SIMS Solutions in Materials and Life Sciences
Room Great Lakes A2-A3 - Session SS-TuM1

Cells and Tissue I

Moderators: Gregory Fisher, Physical Electronics USA, **Sebastiaan Van Nuffel**, Maastricht University

10:00am **SS-TuM1-1 Biological Explorations with NanoSIMS: From Cells to Humans*, Matthew Steinhauser,*** University of Pittsburgh **INVITED**

Measurement of metabolism within individual cells is critical for a functional understanding of heterogeneous cell populations, particularly in complex multicellular tissues. Nanoscale secondary ion mass spectrometry (NanoSIMS) probes sample surfaces at high resolution (< 50nm), yielding multiplexed quantitative images of elemental composition. Tuning of a NanoSIMS instrument to measure two different isotopic variants of a specific element effectively enables quantitative mapping of isotopic ratios. The term "multi-isotope imaging mass spectrometry - MIMS" was coined to describe the merger of stable isotope tracer methodologies with NanoSIMS. Our group and others have demonstrated the power of MIMS as a quantitative window into a wide range of biochemical pathways at subcellular and even sub-organelle resolution. MIMS has been utilized to study processes such as glucose, amino acid, lipid, and nucleic acid metabolism and cell turnover in development, homeostasis, and disease. In this presentation, I will provide an overview of our standard workflow when conducting MIMS biological experiments, incorporating considerations of tracer selection, dosing, sample processing, and NanoSIMS analytical strategies, emphasizing experiment-specific tradeoffs between measurement accuracy and analytical throughput. With a series of specific experimental examples—including studies in murine atherosclerosis, rat pulmonary hypertension, and murine tumor models—I will illustrate how MIMS can be valuable to generate new and unexpected discoveries or as a method to test specific predictions arising from orthogonal experiments. Finally, I will share early experience with human translation to underscore the immense potential of revealing aspects of human biology that are not easily accessible with any other method.

10:40am **SS-TuM1-5 Using Multimodal Mass Spectrometry Imaging to Iron Out the Mechanisms of Ferroptosis in Epithelial Ovarian Cancer*, Michael J. Taylor,*** *J. Lukowski,* Pacific Northwest National Laboratory*; L. Tesfay,* University of Connecticut Health*; J. Cliff,* Pacific Northwest National Laboratory*; S. Torti,* University of Connecticut Health*; C. Anderton,* Pacific Northwest National Laboratory

Introduction: In 2023, 14,000 epithelial ovarian cancer deaths are expected to occur in the United States alone. It is an aggressive disease with a dismal five-year survival rate. In ovarian cancer, iron accumulates in tumor initiating cells making them susceptible to ferroptosis inducing agents. We have developed a method of linking iron accumulation with lipid profiles in a mouse model. High resolution secondary ion mass spectrometry (SIMS) and matrix-assisted laser desorption/ionization mass spectrometry imaging (MALDI-MSI) was performed on tissue sections to identify and correlate biologically important iron pools with lipid composition.

Methods: Mice were injected with FTT ovarian cancer tumor-initiating cells. After 7 days, Group 1 were treated with Erastin, and group 2 with a buffer solution (control). Mice were sacrificed 10 days after treatment, and tumors excised. Serial sections were taken. MPLEx followed by global-lipidomics (LTQ Velos) were performed on the first sections. The thinner sections were thaw mounted and dihydroxybenzoic acid (DHB) applied. MALDI-MSI was performed using a Bruker Scimax 7T. Imaging datasets were uploaded to METASPACE for annotation (SwissLipids). The DHB matrix was washed off (methanol, water, 2 mins). SIMS imaging (IONTOF V TOFSIMS / CAMECA NanoSIMS 50L) was used to semi-quantitatively identify iron regions.

Results: Comparison of lipid distributions between the Erastin treated and control samples revealed a chemically distinct region in Erastin samples Time-of-flight SIMS (TOF-SIMS) imaging of the washed tissue sections detected no DHB peak indicating that the washing steps removed all DHB from the tissue. High-spatial resolution SIMS imaging with the NanoSIMS identified that mineralized pockets of iron and calcium were present in the chemically distinct regions in the Erastin treated sample, whereas no iron pooling was observed in the control sample. The Iron pooling regions were used to specify regions of interest to compare lipid profile changes between iron and non-iron pooled areas. Higher relative abundance of Phosphatidylcholines was observed in non-iron pooled regions, whereas iron pooled regions were rich with sphingolipids. Liquid chromatography tandem mass spectrometry LC-MS/MS) analysis of the non-polar phase of the MPLEx bulk preparation was able to confirm lipid assignments putatively assigned in METASPACE based on MS1.

Conclusion: This preliminary study suggests thatErastin-induced ferroptosis is associated with pooling of iron and metals which correlates with changes in lipid profile composition

11:00am **SS-TuM1-7 GCIB-SIMS of Lipid Trafficking and Turn-Over in Cancer Cells and Spheroids*, K. Dimovska Nilsson, M. Leiva, G. Landberg, John Fletcher,*** University of Gothenburg, Sweden

The tumour microenvironment is extremely heterogenous consisting of different cell types, variation in oxygen supply and different chemical species in the extra cellular milieu. Cancer cells require high amounts of lipids in order to maintain proliferation and meet this demand through *de novo* synthesis. This can result in a deficit of poly-unsaturated fatty acid (PUFA) containing lipids as many of these rely on conversion of dietary *essential* fatty acids. This weakness in the cancer cells has been suggested as a possible therapeutic target.

