MXene is needed. We obtained Raman spectra and assign peaks for M2X members of the family: Nb2C, Mo2C, V2C and Ti2C, as well as M2X3: Ti3C2, Mo2TiC2 and M4X3: Cr2TiC2 and Nb4C3, Mo2Ti2C4. This covers most widely used MXenes to date. We showed the difference between multilayer form (powder), delaminated form (deposited thin film or filtrated free-standing film). In the collected library of MXene Raman spectra, we showed the change in the atomic vibrations with changing or substituting M element. Since 2D MXenes are hydrophilic materials with rich surface chemistry, we used Raman spectroscopy to evaluate surface groups. This information will lay the ground for further investigation of surface reactions mechanisms of MXenes in electrochemical energy storage applications. Moreover, we monitored changes in Raman spectrum of MXenes to show early stages of sample degradation and how to assess the MXene quality by using Raman spectroscopy. This data will help researches to assess the quality of the product of MXene synthesis, understand the structural properties of synthesized material and get information about MXene surface groups.

Rapid Reaction Monitoring for Stopped Flow Instrumentation with Dual Comb Spectroscopy - 220 spectra / second

Presenting Author: Florian Eigenmann- IRSWEEP

Non-Presenting Author: Raphael Horvath, PhD - IRSWEEP

Rapid Reaction Monitoring with subsecond time resolution in MID-IR Stopped Flow - QCL frecquency combs

Understanding the mechanism of a chemical reaction is an important step away from trial-and-error-based reaction optmizaton. By studying the structures of intermediates, for how long they exist, and the ways in which they can and cannot react, reactions can be intelligently designed, controlled and where required, optmized. This work explores the use of the IRis-F1 dual-comb spectrometer in IR stopped-flow experiments. Firstly, we investigate the hydrolysis of methyl chloroacetate (MCA) and this will be followed by the refolding of ubiquitin, which is expected to be a more challenging measurement. Stopped-flow is a powerful technique to study reactions on a millisecond to minute timescale. Simply speaking, it works by rapidly mixing reagents before a spectroscopic cell. When the flow is stopped, the chemical reaction initiated by mixing can be analyzed using a variety of techniques. IR spectroscopy is powerful in this regard because it is sensitive to chemical and structural changes of the analytes and its specificity is outstanding. For this set of experiments, we partnered with TgK Scientific, a leader in stopped-flow IR equipment. The syringe drive unit of the SF-73 stopped-flow system is shown in the picture (top right). It connects via an umbilical to a spectroscopic cell with CaF2 windows and a path-length of 100 μm. For the MCA experiment, a background was acquired of the pure solvent and for the ubiquitin experiment a background was acquired of the already-reacted mixture. This was necessary to subtract the signal from the changing solvent environment. Stopped-flow is a powerful tool to investigate the IR spectra of evolving and transient species and to monitor reactions on the timescale of milliseconds to minutes. It allows for precise, efficient and rapid mixing of a wide range of samples at

low volumes, controlled temperatures and, if necessary, at anaerobic conditions. We have successfully investigated chemical and biochemical reactions with this technique and have shown that good S/N ratios can be obtained even for weak signals and with single repetitions. Combining dual comb spectroscopy with stopped-flow opens up investigations of new reactions and processes that were previously difficult or impossible to measure in the IR.

Simultaneous and Reproducible Detection of Multiple Microcystins Based on Self-Assembly and Aptamer-Driven Core–Satellite SERS-Active Plane Substrate

Presenting Author: Xiaojun Luo- University de montreal

Non-Presenting Author: Xingjuan Zhao- University of Montreal

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An ultrahigh sensitive SERS apatsensor method for multiplex detection of MC-LR and MC-RR.

