Recent Advances in SIMS
Room Great Lakes B - Session RA-ThM3

HR Imaging and Spectrometry

Moderators: Laura Creon, CAMECA, **Albert Fahey**, Corning Incorporated

10:20am **RA-ThM3-11 Highest Resolution Sims Imaging Performed on Focused Ion Beam - Based Platforms***, Jean-Nicolas Audinot, O. De Castro, P. Philipp, A. Biesemeier, H. Hoang, T. Wirtz,* Luxembourg Institute of Science and Technology (LIST), Luxembourg

FIB–SEM platforms are equipped with a Focused Ion Beam (FIB) column and a focussed electron beam column. This combination is used for a vast number of applications, including nano-patterning applications, sample preparation, imaging in various electron microscopy (EM) modes (SE, BSE, EBSD, STEM) and chemical analysis (WDS/ EDX). However, electron beam probes for EDX analysis have a large interaction volume in the sample, and EDX does not allow detection of light elements (H, Li, ...) and trace elements (<1% in at.). Ion sources have seen significant improvements in source brightness in recent years, resulting in better spatial resolution and current densities. The gas field ion source (GFIS) emitting He^+ or Ne^+ ions has a brightness of 10^9 $Acm^{-2}sr^{-1}$ and thus enables a spatial resolution of 0.3 nm or 2 nm for He^+ or Ne^+ beams, respectively[1]. Likewise, a low temperature ion source (LoTIS) working with Cs ions and reaching a brightness of 10^7 $Acm^{-2}sr^{-1}$ can produce nm-sized probe sizes while maintaining high ion currents, which is of great interest not only for their ability to mill a variety of structures, but also to provide structural and morphologic information with (sub)nm resolution[2,3].

Therefore, the prospect of connecting SIMS spectrometers to FIB-SEM platforms to combine high spatial resolution imaging with high sensitivity analytical data has emerged. To maximize the extraction and detection of secondary ions (SI), LIST developed a compact SIMS system based on a double focussing magnetic sector spectrometer that is optimized for state-of-the-art FIB platforms [2,4]. The design of the SIMS system first optimizes the SI collection by placing a small retractable extraction box as close as possible to the surface of the sample. The extracted SI are then post-accelerated and focussed into the mass spectrometer. After mass filtering, SI are detected using either a multi-collector system that allows simultaneous counting of several isotopes or using a newly developed continuous focal plane detector, which allows detection of the full mass spectrum of each sputtered voxel.

Here, we will review the performance of the FIB-SEM-SIMS platforms developed at LIST by showing applications, focusing on specific methodologies, correlative microscopy, and high-resolution 3D chemical imaging. Thus, we will show that the SIMS data correlated with other data obtained on the FIB-SEM platforms, such as SE images, can provide solutions for current and future analytical challenges.

[1] T. Wirtz et al., Annu. Rev. Anal. Chem. 12, 2019

[2] B. Knuffman, A. V. Steele, and J. J. McClelland, J. Appl. Phys. 114, 2013

[3] J. N. Audinot et al., Reports Prog. Phys. 84, 2021

10:40am **RA-ThM3-13 Integrated Spatial Multiomics using Successive $(H_2O)_n$-GCIB-SIMS and C_{60}-SIMS Imaging to Delineate Tissue Heterogeneity at Single-cell Resolution***, Hua Tian,* Pennsylvania State University

Tissue is highly organized with diverse cells that interact and communicate. Together with numerous biomolecules (e.g. metabolites and lipids) of cellular processes, the multilevel heterogeneities drive the biological function and disease-associated discoordination[1-2]. This spatial complexity is often ignored by traditional tissue assay. Mass spectrometry imaging holds the potential to visualize the heterogeneous cell organization and biomolecules in their context. However, it is challenging to achieve high spatial resolution and high chemical sensitivity toward different biomolecules. Moreover, the correlation of spatial omics in a single sample is impossible due to the difficulty of preserving the fast-changing metabolites.

To overcome these analytical hurdles, innovative technology and methodology are developed for omics imaging in single cells. On the same frozen-hydrated tissue, successive $(H_2O)_{n\ (n>28k)}$-GCIB-SIMS and C_{60}-SIMS imaging are employed to profile untargeted metabolites/lipids and targeted proteins by lanthanides antibodies (~ 40 in one acquisition) at 1 µm resolution. The novel ion source, $(H_2O)_{n(n>28k)}$-GCIB enhances chemical sensitivity, improves beam focus, reduces matrix effect, and extends detection ranges up to *m/z* 6000 [3-12]. Coupled with cryogenic analysis, the tissue is analyzed at near nature state, retaining the spatiotemporal distribution of metabolites and lipids. The AI-aided computational processing is used to register the omics in different cell types for further discriminant analysis.

