Applied Surface Science
Room 101B - Session AS+BI-MoA

Practical Surface Analysis I: Advancing Biological Surface Analysis/Imaging Beyond 'Show and Tell'

Moderators: Ian S. Gilmore, National Physical Laboratory, UK, Jordan Lerach, The Pennsylvania State University

1:40pm **AS+BI-MoA1 A Multi-technique Approach for Studying the Effect of Protein G B1 Orientation on Antibody Binding***, Elisa Harrison, G. Interlandi, D.G. Castner,* University of Washington

The orientation of adsorbed proteins on surfaces plays a vital role in the function and performance of biomaterials. Development of diagnostic tools such as sandwich ELISAs have focused on controlling the orientation of each protein layer. A full understanding of adsorbed proteins on surfaces, especially at the molecular level, is therefore essential. Our research address es the challenges for characterizing protein orientation by developing new method s to study multilayer protein systems.

The aims of this study were to control and characterize the orientation of protein G B1, an IgG antibody-binding domain of protein G, on well-defined surfaces and measure the effect of its orientation on antibody binding using a variety of surface-sensitive tools and simulations. We hypothesize that binding selectivity would increase for well-ordered protein films due to high er availability of binding domains.

The surface sensitivity of time-of-flight secondary ion mass spectrometry (ToF-SIMS) enables us to distinguish between different proteins and their orientation by monitoring the changes in intensity of amino acid mass fragments. We have developed ToF-SIMS methods for analy zing the orientation of five different cysteine mutants of protein G B1 covalently attached to a maleimide surface. T his technique was further extended by studying multilayer protein systems, specifically the binding of IgG antibodies to the protein G B1 films.

To study the effect of protein orientation on antibody binding, we utilized self-assembled monolayers (SAMs) to form protein G B1 films with both random and well-defined orientations. Using complementary techniques, such as X-ray photoelectron spectroscopy and quartz crystal microbalance with dissipation monitoring (QCMD), the ratio of bound IgG antibodies to protein G B1 increased from 0.06, when chemisorbed onto bare gold, to 0.2, when covalent ly attach ed to the surface. Further analysis revealed structure/orientation rearrangement of protein G B1 upon adsorption onto bare gold, which is likely responsible for decreased antibody binding.

Additionally, we developed and applied Monte Carlo (MC) simulations to predict protein orientation on a surface. The MC simulations showed that the outermost b-sheet of protein G B1 interacts most frequently with a hydrophobic surface. The predicted orientations were verified using molecular dynamics simulations, QCMD, and sum frequency generation.

The model systems explored in this study are a first step in developing methodology using state-of-the-art tools that can be applied to more complex systems and expand our knowledge and control of biomolecules on surfaces.

2:00pm **AS+BI-MoA2 ME-SIMS Revisited: Attempting to Unlock the Potential using Advancements in Sample Preparation and SIMS Technology***, Nina Ogrinc Potocnik,* Maastricht University, The Netherlands*; C.R. Anderton, L. Pasa-Tolic,* Pacific Northwest National Laboratory*; R.M.A. Heeren,* Maastricht University, The Netherlands

This year marks the 20th anniversary of Wu and Odom first describing the application of a solid organic matrix to improve the ionization efficiency of molecular species in secondary ion mass spectrometry (SIMS) measurements. This so-called matrix enhanced-SIMS (or ME-SIMS) method overcame one of the disadvantages of SIMS analysis, providing the capability of imaging large molecules with high spatial resolution. With increased ionization efficiency and minimized fragmentation caused by the primary ion beam, the method is ideal for detection of intact bimolecular species, where detection of proteins greater than 10,000 Da is feasible. However, the combination of instrumentation limitations of resolving isobaric compounds and lateral diffusion caused by matrix application has pushed this technique into near irreverence. Here, we reevaluate ME-SIMS with new technologies such as parallel MS/MS capabilities on the PHI nano-TOF TRIFT V, and the custom-build FTICR-SIMS capable of unmatched mass resolving power and mass accuracy. We also explore new matrix application techniques to revisit the potential of ME-SIMS and apply it to a number of different biological settings.

Specifically, we reexamined peptide standard profiling with the addition of tandem MS on the nano-TOF TRIFT V . The ability to isolate precursor ions with a 1 Da mass window, followed by a high-energy collision-induced dissociation (CID), enables a very precise fragmentation of molecules. We observe peptide fragmentation through the amino terminus, a_m, providing us with a specific fragmentation pattern for identification of peptide species and opening doors to *de novo* peptide sequencing. Further on, we applied it for characterizing tryptically digested peptides investigating the applicability to bottom-up proteomics. We then imaged model plant and mammalian tissue sections that were subjected to a variety of different matrices *via* supplication using a home-built sublimation chamber. Matrix sublimation produces small, homogenous crystal sizes, without the need for solvents that delocalize molecular species. Consecutive sections were analyzed by FTICR-SIMS, to accurately identify molecular species of interest, and by the nano-TOF TRIFT V for high lateral resolution images and confident identification of said species with tandem MS.