Aquatic blooms of cyanobacteria (blue-green algae) have increased due to changes in climate, eutrophication of water reservoirs and pollution from modern industrial, agricultural, and domestic activities. Microcystins (MCs) are the most widespread algal toxin species released from cyanobacteria and cause a tremendous threat to aquatic ecosystem and public health. MCs family have many variants, Microcystin-LR (MC-LR) and Microcystin-RR (MC-RR) are the most toxic and the most frequently encountered species in the family of MCs. Thus, tracing MC-LR and MC-RR plays important roles in environmental safety and human health fields. We first reported a highly sensitive SERS aptasensor methods based on Au@Ag@Au SERS tags and gold nanoflowers (Au NFs) plane substrate to simultaneous detect MC-LR and MC-RR levels. 4-MBN (4-mercaptobenzonitrile) and 4-NTP (4-nitrothiophenol) were embedded in the junction of Au core and Ag@Au double shell structure and employed as stable and strong SERS tags to achieve ultrasensitive double detection of MC-LR and MC-RR. Then, the BCP-guided branched Au NFs plane substrate can capture SERS tags and form core-satellite configurations in a sandwich hybridization manner. The large-area formation of core-satellite assembled configuration on Au NFs plane substrate can largely intensify the SERS response of Raman labels and maintain a uniform and reproducible SERS performance at the same time. Experimental results indicate that the developed SERS aptasensor offers multiplexing capability for simultaneous detection of the two kinds of MCs in one single SERS experiment, and achieving high sensitivity with the limit of detection (LOD) down to 1 pM for MC-LR and 3 pM for MC-RR, which is much lower than the maximum permitted level of 1 nM of MC-LR in drinking water set up by the WHO. Moreover, the developed methods showed high reproducibility and specificity, and also can be applied to detect MC-LR and MC-RR in tap and river waters with satisfactory recoveries (95.6% to 104.8%). Finally, we monitored dynamic changes of MCs levels in hepatoxic MC-LR producing M. aeruginosa cells production over time. The proposed multiplex bioassay highlight the potential of SERS technology for the multiplex identification of MCs variants and then monitoring the water quality and human healthy in future.

Spectroscopic Studies of Cyclic Alcohols and π-Type Hydrogen Bonding

Presenting Author: Esther J. Ocola, PhD. - Texas A&M University, College Station
Non-Presenting Author: Jaan Laane - Texas A&M University, College Station

Few previous studies have demonstrated the presence of conformations with the π-type hydrogen bonding.

We have investigated theoretically and experimentally the energetics of several different conformations of cyclic alcohols including 3-cyclopenten-1-ol (I), 2-cyclopenten-1-ol (II) and 2-cyclohexen-1-ol (III). The two-dimensional potential energy surfaces (PESs) for the internal rotations and out-of-plane ring vibrations were calculated for each based on ab initio calculations. The PEFs predict six different conformers for each of the molecules. Infrared and Raman spectra were recorded for (I) and (III) while only infrared spectra were recorded for II. The experimental spectra were analyzed with the aid of complementary theoretical calculations of the vibrational frequencies. The infrared and Raman spectra confirm the existence of the predicted conformers for each of these molecules and also provide evidence that the conformer with the π-type hydrogen bonding has the lowest energy. For all three molecules the calculations show that in the vapor phase the conformers with intramolecular π-type hydrogen bonding are the lowest in energy. This weak type of bonding involves the hydrogen of the OH group interacting with the C=C double bond. According to our performed calculations, the conformers with the π-type intramolecular hydrogen bonding are estimated to be 200 to 400 cm-1 (2.4 kJ/mol to 4.8 kJ/mol) lower in energy than the other forms.

Standardization of Microplastics Analysis with Infrared Microscopy

Presenting Author: Sudhir Dahal, PhD - Shimadzu Scientific Instruments
Non-Presenting Author: Ruth Marfil-Vega, PhD - Shimadzu Scientific Instruments
Non-Presenting Author: Liang Zhao, PhD - Shimadzu Scientific Instruments

Method standardization for microplastics analysis using infrared microscopy

Microplastic are released into the environment as a part of manufactured products like cosmetics and industrial products. They can also be generated as a breakdown of larger plastics by weathering process. Concern about microplastics as a major pollutant is growing because of increased attention by scientific community on their occurrence and effects on ecosystems, and the rapid dissemination of information with citizens. Infrared (IR) Microscopy has proven to be a valuable technique for identification and characterization of microplastics; however standard methods are lacking, thus creating challenges for comparing results and research collaborations. This work focuses on systematic evaluation of significant operational and measurement parameters for IR Microscopy conducted using controlled sample sets. The quantitative result for lowest measurable sample size is backed by statistical analyses, with overall goal to set optimal performance parameters that will help in the standardization of microplastics analysis.

