doi:10.1017/S1431927616002609
Microsc. Microanal. 22 (Suppl 3), 2016

Sub-Micron Resolution Imaging with Bio-Molecular Identification by TOF-SIMS Parallel Imaging MS/MS

Gregory L. Fisher[1], Nina Ogrinc Potočnik[2], Anne L. Bruinen[2], John S. Hammond[1], Scott R. Bryan[1], and Ron M.A. Heeren[2]

[1.] Physical Electronics, Chanhassen, MN 55317, USA
[2.] Maastricht Multi-Modal Molecular Imaging Institute, 6211 Maastricht, The Netherlands

TOF-SIMS offers a number of advantages which include high spatial resolution, high abundance sensitivity, and shallow sampling depth. But, TOF-SIMS has had no means for unambiguous molecular identification and this inherent weakness in high *m/z* molecular identification has greatly impeded its adoption into fields of biological research. MALDI is routinely used for molecular identification via tandem MS but has been limited to a practical lateral resolution of greater than 10 μm. Data reproducibility has been problematic with MALDI due largely to variations of the applied matrix, and a sample is consumed in a single multiplexed analysis. The deficiencies of both TOF-SIMS and MALDI drive an enormous analytical need in biological research because many disease states and therapeutics must be understood at the cellular or sub-cellular scale.

A newly developed TOF-TOF tandem imaging mass spectrometer allows conventional TOF-SIMS (MS^1) analysis and product ion (MS^2) analysis to be executed simultaneously and in parallel [1]. Secondary ions for MS^1 and MS^2 analysis are produced from the same area of the surface by a pulsed and digitally raster-scanned primary ion nanoprobe. The sensitivity of the parallel imaging MS/MS spectrometer is high so that the analytical ion dose may be minimized; therefore, precious and one-of-a-kind samples may be probed relentlessly. Fragmentation of the precursor ions, defined by a 1 Da precursor selection window, is accomplished by collision-induced dissociation (CID) at 1.5 keV in an activation cell of Ar gas at high pressure. Lateral resolutions produced in both MS^1 and MS^2 images are demonstrated to be in the range of 100 nm < $\Delta\ell_{80/20}$ < 1 μm. We have employed this new capability to investigate the relationship of lipids and lipid metabolites to neuro-regeneration and blood-borne disease.

Lipids and metabolites play an important role in the early stages and progression of neurodegeneration and disease, and play an equal but different role in brain plasticity and ontogenesis. Our first study concerns song bird ontogeny in male zebra finch (*T. guttata*). Almost all analysis was performed on a single cross-section of brain tissue. Several sulfatides, phospholipids, sterols and fatty acids have been identified as playing a role in song learning. We have employed parallel imaging MS/MS to disentangle the role of specific molecules because the chemical noise, or unresolved chemical interferences, present in TOF-SIMS imaging necessitates the use tandem MS imaging. We have evidence suggesting that specific sulfatides are active primarily within the song nuclei while cholesterol and specific fatty acids are active in signaling between the song nuclei.

In other work, we have probed the role of lipids and metabolites in disease states of zebrafish (*D. rerio*) that have been infected with *M. marinum*, a form of tuberculosis. The bacteria initiate a granulomatous inflammation, and first signs of disease are observed in the spleen. All analysis has been conducted on a single whole-body cross-section which is possible because dozens of repeat analyses can be performed by TOF-SIMS parallel imaging MS/MS without damage or consumption of the sample. We have

observed so far that α-tocopherol is elevated in infected tissue as well as in the granuloma, but is not present in the necrotic cells. Cholesterol is elevated primarily in the granuloma. The role of phospholipids appears to differ, specific molecules being either elevated or depressed in the infected tissue. We also have preliminary evidence of a metabolic source for bacterial growth. For example, we observe PC(16:0/16:0) to be elevated in the granuloma; however, in the necrotic cells surrounding the granuloma we observe elevated signals of FA(16:0). The fatty acid distribution about the granuloma is revealed in Figure 1 with a measurement of the lateral resolution.

[1] P.E. Larson, J.S. Hammond, R.M.A. Heeren, G.L. Fisher, Method and Apparatus to Provide Parallel Acquisition of MS/MS Data, *U.S. Patent 20150090874*, **2015**.

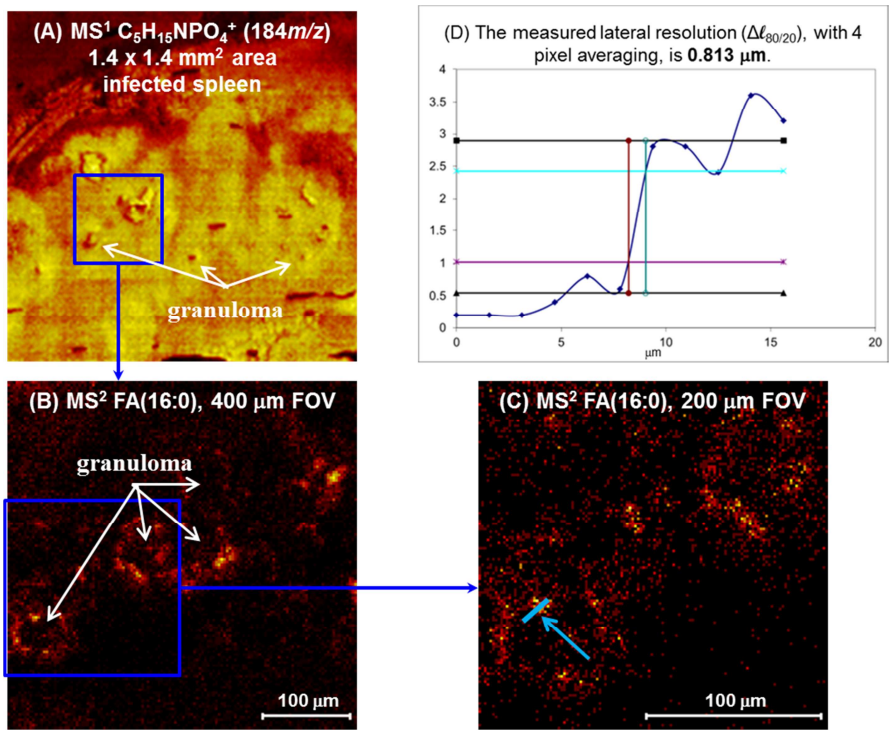

Figure 1. (A) The 1.4 x 1.4 mm^2 map of a portion of the zebrafish spleen 24 hours after infection. In the positive ion polarity MS^1 image of the phosphocholine headgroup, $C_5H_{15}NPO_4^+$ (184 *m/z*), numerous granuloma are observed. (B) A separate analysis was conducted in the negative ion polarity and MS^2 images of the $[M - H]^-$ ion of FA(16:0), $C_{16}H_{31}O_2^-$ (255 *m/z*), were generated. The scale marker is 100 µm. (C) From the large area MS^2 map of the $[M - H]^-$ ion of FA(16:0), a zoom to a 200 µm FOV is shown. A line scan with 4 pixel averaging, indicated by an arrow, is made on a chemical feature. The scale marker is 100 µm. (D) A plot showing the 80% - 20% line scan measurement and revealing a lateral resolution of 0.813 µm. Measurements on several features indicate a lateral resolution of < 1 µm. For all TOF-SIMS parallel imaging MS/MS analyses on the zebrafish cross-section, the analytical ion beam was operated at a dc current of 12 nA.

https://doi.org/10.1017/S1431927616002609 Published online by Cambridge University Press