SIMS Solutions in Materials and Life Sciences
Room Great Lakes B - Session SS-MoA1

Bio Materials

Moderator: Gregory Fisher, Physical Electronics USA

4:00pm **SS-MoA1-13 Spatially Mapping Single Cells in Diseased Tissue with Multiplexed Ion Beam Imaging***, Jay Tarolli,* Ionpath **INVITED**

The multiplexed ion beam imaging (MIBI) platform was designed to bridge the gap between imaging mass spectrometry and the clinical lab, delivering high throughput, subcellular spatial resolution imaging for 40+ protein markers per sample. Time-of-flight secondary ion mass spectrometry (ToF-SIMS) can be a powerful tool for tissue imaging. However, its applications in tissue imaging are often limited by its usability, acquisition time, and complex mass spectra, the latter making data analysis and interpretation difficult. MIBI has overcome these limitations with a high resolution, high throughput ToF-SIMS system to quickly analyze proteins of interest which are labeled using conjugated antibodies. Specifically, heavy metal atoms, which become the reporter ions measured, are conjugated to antibodies that target the proteins of interest and stain the tissue using a protocol much like that for other multiplexed imaging techniques, such as immunohistochemistry and multiplexed immunofluorescence. Antibody clones, which are known to be successful in other pathology research and clinical settings, can often be used to further facilitate the adoption of the MIBI platform in these settings.

The MIBIscope utilizes a high density Xe plasma ion source to enable rapid imaging of tissue with ToF-SIMS, acquiring an 800 µm x 800 µm ROI in as little as 35 minutes. By targeting protein species with labeled antibodies, the resulting mass spectra are less complicated and since the target analyte is not being fragmented, and the image data has higher signal to noise. An increase in throughput, simpler data analysis, and a sample prep procedure consistent with other common techniques have all allowed the MIBI platform to enter applications spaces that traditionally have been unobtainable for ToF-SIMS.

4:40pm **SS-MoA1-17 Single Cell Metabolomics using the 3D OrbiSIMS for Novel Biomaterials Development***, Morgan Alexander,* University of Nottingham, UK

Metabolomics provides the chemical readout that is closest of all the omics to the phenotype of cells. We believe that this level of insight is necessary to interpret the effect of the environmental cues provided to cells by manmade biomaterials.[1]

ToF SIMS struggles with its poor mass resolving power in complex biological systems when faced with myriad possible peak assignments for each secondary ion peak.[2] The 3D OrbiSIMS approach addresses that by combining an OrbiTrap with a time-of-flight SIMS instrument to undertake direct analysis of solid samples.[3]

Application examples from the field of novel biomaterials development will be provided that take advantage of the unique capability of this instrument, focussing on its ability to detect and identify small molecules with a high degree of certainty. Markers of immune cell polarisation for next generation implant materials have been found by assessing single macrophage cells rather than the 6 million cells required previously by LC-MS.[4] Small molecules in complex bacterial biofilms are of interest in understanding the response to novel materials that resist bacterial colonisation and infection.[5]The utility of recently development software to allow chemical filtering to predict molecular formula from SIMS using existing databases[6] is illustrated by reanalysis of the data from Zhang et al, to exemplify the massive increase in the proportion of the spectrum assigned using this automation of data interpretation for OrbiSIMS.[7]

These recent developments enable metabolomic analysis by OrbiSIMS to achieve a label-free, unbiased insight into cellular phenotype at the resolution of single mammalian cells in culture, but ultimately on explanted devices to interpret their responses to different biomaterials.

1. *Single-cell metabolomics hits its stride* **Nature Methods** Caroline Seydel
2. *Mass Spectrometry and Informatics: Distribution of Molecules in the PubChem Database and General Requirements for Mass Accuracy in Surface Analysis* **Anal Chem** 2011, Green et al
3. *The 3D OrbiSIMS - Label-free metabolic imaging with subcellular lateral resolution and high mass-resolving power*. **Nature Methods**2017**,** Passarelli et al.
4. *Single cell metabolomics of macrophages using 3D OrbiSIMS: correlations with phenotype* Suvannapruk et al. under review 2022
5. *Cryo-OrbiSIMS for 3D Molecular Imaging of a Bacterial Biofilm in Its Native State*. **Anal Chem**2020**,** Zhang et al.
6. *Molecular formula prediction for chemical filtering of 3D OrbiSIMS Datasets* **Anal Chem** 2022 Edney et al.
7. *Towards comprehensive analysis of the 3D chemical makeup of Pseudomonas aeruginosa biofilms*Kotowska et al. under review 2022.