Strategies for Application of Loading Space Standardisation for Temperature Correction of Spectra to Improve Concentration Monitoring

Presenting Author: Magdalene Chong- University of Strathclyde

Non-Presenting Author: Thomas McGlone - University of Strathclyde

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Application of temperature correction algorithm to UV data to improve model predictive performance, particularly precision.

Temperature changes can significantly affect spectroscopic-based methods for in situ monitoring of processes. As the use of temperature is inherent to many processes, the changes in temperature cannot be avoided. Therefore, removal of the effects of temperature from spectroscopic data is required and many advanced temperature correction algorithms have been developed for this purpose. Amongst these, a comparatively simpler algorithm to implement is loading space standardisation (LSS). Construction of an LSS model requires a dataset comprising multiple samples (concentration) at a number of temperatures. To collect spectral calibration data relevant to many processes, such as cooling crystallisation, there are likely to be unobtainable concentration/temperature combinations. Spectral data were collected for the construction of calibrations to predict the solute concentration of L-ascorbic acid by UV-visible and infrared spectrometries. For both datasets, application of LSS preprocessing improved the performance of the subsequent partial least squares (PLS) calibration models in comparison to global models constructed without LSS treatment of the spectra. The use of LSS preprocessing reduces the number of latent variables required with better PLS model performance metrics. The datasets have also been used to explore different strategies for parameter and sample selection for construction of LSS models to enable easier implementation of the method for real-time process monitoring.

Surface - Enhanced Raman Spectroscopy For The Single Cell Analysis Of Synechocystis Sp. PCC 6803 In Regards To Industrially Useful Metabolites

Presenting Author: Daniel-Ralph Hermann - Technische Universität Wien

Non-Presenting Author: David Lilek - Austrian Biotech University of Applied Sciences

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Surface - enhanced Raman spectroscopy was successfully applied for the fast assessment of Synechocystis cultures

Cyanobacteria are mostly aerobic photoautotroph microorganisms that make up a large portion of earths biomass. Due to their abundance and their capability of agglomerating carbohydrates like glycogen, they are used for animal feed production. Additionally, model cyanobacteria like Synechocystis sp. PCC 6803 naturally accumulate polyhydroxy-alkanoates, which can be used as biopolymers. These metabolites combined with their CO_2 fixation capabilities make cyanobacteria ideal candidates for a CO_2 neutral feed or plastic manufacturing. As a consequence, microbiological as well as molecular biological approaches are tested to increase the production of glycogen or polyhydroxyalkanoates towards an economically viable manufacturing. Sensitive and stable analytics are vital to correctly assess the success of these approaches. In this study, two sample groups were analyzed, one grown on substrate limited media for an increased polyhydroxyalkanoate production, the other one genetically engineered towards a higher glycogen yield. In both cases, Raman spectra of single cells were gathered and compared to the spectra obtained from the control group. Raman spectroscopy offers information

about the chemical composition of cells, but its signal is typically too weak for high throughput analytics. The incorporation of noble metals, either as structured surfaces or soluble nanoparticles enhances the Raman signal significantly. This process is commonly called surface - enhanced Raman spectroscopy (SERS). In this study we used globular silver nanoparticles for SERS. With the enhancement offered by SERS active particles, the time for one spectrum was reduced to 1 minute (at least 10 spectra per culture were measured). The gathered spectral data was then post processed by principal component analysis (PCA). PCA yielded a good separation of enhanced producer and control group along the first two principal components. This demonstrates that SERS can be employed for rapid and reliable assessment of Synechocystis cultures and as a tool to evaluate and optimize production processes.

