Beyond SIMS

Room Great Lakes A2-A3 - Session BS+FM+SS-TuA1

Cells and Tissue II

Moderators: Peter Sjövall, RISE Research Institutes of Sweden, **Michael J. Taylor**, Pacific Northwest National Laboratory

4:00pm **BS+FM+SS-TuA1-13 Answering Biomedical Questions Using Integrative ToF-SIMS Imaging**, ***Sebastiaan Van Nuffel***, Maastricht University, Netherlands **INVITED**

For the past two decades, cell and tissue imaging using Time-of-Flight secondary Ion Mass Spectrometry (ToF-SIMS) has successfully answered various biological and clinical questions over the past two decades. Because it can visualize the spatial distribution of small molecules (< 2000 Da) in 2D with a spatial resolution comparable to that of a light microscope, it can be used to simultaneously investigate the elemental composition, the metabolome and the lipidome of tissue sections as well as their interaction with non-native compounds such as drugs or toxins. However, it remains a niche technique and there are several issues still hampering its widespread application.

First of all, the data generated is very complex, because the secondary ions of the different compounds present in the sample are all formed together after the impact of the primary ion, which is why SIMS is typically combined with a 'panoramic' detector with high transmission such as a ToF mass analyzer. This property allows for label-free detection, but is a double-edged sword because it also means that a typical ToF-SIMS mass spectrum can be considered a summation of the spectra of the individual compounds present. Multivariate analysis and more advanced machine learning approaches have been successfully used for image segmentation and can help identify positive correlations between various mass peaks. However, spatial colocation does not necessarily mean that these mass peaks all originate from one compound, particularly in the case of complex biological systems. In addition, the secondary ion intensity and fragments produced using desorption-ionization techniques such as ToF-SIMS are highly dependent on the chemical environment of the compounds. This so-called 'matrix effect' has made it very difficult to fingerprint and library approaches have proven largely ineffectual for ToF-SIMS. Luckily, the creation of ToF-SIMS instruments with MS/MS capabilities makes unambiguous identification finally possible. Another issue is the fact that it is difficult to detect large molecules such as intact proteins with a typical ToF-SIMS instrument. It is therefore necessary to integrate ToF-SIMS with other imaging techniques such as other mass spectrometry imaging methods and immunohistochemistry.

Invited speaker Dr. Sebastiaan Van Nuffel will present various examples of his past and ongoing research to demonstrate the power of ToF-SIMS MS/MS and its integration with advanced data analysis techniques such as machine learning. He will also discuss his ongoing research efforts developing methods in order to establish a spatially resolved multi-omics atlas.

4:40pm **BS+FM+SS-TuA1-17 In Situ Matrix Enhanced Secondary Ion Mass Spectrometry for Tissue Analysis**, ***Thomas Daphnis***, *B. Tomasetti, D. Vincent, A. Delcorte, C. Dupont,* UCLouvain, Belgium

During the last decade, mass spectrometry imaging (MSI) has gained substantial interest thanks to impressive instrumental development. MSI can achieve simultaneous detection of hundreds of biomolecules including lipids, proteins but also drugs and xenobiotics directly in tissues and cells. The main advantages of MSI compared to classical imaging techniques are the great lateral resolution and the ability to perform analysis with no prior labelling of the biomolecules of interest. MSI finds therefore applications in the biological and pharmaceutical fields as well as many others [1].

In spite of the recent progress, the sensitivity to molecular species often remains a limiting factor for high resolution 2D and 3D molecular analysis of biological tissues in cluster secondary ion mass spectrometry (SIMS). Recently, in-situ matrix enhanced SIMS, where an acidic MALDI-type matrix is applied to the sample via large gas cluster ion-induced sputtering from a matrix "target" towards the tissue sample surface inside the ToF-SIMS, was proposed to alleviate this shortcoming [2,3]. Here, the interest of the method is demonstrated for a series of matrices and samples including lipid references and tissue sections.

First, seven MALDI matrices were selected and the ability to transfer them was demonstrated using an Ar_{3000}^+ ion beam. Then, the different matrices were transferred onto a phosphatidylcholine (PC) mix layer spin-coated on silicon (PC is an abundant lipid class of cellular membranes). Matrices CHCA, DHB and SA proved to enhance intact lipid ion signals up to one order of magnitude. Interestingly, the matrices not only increase the signals of protonated species $[PC+H]^+$ but also the signal of adducts $[PC+Na/K]^+$. Therefore, the acidic matrix deposition effect is twofold: it brings extra protons to analyte molecules but also provides a favouring environment for their ionisation.

Finally, these three matrices were transferred on real mouse brain tissue sections. As similar tissues have been extensively studied in the MSI community, peak identification was facilitated. The measured lipids ion yields were compared as a function of sample pre-treatment. Our results show that the matrix transfer of CHCA and DHB was highly beneficial to intact lipids detection in these tissue sections. Indeed, some peaks were revealed by the matrix while the signals of others were increased by 10-fold. Moreover, signal enhancement was observed for both Bi_5^+ and Ar_{3000}^+ as analysis beams.

References

[1] M. Noun et al., Microscopy and Microanalysis, pp. 1-26, 2021;[2] K. Moshkunov et al., Analyst 146, pp. 6506-6519, 2021;[3] M. Lorenz et al., Anal. Chem. 93, pp. 3436-3444, 2021.

5:00pm **BS+FM+SS-TuA1-19 Evaluating Topical Product Sensitivity and Distribution Using a Multi-Modal Imaging Approach**, ***Jean-Luc Vorng***, *D. Tsikritsis,* National Physical Laboratory, UK*; P. Zarmpi, V. Tyagi,* University of Bath, U.K.*; A. Dexter, I. Gilmore, N. Belsey,* National Physical Laboratory, UK*; R. Guy,* University of Bath, U.K.

There is a need to characterise non-invasively both the epidermal bioavailability of a topically applied drug and to distinguish correctly between formulations that are bioequivalent, i.e., to measure if a generic formulation performs the same as the branded product. In this study, Time-of-Flight Secondary Ion Mass Spectrometry (TOF-SIMS) was used to detect, characterise, and image the distribution of 4-cyanophenol a drug permeation enhancer[(1)] within a pig skin tissue homogenate and pig skin tissue sections[(2)].

Due to its high sensitivity and the capability to provide chemical mapping of the sample, SIMS enables a step-by-step approach to the problem starting from the screening of the product to investigating the limit of detection within a biological matrix. In this study, we have investigated the distribution of 4-cyanophenol in skin using a multi-modal imaging approach. Correlative mass spectrometry imaging (MSI) measurements with non-invasive Raman spectroscopy on the same sample provides superior chemical specificity and permits the distribution of the compound to be accurately characterised using spatial registration[(3)]. Finally the OrbiSIMS has been used to investigate the contribution of endogenous species that might interfere with the signal of interest in TOF-SIMS[(4)]

In this work, the compound of interest has been successfully detected as an intact molecular ion and a linear response of intensity as a function of concentration has been obtained. Finally, the distribution 4-cyanophenol within a pig skin tissue section was mapped and a strong correlation between SIMS and Raman spectroscopy was demonstrated.

References

(1)*Romonchuk et Al. Skin Pharmacol Physiol, 23(3),* ***2010****, 152–163*

(2)*Summerfield et al. Molecular Immunology 66* ***2015****, 14–21*

(3)*Siy, P. W.et al. BioInformatics and BioEngineering.* ***2008****, BIBE. 2008, 8th IEEE International Conference on, IEEE: 2008; pp 1-6*

(4)*Passarelli et al. Anal. Chem.* ***2015****, 87, 6696-6702*

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