Applied Surface Science Division
Room 204 - Session AS+BI-TuM

Applied Surface Science: From Electrochemistry to Cell Imaging, a Celebration of the Career of Nicholas Winograd

Moderators: Arnaud Delcorte, Université Catholique de Louvain, Belgium, Michaeleen Pacholski, The Dow Chemical Company

8:00am **AS+BI-TuM1 Surface Analysis and Beyond, Using Ion Beams and Lasers**, ***Nicholas Lockyer***, *J.C. Vickerman,* University of Manchester, UK **INVITED**

Applications of secondary ion mass spectrometry (SIMS) have expanded enormously from pure surface science experiments to biology and bio-medicine, driven largely by developments in instrumentation. Polyatomic primary ion beams have resulted in a step-change in the technique's capability to detect and localise molecular chemistry, in biological cells and organic devices etc. This has stimulated new mass spectrometer designs and analytical paradigms for molecular imaging in 2D and 3D. The quest for still greater lateral resolution is a quest for improved sensitivity. Here ion beam chemistry can play a role, increasing the ionized fraction of the sputtered plume. Alternative routes to improved ionization and greater quantification include laser post-ionization. In this talk I will chart the progress made in these areas by our group in Manchester, and draw parallels with the work of the Winograd group, with whom we have had fruitful collaboration over many years.

8:40am **AS+BI-TuM3 A High Resolution Tandem MS Imaging Method to Probe the Composition of Organelles in Single Cells**, ***Gregory L. Fisher***, Physical Electronics; *C.E. Chini,* University of Illinois at Urbana-Champaign; *B. Johnson, M.M. Tamkun,* Colorado State University; *M.L. Kraft,* University of Illinois at Urbana-Champaign

A goal of cellular imaging is to ascertain the composition of organelles, e.g. lipid profiling or pharmaceutical efficacy. To date most MS imaging of organelles is accomplished by stable isotope labeling because the imaging ion beam produces primarily (di)atomic ions. Such analyses are void of desired molecular specificity. We employed a TOF-TOF imaging capability [2] to achieve molecular specificity and conjectured that an ER-Tracker stain would yield characteristic molecular ions with which to image the endoplasmic reticulum (ER) and ER tubules.

We used human embryonic kidney (HEK) cells that had a high number of ER tubules near the plasma membrane (PM). Experimental cells were transfected to express GFP-Kv2.1 fluorescent ion channels. The cells were stained with ER-Tracker which selectively labels the ER. Control specimens were neither transfected nor stained.

We observed by simultaneous MS imaging and tandem MS imaging, in both the positive and negative ion polarities, the atomic and molecular moieties characteristic of an ER-Tracker stain localized to the ER and ER tubule structures. The ion species used for tandem MS imaging of the ER and ER tubules, namely F^-, $C_6H_5^-$, $C_5H_5^+$ and $C_{17}H_{15}N_2O^+$, were shown irrefutably via the product ion spectra to arise solely from the ER-Tracker stain. Two-dimensional (2D) imaging revealed intersection of some ER tubules at the PM. Three-dimensional (3D) visualization via depth profile analysis, carried out to a depth of ≈ 40 nm from the PM, revealed additional ER tubules just under the PM. Some ER-Tracker was observed in the PM indicating ER tubule contact with the PM to form ER-PM junctions. We were able to confirm the presence and position of the PM owing to the presence of characteristic lipids, lipid fragments and fatty acids which were imaged in parallel. The observed tubule features were imaged at an effective lateral resolution of 137 nm and had measured diameters in the range of approximately 500 nm to 2 µm corresponding well with previous studies [3] and present total internal reflection fluorescence (TIRF) observations. More than a dozen control cells were analyzed, and neither atomic nor molecular moieties characteristic of the ER-Tracker were observed to be present. Our next aim is to visualize the ER within entire cells and to assess the lipid composition at different locations within the ER. By extension, with organelle-specific stains, we can apply this TOF-SIMS tandem MS imaging method to aspects of pharmaceutical delivery and metabolism.