Thermal Infrared Hyperspectral Imaging for Detection, Identification, and Quantification of Industrial Stack Gas Emissions

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Non-Presenting Author: Vince Morton - Telops

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Identification and quantification of gas emissions from remote imaging measurements

Thermal infrared hyperspectral imaging is a completely passive optical analysis technique generating rich informational content that can be exploited for industrial gas emissions monitoring activities. The three-dimensional HSI data product is called a datacube and can be thought of as a traditional 2-dimensional infrared image with a continuous infrared spectrum associated with each individual pixel. This type of spatially distributed spectral data is well-suited for the analysis of gas plumes generated from industrial smokestack emissions. In this presentation, we will describe an industrial gas emissions monitoring campaign performed using the Telops Hypercam, a commercially-available, FTIR-based thermal infrared hyperspectral imaging instrument. Experimental details will be presented along with a discussion of gas detection, identification, and quantification data processing strategies. Finally, results from three different industrial emissions sites around a commercial shipping port will be described as an illustration of the utility of thermal infrared hyperspectral imaging.

Towards Fiber Optic Near-Infrared Ethanol-Water Probe Sensor

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Providing guidance for optimal design on a fiber probe sensor.

Near-infrared (NIR) spectroscopy enables non-invasive, non-destructive, and rapid analysis of chemical properties of a variety of samples which is attractive to a wide range of applications such as alcohol industry, food, pharmaceuticals, and medical applications. Routine analysis and detection using traditional techniques can be time-consuming and complicated. In addition, real-time monitoring cannot

be easily realized. Through NIR spectroscopy, information of the chemical properties can be obtained timely and easily. Our aim is to develop a fiber-based sensor for accurate detection of ethanol in aqueous solutions using NIR spectrometer. As a first step, we measure NIR spectra of ethanol – water solutions and analyze the important factors such as optimal pathlength and reference spectrum, which significantly affect the sensitivity and performance of NIR spectroscopy and have not been well studied. Absorbance spectra of various ethanol concentrations are recorded and analyzed using cuvettes with different pathlengths, and different reference spectra, to study the signal-to-noise-ratios and effect on sensitivity. Our results will guide the design on a fiber probe sensor which could be used for real-time monitoring the fermentation process in alcohol industry, microdialysis in biomedical applications and quality control in food production process.

Ultrafast Dynamics of Carbon Dioxide Diffusion Through Polymeric Membranes

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An understanding of ultrafast dynamics can improve the design of gas-trapping structures, yielding environmental benefits.

Carbon dioxide is the most abundant of anthropogenic greenhouse gases that contribute toward climate change. Multi-disciplinary research aimed at capturing and trapping CO2 presents a potential solution to the planet's CO2 crisis. Many potential systems that could effectively trap and store this gas feature a nanoporous structure embedded within a polymeric membrane. The design and optimization of these hybrid materials require an intimate understanding of how CO2 behaves when dissolved inside each component of these systems. To address this question, we present the ultrafast dynamic behavior of CO2 in three ubiquitous polymers: poly(methyl methacrylate), poly (methyl acrylate), and poly(dimethylsiloxane). Fourier transform infrared, pump-probe polarization anisotropy, and two-dimensional infrared spectroscopies were used to study the steady-state and time-dependent behavior of CO2 in the three systems. For each study, the CO2 asymmetric stretch served as the vibrational probe. We determined that the rate of molecular reorientation is sensitive to Lewis acid-base type interactions with the surrounding matrix, as well as polymer phase (rubbery vs glassy). Additionally, we found that the rate of spectral diffusion (energetic decorrelation) for the asymmetric stretch depends heavily on the interactions with the matrix and less so on the matrix phase. CO2 dissolved inside glassy poly(methyl methacrylate) exhibits minimal spectral diffusion. The energetic decorrelation of the vibrational probes is more prevalent in poly (methyl acrylate), which is rubbery at room temperature. Significant spectral diffusion only occurs for CO2 inside poly(dimethylsiloxane), an environment in which the surrounding matrix is both rubbery and lacks significant interaction. Subsequent frequency-frequency correlation function (FFCF) analysis was used to parse the observed dynamics into homogeneous and inhomogeneous contributions. The homogeneous linewidth was found to directly correlate to the diffusion constant of CO2 in each polymer. We then extend these methodologies toward characterizing CO2 inside the metal organic framework MIL-53(Al). MIL-53(Al) in particular exhibits both selectivity and impressive storage capacity for CO2, making it an important system of study. The data herein can help create quantitatively accurate computational models that allow for both identification and design of hybrid membranes for gas trapping and storage.