With the new development, a number of tissues are imaged. On breast cancer tissue, the high population of macrophages (CD68) and less infiltration of immune cells (CD45, CD4) are observed, as well as the variation of the metabolic state in different cells. Several phosphatidylinositol species are concentrated in the epithelial tumor cells (pan-cytokeratin), along with desaturated lipids and GSH, indicating the mechanism of immune resistance and antioxidation for tumor survival [27]. Eight ganglioside GM3s correlate with the Ki-67 expressing cells, likely the markers of neoplastic transformation of breast tissue[37]. On liver tissue, distinct lipid clusters colocalize with periportal and pericentral proteins, and metabolic and lipidomic signature varies in distinct liver cells (*e.g.,* sinusoidal, Kupffer, hepatocytes, Ito stellate, immune cells). Similar to protein markers, further clustering analysis shows that metabolites and lipids classify the cell types for the first time. The multimodal SIMS imaging opens broad applications for exploring various biological phenomena of cellular/biomolecular interactions in health/disease.

11:00am **RA-ThM3-15 Preliminary Development of Microscope Mode Secondary Ion Mass Spectrometry Imaging***, Felicia Green,* Rosalind Franklin Institute, UK*; M. Castellani, A. Eyres, Y. Jia, M. Brouard,* Oxford University, UK*; J. Bunch, Z. Takats,* Rosalind Franklin Institute, UK*; S. Thompson, P. Blenkinsopp,* Ionoptika Ltd, UK

We want to achieve parallel detection of hundreds of thousands of chemical species, to enable rapid localisation of molecular interactions in tissues. Microscope-mode imaging mass spectrometry has the potential to provide rapid imaging of the many chemical constituents present at a surface in a variety of applications; it decouples acquisition time from spatial resolution and is a promising route to attaining MS images in seconds rather than days. This project aims to construct a unique stigmatic imaging secondary ion mass spectrometry (SIMS) instrument, which allows the rapid molecular mapping of biological tissues at unprecedented speed, with good mass range and mass resolution.

Here we describe the development and initial results from a secondary ion mass spectrometer coupled with microscope mode detection. Stigmatic ion microscope imaging enables us to decouple the primary ion beam focus from spatial resolution and is a promising route to attaining higher throughput for mass spectrometry imaging (MSI). Using a commercial C_{60}^+ primary ion beam source, we are able to defocus the probe beam to give uniform intensity across a 1 mm area. This allows simultaneous desorption of ions across a large field of view and with the potential to get an MSI of a 1mm area in a matter of seconds. Moreover, we can distinguish features with better than 20 µm spatial resolution. Then coupling the probe to a position sensitive detector, we are able to show the detection of a range of positive and negative secondary ions from both metals and dyes. Each of the spectra took less than a minute to acquire and although the present mass resolution is sufficient, we can see the scope for significant improvements with planned upgrades in the instrumentation. Simulations show that we have the potential of achieving >10,000 mass resolution over a 400 m/z range, and up to 2 µm spatial resolution simultaneously. As we move to complex biological samples this will become increasingly important, but will not incur any loss in speed. These initial experiments suggest a very promising set up to achieve rapid secondary ion mass spectrometry imaging with data comparable to microprobe mode SIMS.

11:20am **RA-ThM3-17 Implementation of an OTOF-SIMS on a FIB/SEM UHV Workstation for Correlative Imaging at High Spatial Resolution and High Mass Resolution***, Jean Almoric Almoric,* Orsay Physics, France*; T. Genieys,* CIMAP, France*; A. Houel,* Orsay Physics, France

Due to technology advances in most of the science field (microelectronics, metallurgy, life science), the need of highly resolved analysis technics is rising. In this context, the instruments used to analyze materials at nanoscale are driven by a constant optimization of their spatial resolution. The latest generation of Focused Ion Beam (FIB) using Ga^+ ion source and developed by Orsay Physics can go down to an imaging resolution of 2.5 nm [1]. Integrated into a workstation and coupled with an electron microscope (SEM), this equipment allows nano-patterning [2], implantation [3], preparation of TEM coverslips, cross sectioning and 3D tomography. In addition to conventional detectors (SE, BSE…), advanced analytical tools can be added to the workstation such as an orthogonal time-of-flight secondary ion mass spectroscopy (OTOF-SIMS).