9:00am **AS+BI-TuM4 SIMS and MALDI-MS. Competitive, Complimentary or Complementary Techniques for Bio-imaging?**, ***John Stephen Fletcher***, *I. Kaya,* University of Gothenburg, Sweden

Despite imaging SIMS being a much older technique than MALDI in the bio-MS imaging area MALDI has enjoyed considerably more widespread success. The advantage of higher resolution imaging that is possible with SIMS has generally been outweighed by the low signal for intact molecular ions that are routinely delivered by soft ionisation techniques like MALDI – and MALDI is cheaper. However, advances in ion beams and mass spectrometry for SIMS analysis in parallel with new matrices, sample preparation and analysis approaches for MALDI have brought the two techniques closer together with significant overlap in the 1-10 µm "small molecule" imaging range.

In this presentation the benefits (if any) of multimodal MS imaging are discussed using examples from cancer, cardiovascular and neurological studies. Analysis was performed using high energy (40 keV) gas cluster ion beams (GCIBs) for SIMS analysis on the Ionoptika J105 and different MALDI approaches including gentle/static MALDI on the Bruker Ultraflextreme. On tissue derivatisation strategies applicable to both techniques will also be presented.

9:20am **AS+BI-TuM5 High Spatial Resolution Metabolic Imaging using the 3D OrbiSIMS - Fundamentals of Metabolite Fragmentation and Biological Applications**, *C. Newell, Y. Panina,* Francis Crick Institute, UK; *L. Matjacic, V. Cristaudo,* National Physical Laboratory, UK; *A.P. Bailey,* Francis Crick Institute, UK; *R. Havelund,* National Physical Laboratory, UK; *M. Yuneva, A.P. Gould,* Francis Crick Institute, UK; ***Ian S. Gilmore***, National Physical Laboratory, UK

Ground-breaking advances in single-cell genomics and transcriptomics are revealing the heterogeneity of cells in tissue and are transforming biological understanding. There is a great need for metabolomics with single-cell resolution. Recent advances in both SIMS and MALDI imaging have pushed the spatial resolution boundary to a few micrometres [1-3].

Here, we report on the 3D OrbiSIMS [1] which combines a gas cluster ion beam (GCIB) that is able to simultaneously achieve a spatial resolution of < 2 µm with high mass resolving power (>240 k) and mass accuracies of ~1 ppm. The GCIB significantly reduces fragmentation of metabolites compared with small cluster ion beams and we provide fragmentation data for a variety of metabolites for different energy per atom conditions.

We demonstrate the OrbiSIMS capability with two biological examples. Firstly, a study of the cuticular lipid composition and distribution of drosophila and how these change with various environmental and genetic manipulations. Drosophila secrete many different classes of lipids to form a protective surface barrier against environmental challenges and hydrocarbons which play a separate role as pheromones that influence sexual behaviour. Secondly, to identify metabolic heterogeneity in mammary gland tumours. One of the hallmarks of cancer is deregulated metabolism, often characterised by increased glucose and glutamine uptake for energetic and anabolic purposes. Metabolic changes contribute to well-established tumour heterogeneity, which is a major challenge for anti-cancer therapeutics. We demonstrate a protocol to co-register high-resolution OrbiSIMS metabolite images with immunohistochemistry microscopy images of the same sample.

References

1 Passarelli, M. K. *et al.* The 3D OrbiSIMS-label-free metabolic imaging with subcellular lateral resolution and high mass-resolving power. *Nature Methods***14**, 1175, doi:10.1038/nmeth.4504 (2017).

2 Kompauer, M., Heiles, S. & Spengler, B. Autofocusing MALDI mass spectrometry imaging of tissue sections and 3D chemical topography of nonflat surfaces. *Nature Methods***14**, 1156, doi:10.1038/nmeth.4433 (2017).

3 Dreisewerd, K. & Yew, J. Y. Mass spectrometry imaging goes three dimensional. *Nature Methods***14**, 1139, doi:10.1038/nmeth.4513 (2017).