Understanding the surface of SERS-active colloidal anisotropic nanomaterials: stabilizers adsorption and signal enhancement

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A hybrid experimental-computational approach provided SERS-active nanostars with surface chemistry control for straightforward protocol development.

Surface enhanced Raman spectroscopy (SERS) is an analytical technique in which nanostructured substrates amplify the inherently weak Raman signal of an adsorbed species by several orders of magnitude, enabling the detection of trace compounds, up to the single molecule level. While this may be an exceptional tool for any analytical scientist, SERS is at present relegated to the role of academic sensation, and is underutilized in everyday analytical practice. The SERS community is increasingly attributing this setback to a poor understanding of nanoscale surfaces and their chemical environment; since molecular adsorption at the nanostructured surface enables SERS detection, uncertainty about what happens at the surface makes SERS experiments convoluted and often inaccessible. Therefore, there is a pressing need to further nanoscale surface chemistry studies: they are the key to effect the transition of SERS from academic sensation to benchmark technique for routine diagnostics. The present research takes this call by developing a library of SERS-active bimetallic nanostars, and utilizing them to systematically study the interplay between colloidal stability and SERS performance. Particular emphasis is given to elucidating the adsorption process of capping species to the nanoparticle surface, which was studied utilizing a multi-analytical approach. In addition, DFT calculations were performed in support of mechanistic and structural hypotheses. A population of structures for a simplified all-gold cluster system were obtained at the B3LYP/LANL2DZ level of theory, using both explicit solvent molecules and the continuum solvent model SMD. Our experimental results suggest the driving force in the nanoparticle capping process is not purely electrostatic in nature, with a pattern that favors capping by carboxylate-bearing molecules. Our DFT results provided a population of possible gold-ligand structures, which show potential significance of gold-ligated water in the adsorption of carboxylates. Preliminary results on a bimetallic system suggest a similar trend as well. This multi-analytical, theory-assisted approach to colloid development allowed for the formulation of a set of well characterized SERS-active colloidal surfaces, capable of providing a high level of surface control during SERS measurements. This ultimately allows for straightforward protocol development, setting foundations for the establishment of SERS as the next golden standard analytical technique.

Utilizing Raman spectroscopy and machine learning for developing a novel universal method for medical diagnostics

Presenting Author: Nicole M. Ralbovsky- University at Albany, SUNY

A novel method for achieving more accurate, reliable, and objective medical diagnostics is explored.

Receiving a diagnosis for an ailment is an event nearly every individual will experience at least once in their lifetime. Just as the diseases a person can contract are diverse, so too are the methods utilized for diagnosing them. Within these various techniques lies significant room for improving measures such as the tests' sensitivity and specificity, its availability and definitiveness, as well as reducing the cost and invasiveness associated with the test. To address these needs, there has been a strong push toward development of a minimally invasive test which can be used for accurate and rapid universal medical diagnostics. Raman spectroscopy in combination with machine learning is proposed here as a method which can satisfy this massively important role. This method has been applied for analyzing several different diseases including Alzheimer's disease, Duchenne muscular dystrophy, and Celiac disease in proof-of-concept studies. In each individual case, Raman spectral data was collected from bodily fluids of healthy and diseased donors. Machine learning algorithms were built and validated, each achieving over 95% diagnostic accuracy. The studies reported herein support the hypothesis that Raman spectroscopy in combination with machine learning analysis could be explored as a non-expensive, minimally invasive, accurate and rapid universal medical diagnostic method.

Characterization of Porcine Articular Cartilage and Subchondral Bone in the Near Infrared Region

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This research paves a way for novel nondestructive assessment of articular cartilage and subchondral bone.