2:20pm **AS+BI-MoA3 Improvements in SIMS Methods and Instrumentation in Effort to Make Measurements Biologists Can Use***, Christopher R. Anderton,* Pacific Northwest National Laboratory **INVITED**

The ability of mass spectrometry imaging (MSI) to visualize chemical distributions within samples has made it an increasing popular method in many biological fields, including medicine, pathology, and microbial ecology. Secondary ion mass spectrometry (SIMS) is a surface sensitive MSI technique that offers extensive versatility in its ionization and analysis modes, requires relatively minimal preparation, and can achieve the highest lateral resolution of any MSI method. Early bio-applications of SIMS routinely focused on pursuing the molecular information attainable by softer ionization methods (e.g., matrix assisted laser desorption/ionization), but with the added benefit of achieving subcellular lateral resolution. Even though primary ion beams used in SIMS measurements afford smaller probing areas than other ionization methods, their excessive energy typically causes extensive fragmentation of most biorevelent molecules. This renders identification of parent molecules from the detected secondary ions a nontrivial endeavor. Nevertheless, recent improvements in SIMS instrumentation, methods, and data analysis approaches have unlocked biochemical information that was previously unattainable. Here, I will discuss our efforts in improvements in sample preparation methods and the employment of unique mass spectrometer technology for analyzing biological material. Stable isotope probes were used to decode lipid distributions within model and cellular membranes, to reveal the intercellular delivery of drug-loaded polymeric nanoparticles, and to elucidate metabolic processes of phototrophic communities. The use of Fourier transform-based mass spectrometers, which have unparalleled mass accuracy and mass resolving power, and tandem mass spectrometry methods have allowed us to unravel the extreme spectral complexity of biological SIMS measurements, while increasing the confidence in our measurements. Lastly, we have revisited previously reported sample preparation routes that were never fully adapted by the SIMS community, in part because they were shackled by the limited ability of more commonly employed mass analyzers.

3:00pm **AS+BI-MoA5 Towards Bacterial Differentiation with Quantitative SIMS***, Christopher Szakal, S. Da Silva,* National Institute of Standards and Technology (NIST)*; N. Olson,* National Institute of Standards and Technology(NIST)

Large geometry secondary ion mass spectrometry (LG-SIMS) has been used extensively for particle analyses and geochemical analyses, owing to its ability to maintain adequate mass resolution while operating at high secondary ion transmission. Efforts will be presented that extend the knowledge acquired in these application areas to single bacterial cell analyses of elemental species. To be useful, LG-SIMS results need to be quantitative for the amounts of a given element per cell and/or in ratios of different elements within each cell. Approaching this level of detail requires the establishment of the natural variability of such data from cell-to-cell, the reproducibility of the measurement technique, and whether the data is relevant to pertinent questions about the cellular population. Progress will be shown towards achieving these aims for single bacterial cells within different known growth conditions, including analytical figures of merit for LG-SIMS elemental ratios. Prospective application areas will be presented, along with potential pitfalls of such an approach.

3:20pm **AS+BI-MoA6 New Insights into the Microenvironment of Cancerous Tissue by Combined Mass Spectrometry, Microscopy and Multivariate Analysis**, ***Tina Angerer,*** University of Gothenburg, Sweden*; Y. Magnusson, G. Landberg,* Sahlgrenska Cancer Center, Sweden*; J.S. Fletcher,* University of Gothenburg, Sweden

Introduction

Mass spectrometric imaging is of growing interest for the medical field, both in applied and basic research[1]. Particularly, imaging secondary ion mass spectrometry (SIMS) is becoming of increasing value to clinicians and has been used on a number of tissues samples to successfully identify and localize different chemical components to various areas of the tissue and answer disease related questions[2]. Fatty Acid Synthase (FAS) has been shown to be increased in many cancer types and is of growing interest as therapeutic target[3]. The changed lipid composition due to increased FAS activity is an ideal ToF-SIMS study target.

Methods

With the J105- *3D Chemical Imager* (Ionoptika Ltd), fitted with a 40 kV gas cluster ion gun[4], we are now able to overcome some previous limitations of ToF-SIMS analysis and image large intact molecular species at high spatial and high mass resolution simultaneously. To capitalize on these improved capabilities we performed imaging SIMS on fresh frozen hydrated and freeze dried, ductal mammary breast cancer sections, followed by H&E staining of the analysed sections.