9:40am **AS+BI-TuM6 Small Molecule Imaging in Single Frozen-Hydrated Cells using High-Resolution Gas Cluster Ion Beam Secondary Ion Mass Spectrometry (GCIB-SIMS)**, ***Hua Tian***, *N. Winograd,* Pennsylvania State Univeristy

Cell heterogeneity leads to the development of antibacterial resistance and tumor relapses in response to drug treatment. Cell-to-cell differences have been extensively investigated at the DNA level. The study of rapid and dynamic small molecule fluctuations in single cells has lagged. However, the complete spectrum of biomolecules can be a direct indicator of cell phenotype and a reflection of immediate response to environment and chemical stress. There is currently no method to directly detect small molecules in their original state because of the rapid and dynamic nature of these molecules and impossibility of amplifying the metabolites. Previously, the characterization of drug and small molecules in cells are conducted using ensembles of cells, with which the spatial distribution, a

vital piece for understanding biological processes is lost. The development of high resolution CCIB-SIMS in our lab has positioned us to directly image small molecule fluctuation in single cells under cryopreservation. The approach takes advantage of three aspects of GCIB-SIMS - low chemical damage, high yield of intact biomolecules, and the possibility of sub-micron lateral resolution. In this work, we utilize a DC beam buncher-ToF SIMS instrument to achieve high lateral resolution. Moreover, this configuration simplifies depth profiling since erosion and spectral acquisition are performed with a single beam. In addition, the flexibility of gas candidates for GCIB allows us to explore tailored beam for ionization enhancement, such as HCl, CH_4, CO_2 or H_2O (1~100%) doped Ar cluster beams. To illustrate this instrumental protocol, chemically resolved 3D images of single cells, HeLa cells and drug treated A549 (Carcinoma) and A673 (Ewing tumor) cells are imaged using a novel 70 keV $(CO_2)_{14000}$+ beam with a spot size of 1 µm. The stable intermediates from various biochemical pathways are visualized in single HeLa cells, demonstrating the sufficiency of the chemical sensitivity using GCIB. The drug propranolol is localized within the cellular structure of A673 and A549 cells, while no sign of fexofenadine is observed. This indicates that propranolol has high passive permeability in contrast to low passive permeability expressed by fexofenadine. Surprisingly, a lipid composition change is shown in A673 cells, particularly the depletion of phosphatidylinositol species after treatment. The approach provides a complete chemical picture of single cells at near original physiological and morphological state, opening the opportunities for single cell metabolomics and heterogeneity studies using SIMS.

11:00am **AS+BI-TuM10 Pushing the Limits of Measurement Science with SIMS**, ***Christopher Szakal***, *D.S. Simons, J.D. Fassett, T.P. Forbes,* National Institute of Standards and Technology (NIST) **INVITED**

The career of Dr. Nicholas Winograd was exemplified by an unwillingness to accept the scientific status quo. Both in words and actions, he set a tone that encouraged everyone he worked with to 'push the limits' of what they thought was possible within their research endeavors. In this presentation, several topics will be explored where this mindset has been directly put into practice. A mix of historical examples, recent advancements, and new science that fit within the theme will be presented. Specifically, topics related to cluster ion beams, single cell imaging, single cell quantification, precision isotopic measurements, secondary ion mass spectrometry (SIMS) standard generation, and precision atmospheric pressure ionization MS measurements will be described in terms of how the measurement science boundaries were assertively targeted. Since Dr. Winograd had a fondness for cutting-edge instrumentation throughout his career, the presented efforts will focus on pushing the limits within time-of-flight (TOF)-SIMS, large geometry (LG)-SIMS, and ultra-high-resolution (UHR)-MS quadrupole(Q)-TOF technologies.

11:40am **AS+BI-TuM12 Multiplexed Ion Beam Imaging: Cell and Tissue Imaging using Secondary Ion Mass Spectrometry for Pathology**, ***Jay Tarolli***, *R. Finck, M. Aksoy, D. Stumbo,* Ionpath, Inc.

