1018
doi:10.1017/S1431927618005585
Microsc. Microanal. 24 (Suppl 1), 2018

Surface Characterization of Biologically Related Systems with Imaging TOF-SIMS and Complementary Techniques.

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Imaging time-of-flight secondary ion mass spectrometry (TOF-SIMS) can provide sub-micron resolution images of cells, tissues, and other biomedically relevant samples with chemical and molecular specificity. Applications include spatially identifying chemical changes as a function of an applied stress (delivered drug or biomaterials) or as a result of disease and tracking the spatial distribution of metabolites and lipids in tissue. Similarly, TOF-SIMS images of biomaterials (e.g., polymer scaffolds) can give insight to the biological response to biomaterials and determine distribution of surface modification of three-dimensional scaffold structures.

With TOF-SIMS the surface chemistry is probed by bombarding a sample with primary ions which leads to secondary ions being ejected, representing the samples chemical composition in the bombarded area. This primary ion beam can be rastered over the surface, acquiring individual mass spectra for each image pixel. Since TOF-SIMS is a label free, semi quantitative analysis method, this generates a chemical map of the surface providing information on localization and intensity of a large number of chemical species in one data set. A variety of primary ion beams is currently available, all with different properties concerning achievable spatial resolution in images as well as the intensity of high mass signals in the spectra. As a rule of thumb: the higher the spatial resolution the lower the mass signals, in part because primary ion sources capable of submicron spatial resolution in images tend to fragment molecules but also, larger molecules need a bigger analysis area per pixel to produce intense enough signals to be imaged.[1]

The NESACBIO Center has two TOF-SIMS imaging mass spectrometers: **1.** IONTOF ToF SIMS 5 (ION-TOF GmbH, Münster, Germany); and **2.** J105 – *3D chemical imager* (Ionoptika Ltd, UK). We use both instruments for 2D and 3D chemical imaging of biologically relevant samples. Imaging on *Phagocata gracilis* (planarian flatworms) was done with the J105. Unlike conventional TOF-SIMS instruments the J105 uses a quasi-continuous primary ion beam to produce secondary ions that are sampled by a linear buncher prior to TOF measurement.

Multivariate analyses were performed in MatLab (The MathWorks Inc.) using the NESACBIO NBToolbox (https://www.nb.uw.edu/mvsa/nbtoolbox). To reduce the volume of data, mass channels were reduced to a peak-list of 1000 – 1500 peaks (generated from each dataset). In the first step, principal component analysis (PCA) was performed on the whole image on mean centred and square rooted data.

In order to get an initial idea of the lipidomic landscape in planarian worms, longitudinal sections of planaria were analyzed with imaging TOF-SIMS, and the data contained in the subsequent MS images were analyzed with PCA in order to identify chemically unique areas in scores images as well as the MS signals (peaks) associated with those areas in loading plots. Comparing light microscopy images of the sections and MS/PCA scores-images, we were able to identify several organ structures in the sections: brain (CNS), intestines, pharynges (different to other planarian species, *Phagocata gracilis* has multiple

small pharynges instead of one central pharynx), testes, and several parts of the male reproductive system. Studying the loadings of the PCA analysis we were able to identify unique lipid species present in each organ system.[2]

References:

[1] E. R. A. van Hove, D. F. Smith, and R. M. A. Heeren, J Chromatogr A **1217** (2010), p. 3946.
[2] Work done at NESACBIO with funding provided by NIH P41 EB002027.

https://doi.org/10.1017/S1431927618005585 Published online by Cambridge University Press