In this work, we studied the possibilities offered by a correlative approach, combining several analysis and imaging tools (O-TOF SIMS, SEM, FIB and GIS) in a single versatile and customizable UHV platform called "NanoSpace". Chemical mapping combining a high spatial resolution (<30nm) and a high mass resolution (FWHM 4500 on ^{28}Si) was reached by pulsing the secondary ion beam instead of the primary one. The injection of reactive precursors near the surface using a gas injection system was done to increase the secondary ionization and allowed to achieve high useful yields despite the use of a primary Ga^+ ion source. This great improvement of the transmission and mass resolution was able thanks to the redesign and the optimization of the secondary ions extraction column from an O-TOF spectrometer. Correlative analysis and innovative approach had been applied to the study of different materials, in particular a nickel-based superalloys. These materials contain precipitates of a few tens of nanometers which have been chemically characterized by a semi-quantification protocol, which is not possible by SEM-EDX [4].

[1] S. Guillous et al; A new setup for localized implantation and live-characterization of keV energy multiply charged ions at the nanoscale; Review of Scientific Instruments 87, 113901 (**2016**)

[2] A. Benkouider et al; Ultimate nanopatterning of Si substrate using filtered LMAIS-focused ion beam; Thin Solid Films (**2013**)

[3] M. Lesik et al; Magnetic measurements on micrometer-sized samples under high pressure using designed NV centers; Science 366, 1359-1362 (**2019**)

[4] J. Almoric et al; Implementation of Nanoscale Secondary-Ion Mass Spectrometry Analyses: Application to Ni-Based Superalloys, Physica Status Solidi (a), 2100414 (**2022**)

11:40am **RA-ThM3-19 Polyamide Chemical Bonding with Titanium and Aluminum Probed with ToF-SIMS and XPS***, P. Hirchenhahn,* ***Laurent Houssiau,*** University of Namur, Belgium

Structures joining metals and polymers are increasingly demanded as they combine the high mechanical resistance of the metal with the functionality and low weight of the polymer. Laser welding turns out to be a fast and efficient method to bind directly metals to polymers with no need for adhesives or mechanical fastening, but the root cause for adhesion is still poorly understood. In this contribution, we show how titanium alloys and polyamide-6.6 (PA or Nylon™) can be easily joined by shining a laser on the metal side. The very nature of the chemical bond was then probed with ToF-SIMS and XPS [1]. The first batch of samples was made of raw materials directly welded to each other, then disassembled. The fracture was both cohesive and adhesive, so that surface analysis could be directly performed on the adhesive fracture sites. Signals from the polymer and the metals were recorded simultaneously on the Ti side, proving that a very thin layer of PA was still present after fracture. Hybrid ions containing Ti and PA elements (C,H,N,O) were observed and only two of them exhibited a sharp increase in the welded area, i.e. $CHNOTi^+$ and $CHNOTi^-$, clearly pointing at the formation of C-O-Ti bonds at the interface. The second batch of samples were made by spincoating thin PA layers on Ti plates, followed by laser welding and subsequent dissolution of the polymer with trifluoroethanol. Interestingly, a very thin polymeric film remained on the Ti substrate after dissolution on the welded area but also on the non-welded area, allowing an assessment of the heat effect on binding. A PCA analysis was ran to identify which ions intensities were most changing in the weld. Ions from the substrate decreased while ions from the polymer increased in the weld, hinting at a thicker polymer residual layer. Moreover, most hybrid ions decreased in the weld, with the exception of ions containing C,H,N,O and Ti (e.g. $CHNOTi^-$ or $CHNO_2Ti^-$), confirming the formation of C-O-Ti bonds assisted by laser heat. A mechanism is proposed, with amide groups reacting with Ti hydroxide groups to create covalent C-O-Ti bonds along with imine formation in the polymer chain. This is also supported by a weak contribution of imine groups in XPS high resolution N1s spectrum. The results will be briefly compared with a previous work [2] carried out on Al laser welded to PA, which led to a similar conclusion: the formation of C-O-Al bonds upon heating with the laser beam.

[1] P. Hirchenhahn, A. Al Sayyad, J. Bardon, P. Plapper, L. Houssiau; *Talanta***2022**, 123539

[2] P. Hirchenhahn, A. Al Sayyad, J. Bardon, P. Plapper, L. Houssiau; *ACS Omega***2021**, 6, 33482-33497

Author Index

Bold page numbers indicate presenter