5:00pm **SS-MoA1-19 Investigation of Changes in the Cell Envelope of E. *coli* Mutants with a Deficient Conjugation Efficiency Using TOF-Sims.***, ***Alfred Fransson****, K. Nilsson, M. Palm, A. Farewell, J. Fletcher,* University of Gothenburg, Sweden

The spread of antibiotic resistance is an increasingly difficult problem to deal with as more bacterial infections survive treatments with commercial antibiotics. One of the main routes for the spreading of resistance among bacterial population is horizontal gene transfer, mainly through conjugation where mobile genetic elements are transferred from a donor cell to a recipient cell through a conjugative pilus. One way to deal with the increasing levels of antibiotic resistance in bacteria is to develop new antibiotics for which resistance has not yet emerged, which can be both laborious and not always a lucrative market. An alternative is to inhibit the conjugation itself so that the rate at which new resistance genes spread between populations is reduced and the usefulness of existing and new antibiotics is extended. A previous study done in our lab used a high-throughput screen to identify chromosomal Escherichia coli genes in the donor cells that were important for conjugation of the F-plasmid and could be potential targets to reduce conjugation. Among these hits were several genes that are involved in the cell envelope through stress response pathways, biogenesis, outer membrane protein assembly and homeostasis, which formed an interest into the role and importance of the cell envelope for conjugation(1). Using a J105 ToF–SIMS instrument (Ionoptika Ltd) fitted with a 40 keV GCIB of (CO2)6k+(2,3), our group have previously investigated fabF and lpp E. coli deletion mutants and identified changes in lipid composition and by performing depth profiling to detected changes not specific to the surface of the cell envelope(4,5). Here we present recent data on how different sample preparations affect the cells as some of these mutants have compromised cell envelopes and it's important that we are able to preserve the samples before the SIMS analysis. In addition to how conjugative F-plasmid affects the cells on its own without any genetic deletions.

(1) Alalam, H.; Graf, F. E.; Palm, M.; Abadikhah, M.; Zackrisson, M.; Bostrom, J.; Fransson, A.; Hadjineophytou, C.; Persson, L.; Stenberg, S.; Mattsson, M.; Ghiaci, P.; Sunnerhagen, P.; Warringer, J.; Farewell, A. Msystems 2020, 5.

(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) Angerer, T. B.; Blenkinsopp, P.; Fletcher, J. S. Int. J. Mass Spectrom. 2015, 337, 591-598.

(4) Nilsson, K. D.; Palm, M.; Hood, J.; Sheriff, J.; Farewell, A.; Fletcher, J. S. Anal. Chem. 2019, 91, 11355-11361.

(5) Nilsson, K. D.; Granden, J.; Farewell, A.; Fletcher, J. S. Surf. Interface Anal. 2021, 53, 1006-1012.

5:20pm **SS-MoA1-21 Collimated Beam Imaging with MeV TOF-SIMS***, **Marko Brajkovic****, I. Bogdanovic Radovic, M. Barac, Z. Siketic,* Ruder Boskovic Institute, Croatia

In MeV TOF-SIMS, heavy primary ions with higher energy produce higher secondary ion yield of heavy molecules, an important parameter for molecular imaging. These primary ions (such as 14 MeV copper ions) cannot be focused with magnetic lenses available at the RBI accelerator facility. For this reason, a new setup is developed that uses a simple round aperture with a 5 – 10 um opening to collimate the primary beam independently on primary ion mass. As the beam current is significantly reduced after collimation with the aperture, a common beam pulsing method for triggering the TOF measurement could not be utilized. Instead,

two different options for the start signal for a continuous primary beam are available: for thin samples, a particle detector placed behind the target that detects primary ions that pass through the target, and for any target thickness an electron multiplier that detects secondary electrons created in the interaction of the primary ions with a 5 nm thick carbon foil placed over the aperture. The samples interesting for forensic (ink deposited on a paper and fingermark) and biological (section of brain tissue) applications of MeV TOF-SIMS were analyzed to show the imaging capabilities of the presented setup.

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