Imaging MS, including ToF-SIMS, studies have illustrated that while the tumour may be depleted in PUFAs the surrounding regions can actually be high in these species, especially when inflammatory cells are present in the surrounding stroma.[1]

In this study we use a J105 SIMS instrument with a 40 keV water cluster ion beam[2,3] to investigate the ability of breast cancer cells and spheroid tumour mimics to take up and process omega-3 and omega -6 fatty acids.

(1) Angerer, T. B.; Magnusson, Y.; Landberg, G.; Fletcher, J. S. *Anal. Chem.***2016**, *88*, 11946-11954.

(2) Fletcher, J. S.; Rabbani, S.; Henderson, A.; Blenkinsopp, P.; Thompson, S. P.; Lockyer, N. P.; Vickerman, J. C. *Anal. Chem.***2008**, *80*, 9058-9064.

(3) Dimovska Nilsson, K.; Karagianni, A.; Kaya, I.; Henricsson, M.; Fletcher, J. S. *Anal. Bioanal. Chem.***2021**, *413*, 4181-4194.

11:20am **SS-TuM1-9 Correlative Microscopy of SIMS, Helium Ion Microscopy and XPS*, Jake Sheriff,*** *I. Fletcher,* Newcastle University, UK*; P. Cumpson,* University of New South Wales, Australia, UK

Secondary ion mass spectrometry (SIMS) is a widely used surface analytical technique to interpret surface composition. A primary beam is raster-scanned across a surface to create a total ion image from the secondary ions ejected [1]. The Ionoptika J105 is equipped with two ion beams; C60 and GCIB, the resolution of the images generated by the J105 is dictated by the spot size of these beams.

The Helium ion microscope (HIM) developed by Zeiss uses a beam of He ions to generate a secondary electron image of a surface. The use of He ions as the imaging beam allows for a spot size down to <0.5nm [2]. This has allowed the HIM to take high resolution images on a submicron scale without the need for specimen coating. At Newcastle we use a magnetic-sector analyser to allow SIMS mapping of the surface as pioneered by LIST [3], giving potentially the highest spatial resolution of any SIMS instrument.

The Axis Nova X-ray photoelectron spectrometer (XPS) is capable of parallel imaging. This is done by illuminating the sample surface with x-rays and then either electrostatically or magnetically projecting the electrons into a detector [4]. Using this type of imaging one can acquire a quantifiable image of the elemental distribution from a sample's surface.

All of these techniques only tell a part of a surface's story. The HIM can show an accurate picture of surface morphology with nanometre resolution, while SIMS can give the composition of the surface at the submicron scale and XPS can quantify the elemental distribution. By combining these techniques one can put these parts together and gain a better understanding of the surface structure, be it a bacterial colony or a piece of Martian rock.

We have developed a methodology to be able to co-localise areas of interest when transferring samples between multiple different surface techniques. Then automatically correlate all images to form an accurate representation of a surface [5]. Correlative microscopy with SIMS, XPS, and HIM, allows an unprecedented level of surface detail to be found.

References

[1] J.C. Vickerman et al, *Surface Analysis The Principal Techniques*, 113-199, 2009

[2] D.C. Joy *Helium Ion Microscopy Principles and Applications*, 9-15, 2013

[3] T Wirtz *et alNanotechnology*26, 434001, 2015

[4] P. Van der Heide *X-ray Photoelectron Spectroscopy An introduction to Principles and Practices*, p54-56 2012

[5] J. Sheriff, *Ultramicroscopy", 228, 113322, 2021*

11:40am **SS-TuM1-11 Direct Observation of Drug Localization to Corneocytes Versus Lipid Matrix in Stratum Corneum – Differences between Caffeine and a Jasmonic Acid Derivative***, **Peter Sjövall,*** RISE Research Institutes of Sweden*; S. Gregoire,* L'Oréal Research and Innovation, France*; L. Skedung,* RISE Research Institutes of Sweden*; G. Luengo,* L'Oréal Research and Innovation, France

Understanding the penetration of molecules into stratum corneum (SC) is critical for the development of safe and effective drugs and cosmetics [1]. Proposed mechanisms describe the penetration as diffusion/migration of the active molecule either entirely in the lipid phase of the SC structure, or through the corneocyte bodies. However, experimental verification has been limited to indirect methods, or lacked the spatial resolution/sensitivity/specificity sufficient to reliably monitor the lipid phase and corneocytes separately. In this work, time-of-flight secondary ion mass spectrometry (TOF-SIMS) was used to monitor the 3D distribution of two actives with different properties in tape strips sampled after topical application of a mixture of these actives on ex vivo human skin samples. Alternating TOF-SIMS imaging of the sample surface and gradual removal of material from the same surface, by argon gas cluster ion sputtering, provides spatially resolved mass spectrometry data from the surface of the tape strip sample, through the lipid and corneocyte layers and into the tape. The results indicate that the spatial distribution of caffeine is closely associated to proteins, indicating a localization mainly in the corneocytes. In contrast, the distribution of a jasmonic acid derivative (JDA) is more inhomogeneous and indicates considerable localization to both the lipid phase and the corneocytes. Specifically, the JDA was found to be partially colocalized with C18:1 and C16:0 fatty acids at the interface between the corneocyte bodies and the underlying tape substrate. Based on previous results, we hypothesize that the C18:1 and C16:0 fatty acids represent cholesteryl esters, which are localized at the interface between the corneocyte bodies and the lipid phase of the SC structure, and that the JDA is partially localized to this interface.

(1)Sjövall, P.; Skedung, L.; Gregoire, S.; Biganska, O.; Clément, F.; Luengo, G. S. Imaging the Distribution of Skin Lipids and Topically Applied Compounds in Human Skin Using Mass Spectrometry. Sci. Rep. 2018, 8, 16683.

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