Arthroscopic assessment of articular cartilage using a tactile probe is highly subjective, and quantification of tissue properties is not possible. NIR spectroscopy has been utilized in the investigation of articular cartilage and subchondral bone. However, interpretation of the NIR spectra of these tissues in vivo is challenging due to the influence of fluid, and overlapping absorbances from bone and cartilage. NIR spectroscopy via a fiber optic diffuse reflectance probe has been used to study subchondral bone properties, where the NIR first optical window (650 – 950 nm) was used for characterization. However, further explanation of absorbances in this window is required to understand spectra when cartilage and bone are present simultaneously. The objective of this project is to identify biochemical markers of articular cartilage and underlying tissues in the VIS/NIR spectral region of 500-1500 nm, when layered together, as is the case in the natural anatomic configuration. Freshly harvested stifle joints from Yorkshire pigs (Animal Biotech) were collected and the femoral head was cut into two halves. From one half, cartilage plugs were taken using a 6 mm biopsy punch. They were then cut into flat 1.8 mm sections after removal of subchondral bone tissues. From another femoral condyle, 6 sections of bone (5 section each of 1 mm thicknesses and 1 section of 5 mm thickness) were cut from the proximal end of the condyle using a bone saw. The NIR spectra of the tissues were collected using an ASD Labspec 4 spectrometer (Malvern Panalytical) with a diffuse reflectance probe, with 50 co-added spectra (130 ms integration time) and a 2 mm spacer on the tip of the probe. Three spectra were collected from cartilage pieces only, the subchondral area of the bone sections alone, and with cartilage

on top of sections of bone with different thicknesses. A principal component analysis (PCA) separated the NIR spectra of bone sections with and without the cartilage on top, with the bone section with cartilage have higher absorbances at ~ 530 and 560 nm, and 930 nm. These absorbances likely arise from cartilage proteins, which warrants further investigation.

Pharmaceutical Analysis and Process Analytical

Bulk Versus Surface Crystallization Kinetics Within Amorphous Solid Dispersions Measured By Second Harmonic Generation

Presenting Author: Nita Takanti- Purdue University

Non-Presenting Author: Scott Griffin- Purdue University

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Single particle tracking second harmonic generation analyzed in situ ritonavir surface and bulk crystallization kinetics.

The bioavailability and shelf-life of the final dosage form of amorphous solid dispersion (ASD) are often determined by physical instabilities, such as active pharmaceutical ingredient (API) crystallization. When the API is exposed to environments that have higher relative humidity (RH) or temperature, there is a supposed increased water content near the surface of the ASD that can lead to higher crystal growth and nucleation rates when compared to the decreased water content in the bulk. However, previous studies comparing these crystal kinetics at surface versus bulk rely on ensemble-averaged measurements, which are unable to probe crystal kinetics deep into the powder and accurately report bulk crystallization kinetics. In addition, many of the previous studies that analyzed the difference in the bulk and surface growth rates were predominantly API, which is not representative of the final dosage form in ASDs. Accelerated stability testing paired with single particle tracking second harmonic generation (SHG), a non-linear optical technique that is able to quantitatively assess trace amounts of API crystallization, can be used to sensitively measure and assess the difference for crystal kinetics for surface and the bulk. Growth and nucleation rates at the bulk versus surface were able to be obtained due to the CEiST, which allowed for continuous monitoring of the crystals at 50 degrees Celcius and 75% RH for 48 hours, allowing for in situ accelerated stability testing measurements. SHG microscopy produced average growth rates of 3.8 µm/hr for bulk columnar crystals with a particle to particle deviation of 0.9 µm/hr. In addition, columnar crystal growth rates for surface particles were measured to be 1.3 µm/hr and radiating crystal growth rates for surface particles were measured to be 1.0 µm/hr, both with a particle-to-particle variance of 0.4 µm/hr. As SHG microscopy was able to continuously monitor the same samples in CEiST over the same fields of view (FoV) non-destructively, the signal-to-noise of crystal growth rate improved with a lower amount of samples. Future steps include in situ high-throughput experimentation, where in a single particle tracking SHG run, multiple crystal growth and nucleation rates can be obtained for various temperatures and humidities.