Results

SIMS enables us to distinguish between different areas of the diseased tissue. Multivariate analysis facilitates localizing and grouping the up to 10,000 different signals generated from the tissue to produce comprehensive chemical profiles assigned to different areas in the tissue revealing underlying structures. We have identified a number of molecules which can be, due to high spatial resolution, clearly assigned to the cancerous regions, characterized by conventional histological staining, in different breast cancer sections. Additionally, studying the distribution of specific single ions reveals reoccurring patterns of changes and gradients within the cancerous areas which cannot be observed in the conventionally stained image. Therefore ToF-SIMS can provide deeper insights into tumor metabolism and progression. Our results agree with findings from experiments using different methods, which confirm these molecules to be cancer markers while more importantly elucidating new information form the tissue with cellular resolution.

Conclusions

Imaging ToF-SIMS is a valuable tool for cancer research and can provide new insights into chemical changes within tumors. Further application of ToF-SIMS imaging will be used to study different modes of disease progression and treatment response.

[1] J. L. Norris et al., *Proteom Clin Appl* **2013**, 7, 733-738.

[2] A. Brunelle et al., *Curr Pharm Design* **2007**, 13, 3335-3343.

[3] P. M. Alli, et al., *Oncogene* **2004**, *24*, 39-46.

[4] T. B. Angerer et al., *Int J Mass Spectrom* **2015**, 377, 591-598.

4:00pm **AS+BI-MoA8 Super-resolution Mass Spectrometry Imaging of Biological Materials with the New 3D nanoSIMS**, ***Ian S. Gilmore**, M.K. Passarelli,* National Physical Laboratory, UK*; A. Pirkl, R. Moellers, E. Niehuis,* ION-TOF GmbH, Germany*; A.A. Makarov,* Thermo Fisher Scientific*; H.F. Arlinghaus,* ION-TOF GmbH, Germany*; R. Havelund, P.D. Rakowska, A.M. Race, A.G. Shard,* National Physical Laboratory, UK*; A. West,* GlaxoSmithKline*; S. Horning,* Thermo Fisher Scientific*; P. Marshall,* GlaxoSmithKline*; M.R. Alexander,* The University of Nottingham, UK*; C.T. Dollery,* GlaxoSmithKline

SIMS has become an important technique for the surface analysis of biological materials. However, critical challenges have hampered the uptake into the life-science industry and biomedical discovery. To succeed in this important sector, it has to progress beyond "Show and Tell". Biological samples have complex chemistry and an extraordinarily large dynamic range of concentration. The present state-of-the-art struggles to identify unknowns owing to insufficient mass resolving power and mass accuracy of time-of-flight analysers. The situation is further complicated by sample form and vacuum compatibility.

To address this issue, we have developed a powerful new hybrid SIMS instrument combining an Orbitrap™-based Thermo Scientific™ Q Exactive™ HF instrument and a dedicated ToF-SIMS 5. The instrument is equipped with high-resolution ion beams including a new micron resolution argon cluster ion beam for biomolecular imaging and 3D analysis of organics and an ultra-high resolution Bi cluster focussed ion beam with < 80 nm resolution. The ToF analyser allows high-speed imaging needed for 3D analysis and the High Field Orbitrap analyser allows high mass resolution, mass accuracy and MS/MS for chemical identification. The instrument is designed for life-sciences applications including sub-cellular 3D imaging of metabolites, imaging of bacteria and biofilms and imaging of medical devices with complex topographies that confound traditional instrument designs.

We show data demonstrating the unique advantages of this novel instrument. Imaging with large argon clusters provides rich biomolecular spectra including intact lipids and metabolites. Existing state-of-the-art instruments are limited to a mass resolving power of around 6,000 which is insufficient to allow unique identification. We show images of mouse brain with a sub-cellular spatial resolution of less than 2 microns simultaneously with a mass resolving power of over 100,000 for intact lipids. We fully separate the (3'-sulfo)Gal-Cer(d18:1/24:1(2-OH)) and (3'-sulfo)Gal-Cer(d18:1/25:0) sulfatides, which reveals a difference in spatial distribution. In the low mass region, mass resolving powers of >400,000 are achieved allowing clear separation of the low abundance metabolite dopamine from other peaks. We show the ability to image the drug amiodarone with sub-cellular resolution and show that the mass spectra are not affected by sample topography. The instrument is also equipped with state-of-the-art cryogenic sample preparation specifically designed for high-resolution biological imaging.

