Advances in mass spectrometry imaging instrumentation and sample treatment protocols for in situ chemical characterization of biological samples

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Project Goals: We are developing new instrumentation and complementary sample preparation methods which allow visualization of chemical composition and physical structure across multiple size scales. To this end, we have constructed a hybrid mass spectrometer capable of imaging in three distinct, complementary modes: matrix assisted laser desorption/ionization mass spectrometry imaging (MALDI MSI), secondary ion mass spectrometry (SIMS) imaging, and secondary electron microscopy (SEM). Here we explore mutually compatible sample treatments including sublimation of conventional organic MALDI matrix, and spray deposition of both matrices and metal nanoparticles, in order to enhance molecular coverage, ionization efficiency, and image contrast. We apply this new instrument and the developed treatments to visualize multi-scale chemical heterogeneity in bacterial biofilms to further understand the role of chemical signals in bacterial biofilm formation.

Microprobe mass spectrometry imaging (MSI) utilizes spatially-resolved ionization to interrogate a sample surface at regularly spaced positions. Each point generates a mass spectrum, containing information on the chemical species present at that location. Selected ion intensities at each position can be mapped to pixel color to display spatial information of the analyte abundance. Our lab has developed a mass spectrometer equipped with both a UV laser and a buckminsterfullerene (C60) ion source for MALDI- and SIMS-mode imaging, respectively.1 Interfacing these two ionization techniques with a commercial quadrupole/time-of-flight (QToF) mass spectrometer allows us to obtain in-situ molecular identification and the combined benefits of both MALDI and SIMS ionization. MALDI offers superior molecular coverage for high mass analytes such as lipids and peptides, whereas cluster SIMS offers superior spatial resolution, but tends to fragment molecules larger than ca. m/z 2000. We have further extended the imaging capabilities of our hybrid mass spectrometer by incorporating a secondary electron detector, which enables inline electron microscopy in conjunction with the MSI functionality. With a 5 µm spatial resolution, this microscope rapidly provides architectural and topographical information to overlay with chemical features from MSI.

Improving spatial resolution with MSI necessitates reducing microprobe size and therefore the quantity of sample available for analysis. Decreasing the limits of detection is imperative for extracting useful chemical information at higher spatial resolutions. One method for improving SIMS sensitivity is to apply a matrix or nanoparticles that more efficiently dissipates projectile energy and increases ionization yield. However, application of the matrix solution leads to analyte migration as well as extraction. The importance of these two effects needs to be balanced to achieve the best spatial resolution. We have observed increased signal intensity when the sample is coated with the MALDI matrix, 2,5-dihydroxybenzoic acid (DHB), or nanoparticles. The matrix application method also greatly affects analyte extraction and imaging spatial resolution, and we are currently adapting two matrix application methods, sublimation and robotic spray coating, for MALDI and SIMS analysis of bacterial biofilms.
We have optimized methods for DHB sublimation onto biofilms, for both SIMS and MALDI imaging. We first test the methods on rat spinal cord, a well-characterized bioanalytical standard for MSI, and then transition to biofilm analysis. Our results show that DHB sublimation is a powerful method for the analysis of lipid distributions on biofilm surfaces. Analyte delocalization is limited due to the dry coating and the small crystal size. A number of lipid-associated ions are detectable, with many potassium or sodium adducts. For MALDI, the optimal DHB coating is limited in the range of 0.1 to 0.3 mg/cm², while for C60-SIMS the optimal coating is between 0.1 and 0.2 mg/cm². Sublimation offers a promising avenue for matrix enhancements, however substituting nanoparticles would eliminate interference from DHB while still improving SIMS sensitivity. To uniformly deposit nanoparticles, we have developed a robotic matrix application system. Early trials with nanoparticle coatings have demonstrated speed, uniformity and high transfer efficiency. Two further modifications under development are electrospray and heated nebulizing gas.

We are investigating additional modifications to improve the biofilm-imaging capabilities. A concern with any analysis is that sample preparation will perturb the system, causing loss of information from the pristine sample. The current room-temperature vacuum analysis chamber requires complete desiccation of biofilms which may cause some analyte degradation or redistribution. A future goal is to begin SIMS imaging of hydrated biofilms by maintaining the vacuum chamber at cryogenic temperatures. MSI on our custom system presently utilizes stepper motors to move the sample in a regular array. Along with the SED upgrade, we can now control the ion beam location by electrical deflection. Instead of moving the sample, we can raster the ion beam to generate higher resolution images. We are developing methods to couple MS acquisition with the ion beam raster to achieve a lateral resolution of approximately 2 microns. Achieving sub-micron resolution, which is necessary for sampling sub-cellular components and cell-cell signaling molecule exchange, will require increasing our beam energy to 40 kV through a commercial source upgrade.

References

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