Cannabidiol enhances intracellular calcium in glioma single-cell measured using a single-cell biochip

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Cannabidiol showed promising anti-cancer properties by releasing intracellular calcium from single glioma cell

Cannabidiol (CBD) is one of the important Cannabis derived cannabinoid with a diverse range of pharmacological properties. In our experimental design using the single-cell microfluidic approach, different concentrations of CBD (9.5, and 19 µM) were evaluated to excite the sustained [Ca2+]i levels in a human U87MG glioma single-cell. Ionomycin was used as a control to saturate intracellular calcium required for [Ca2+]i calibration. Results suggested that CBD produced a sustained increase in intracellular [Ca2+]i in a dose-dependent manner, signifying CBD as a potential starting point for future development of novel anti-cancer therapeutics.

Discrimination of ofloxacin formulation tablets using frequency-domain terahertz spectrometer

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The spectrometer with an injection-seeded THz generator was successfully applied to discriminate drug products.

The analysis of chemical constituents and their crystal properties (e.g., crystallinity and crystal polymorphs) is important for the quality control of pharmaceutical tablets. Terahertz (THz) transmission spectroscopy is suitable for such analysis since the vibration of lattice phonon modes originating in a crystal structure can be measured and major pharmaceutical additives are transparent or semi-transparent in the THz frequency region. Many active pharmaceutical ingredients (APIs) have characteristic absorption peaks in this frequency region. In addition, quantitative analysis of the crystal forms of APIs has already been reported. Therefore, we expect that formulation tablets with small deviation of the constituents or crystal properties can be analyzed with THz spectroscopy. We have developed a stable frequency-domain (FD) THz spectrometer that uses a tunable THz source, called an injection-seeded THz parametric generator (is-TPG), and applied it to the discrimination of nine ofloxacin tablet products. These tablets are commercially available in Japan and contain 100 mg (approximately 50 wt%) of ofloxacin in each tablet. We used six tablets for each product and filed them down to a thickness of 1.2 mm to obtain transmission spectra over the wide spectral range of 0.8 to 2.5 THz. The absorption spectra obtained by the spectrometer were preprocessed by the second derivative with a Savitzky-Golay filter and then principal component analysis (PCA) was conducted on the results to reduce the data dimensions before applying discriminant analysis (DA). The PCA results showed that at

least three components were necessary to explain the variation of the absorption spectra precisely. Next, quadratic DA was performed on the scores of the three PCA components and a confusion matrix was obtained. The accuracy of the DA was 98.1%. The small differences in the formulation of commercial ofloxacin tablets were clearly distinguished using the THz absorption spectra obtained by the novel spectrometer. The spectrometer system combined with the data analysis shows good potential for applications such as monitoring the stability of production processes, evaluating the stability of formulations during storage, and inspecting the counterfeit drugs on the market.

Nonlinear Optically Guided Analyses of Pharmaceutical Materials

Presenting Author: Garth Simpson - Purdue University

Guided analysis addresses key bottlenecks in stability testing and dissolution analysis of amorphous solid dispersions.

Second harmonic generation (SHG) microscopy is highly selective to crystals of homochiral active pharmaceutical ingredients (APIs), providing opportunities to significantly enhance conventional PAT methods guided by nonlinear optical imaging. These advantages are arguably brought to the forefront in amorphous solid dispersions of poorly soluble APIs, in which stability and dissolution can be profoundly affected by trace residual crystallinity. In Raman analysis, localizing spectroscopic characterization to regions of interest identified by SHG imaging can lower the limits of detection for residual crystallinity into the ppm regime. Comparable benefits arise in powder X-ray diffraction (XRD), enabling ppm limits of detection using SHG to guide synchrotron XRD. Coupling SHG imaging with differential scanning calorimetry (DSC) has enabled stochastic DSC, in which phase transformations within individual particles are connected to the ensemble-averaged heat transfer measured by DSC. These "hyphenated" methods provide numerous practical advantages by coupling conventional PAT methods with guided analyses enabled by nonlinear optical imaging.