All animal studies were ethically reviewed and carried out in accordance with Animals (Scientific Procedures) Act 1986

4:20pm **AS+BI-MoA9 High-resolution, Sub-cellular Imaging of Pharmaceutical Localization by Correlative SIMS and TEM**, ***Paulina Rakowska,*** National Physical Laboratory, UK*; H. Jiang,* University of Western Australia*; I.S. Gilmore,* National Physical Laboratory, UK

To accurately predict the pharmacological effect of potential drug candidates, there is a strong need in the pharmaceutical industry to image the disposition of drugs at the sub-cellular level and even within specific organelles. This is needed to answer long-standing questions about whether drug concentrations are sufficiently high in the right places to have a therapeutic effect, or if the medicine is lodging within cellular components and causing toxicity. If anomalies were spotted earlier, it might help to explain toxicities or lack of efficacy of a medicine and reduce costly late-stage failures.

Mass spectrometry imaging techniques are well-suited to measure drug distribution in biological samples and have the advantage of label-free analysis. The CAMECA nanoSIMS (secondary ion mass spectrometry) can provide elemental images with high lateral resolution of 50 nm. These high-resolution ion images can be correlated to electron microscopy images. This combination of techniques provides very precise and detailed information of cell morphology, subcellular processes and localization of different molecules within the cells. However, these high-performance instruments require high vacuum and complex sample preparations. Therefore, the sample handling needs careful consideration. Biological samples can suffer from ultrastructural reorganization or the loss or translocation of molecules, which can occur with dehydration under high-vacuum conditions. Chemical fixation of the samples followed by embedding in resin are common in the studies of cell biology by TEM but the solvents used for sample dehydration have a severe effect, translocating or even removing the drug from the cell all together. This has been a fundamental barrier for the use of the technique for intracellular drug localization measurement.

We present a correlative nanoSIMS and TEM imaging of a highly lipophilic drug – amiodarone within lung macrophages dosed at therapeutic concentrations. The protocol used for the fixation and resin-embedding of the cells prevented the drug from being removed from the organelles during solvent treatment. We are able to show, with unprecedented detail, the drug accumulating in lysosome organelles.

4:40pm **AS+BI-MoA10 Sub-Micron Imaging and Identification of Molecular Chemistry by TOF-SIMS Parallel Imaging MS/MS**, ***Gregory Fisher**** , Physical Electronics*; N. Ogrinc Potocnik, A.L. Bruinen,* Maastricht University, The Netherlands*; J.S. Hammond, S.R. Bryan,* Physical Electronics*; R.M.A. Heeren,* Maastricht University*; S. Iida, T. Miyayama,* ULVAC-PHI **INVITED**

A recently introduced TOF-TOF imaging mass spectrometer allows conventional TOF-SIMS (MS^1) analysis and product ion (MS^2) analysis to be

* **ASSD Peter Sherwood Award**

achieved simultaneously and in parallel. Secondary ions for MS^1 and MS^2 analysis are produced from the same area of the surface by a pulsed and digitally raster-scanned primary ion nanoprobe. The sensitivity of the parallel imaging MS/MS spectrometer is high so that the analytical ion dose may be minimized; therefore, precious and one-of-a-kind samples may be probed without significant damage or degradation. Fragmentation of the molecular precursor ions, defined by a 1 Da precursor selection window, is accomplished by collision-induced dissociation (CID) at 1.5 keV in an activation cell of Ar gas at high pressure. Lateral resolutions produced in both MS^1 and MS^2 images are demonstrated to be in the range of 100 nm < $\Delta I_{80/20}$ < 1 µm. This tandem MS imaging capability has been brought to bear for straightforward identification as well as multifaceted studies involving biological, material, and polymer specimens. We will summarize here some of our ongoing biological research, revealing molecular identification at sub-micron practical lateral resolution.

One study concerns song bird ontogeny in male zebra finch (*T. guttata*). Several sulfatides, phospholipids, sterols and fatty acids have been identified as playing a role in song learning. We have employed parallel imaging MS/MS to unravel the roles of specific molecules because the shortcomings of TOF-SIMS imaging alone does not permit conclusive molecular identification and imaging. We have evidence suggesting that distinct sulfatides are active primarily within the song nuclei while cholesterol and specific fatty acids are active in signaling between the song nuclei.

In other work, we have probed the role of lipids and metabolites in disease states of zebrafish (*D. rerio*) that have been infected with *M. marinum*, a form of tuberculosis. The bacteria initiate a granulomatous inflammation, and first signs of the disease are observed in the spleen. We have observed so far that α-tocopherol is elevated in infected tissue as well as in the granuloma, but is not present in the necrotic cells. Cholesterol is elevated primarily in the granuloma. The role of phospholipids appears to differ, specific molecules being either elevated or depressed in the infected tissue. We have preliminary evidence of a metabolic source for bacterial growth. For example, we observe a phosphocholine, PC(16:0/16:0), to be elevated in the granuloma. However, in the necrotic cells surrounding the granuloma we observe elevated signals of a fatty acid, FA(16:0).

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