Traditional techniques for protein imaging in tissue sections based on light microscopy are limited in the number of simultaneous targets that can be analyzed in a single sample. The need exists in pathology, however, for concurrent imaging of more than just a few of these biomolecules to determine localization of cell types in tissue biopsies. Multiplexed ion beam imaging (MIBI) uses secondary ion mass spectrometry (SIMS) to overcome these limitations and is capable of imaging over 40 biomolecules simultaneously with a spatial resolution greater than many traditional optical and fluorescence microscopy techniques.

In a typical MIBI analysis, a tissue sample is stained with target antibodies conjugated to isotopically pure lanthanide metals. The resulting mass spectra of monoatomic heavy metals exhibit a more characteristic response than the complex mass spectra of fragmented biomolecules typically acquired when analyzing tissue samples with SIMS, the benefit of which being twofold: First, as ion counts from the target analytes are preserved instead of lost due to fragmentation into uncharacteristic species, the generated images have a higher signal to noise ratio. Secondly, now that fragmentation of target analytes is not a limitation, a high current primary ion beam with a tight focus can be employed to image tissue samples with a high spatial resolution at a high throughput rate. Specifically, an oxygen duoplasmatron primary ion beam, focusable down to a spot size of 350 nm, is used in conjunction with a time-of-flight mass analyzer to enable to the simultaneous detection of more than 40 labels at a resolution where individual cells can be differentiated in tissue samples.

12:00pm **AS+BI-TuM13 Combined ToF-SIMS and AFM Protocol for Accurate 3D Chemical Analysis and Data Visualization**, ***Maiglid Andreina Moreno Villavicencio***, *N. Chevalier, J.-P. Barnes, I. Mouton,* Univ. Grenoble Alpes, CEA, LETI, France*; F. Bassani,* Univ. Grenoble Alpes, CNRS, LTM, France*; B. Gautier,* Université de Lyon, INSA Lyon, Institut des Nanotechnologies de Lyon, UMR CNRS 5270, F- 69621 Villeurbanne cedex, France

In dual-beam time-of-flight secondary ion mass spectrometry (ToF-SIMS) depth profiling, a succession of two-dimensional chemical images is acquired. The provided images can be used to generate a three-dimensional (3D) visualization of the sputtered volume. However, standard reconstruction methods do not take into account the initial sample topography or lateral variations in sputter rates.

Due to geometry and the diversity of materials the resulting 3D chemical visualization of heterogeneous and non-planar samples may be distorted. To address this issue ToF-SIMS analysis was combined with atomic force microscopy (AFM). This combination supplies the missing sample topography of the ToF-SIMS images and allows the calculation of sputter rates for the materials present in the sample.

To achieve an accurate 3D ToF-SIMS reconstruction a protocol was developed that combines AFM topographical images, crater depth measurements and sequences of ToF-SIMS images, all acquired on the same area of the sample. This combined ToF-SIMS/AFM methodology was applied to a sample consisting of GaAs selectively grown in SiO_2 patterned structures using MOCVD. The initial topography revealed that the GaAs areas were higher than SiO_2 patterns, and the large sputter rate differences (up to a factor 2) mean that a simple reconstruction (flat surface and constant sputter rate) leads to severe distortions in the 3D ToF-SIMS reconstruction.

Using the combined methodology, a 3D overlay between AFM and ToF-SIMS images at each interface can be made and the local sputter rate can be mapped. Finally, a protocol was developed for the correction of the 3D ToF-SIMS reconstruction and depth-profiles within a rendered volume defined by successive AFM imaging.

This work was carried out on the nanocharacterisation platform (PFNC) of the CEA Grenoble and this project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 688225.

References:

Moreno, M. A. et al. Combined ToF-SIMS and AFM protocol for accurate 3D chemical analysis and data visualization. J. Vac. Sci. Technol. B Nanotechnol. Microelectron. Mater. Process. Meas. Phenom. 36, 03F122 (2018).

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