Novel Laser-Based Mid-IR Liquid Flow Analyzer for Real-Time Biophysical Characterization

Presenting Author: Craig Magee, PhD - DRS Daylight Solutions
Non-Presenting Author: Santosh Hodawadekar - DRS Daylight Solutions
Non-Presenting Author: Jeremy Rowlette - DRS Daylight Solutions

Eliminate PAT bottlenecks by analyzing Critical Quality Attirbutes (CQAs) in real-time via laser-based Mid-IR spectroscopy.

Process analytical technology (PAT) has quickly gained importance in the biopharmaceutical industry for monitoring and controlling critical process parameters in the manufacturing of biologics drugs. Widely accepted PAT has now become a regulatory initiative to maintain the critical quality attributes (CQAs) to build quality into 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. 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.

Three-Dimensional Spectroscopic Chemical Imaging of Pharmaceutical Tablets

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Preliminary vibrational and elemental spectroscopy for a new approach to 3-D imaging of pharmaceutical tablets.

Pharmaceutical tablets are the most widely used solid dosage form for delivering therapeutic agents. There are knowledge gaps in current pharmaceutical manufacturing processes; particularly those related to the relationship of components in the formulation, processing conditions and the final product characteristics. By providing a means of visualising the microstructure of a tablet matrix these areas may be better understood. There are several techniques that can generate high resolution chemical images of component distribution within a tablet system but generally cannot go beyond an exposed surface layer. Three-dimensional imaging has been explored by using a Raman microscope to obtain a depth profile of a sample, however, this is typically less than 50 microns. The particle size of common excipients and active ingredients often exceed this depth range making it unsuitable for pharmaceutical products. Instead, this study explores an alternative method to obtain three-dimensional images of a sample using Raman and near-infrared chemical imaging. This involves stacking two-dimensional chemical images obtained at different penetration depths by physically milling the sample and may hold the key to improved fundamental understanding of solid dosage forms. This investigation has also assessed the differences in the chemical images obtained by Raman and near-infrared chemical imaging. Both techniques are closely related tools for characterising the chemical composition of a sample. Scanning electron microscopy with energy dispersive X-ray microanalysis were employed as alternative imaging techniques to evaluate which spectroscopic imaging method provided the most comparable and therefore representative image of the sample.

Toward Real-Time Monitoring Of Metabolism In Biomanufacturing: In-Cell Metabolite Detection And Identification By 31-Phosphorus Nuclear Magnetic Resonance

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Method for optimizing the collection of NMR for intracellular metabolites of intact CHO cells

In recent years, the biologics industry has integrated process intensification (PI) into their manufacturing practices, which seeks to improve the overall productivity of the manufacturing process relative to its scale. To facilitate PI, adequate understanding and monitoring of cellular metabolism is required. Unfortunately, current monitoring practices are typically limited to cell viability and basic nutrient concentrations in the growth media, which does not provide adequate information for understanding cellular metabolism. Nuclear magnetic resonance (NMR) spectroscopy is proposed as a technology for monitoring cellular metabolism. NMR has the potential to non-destructively detect the presence and fluxuations of intracellular metabolites inside intact cells with relatively simple sample preparation. Combined with other technologies, such as mass spectrometry, NMR can potentially provide a wealth of information in real-time. To demonstrate the capability of NMR for metabolism monitoring, this study performed 31-phosphorus NMR (31P NMR) on intact Chinese hamster ovarian (CHO) cells. Cells were chemically fixed in 4% paraformaldehyde to halt cellular metabolism during data collection. The free induction decay (FID) of intact fixed cells were collected on a Bruker 400 MHz NMR at 161 MHz with a 45o pulse. An adequate signal-to-noise ratio (SNR) is required in NMR spectra to facilitate metabolite observation and identification. To maximize SNR, NMR parameters were optimized based on T1 relaxation times of metabolites as determined by an inverse-recovery experiment. Several chemical peaks were observed and assigned based on literature values. The phosphorus metabolites identified included redox agents and energetic nucleotides, as well as metabolites present in glycolysis and the CDP-choline pathway. This study demonstrated several metabolites relevant to cellular metabolism can be captured and observed inside cells by NMR. Monitoring and modeling of the intracellular metabolites promotes understanding of variations in metabolism over the course of a culture and how that metabolism variation affects the overall productivity.