67. In Situ Correlated Imaging of Chemically Communicating Microbial Communities

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Project Goal: Our goal is to develop correlated mass spectrometry imaging (MSI) and confocal Raman microscopy (CRM) analytical techniques and apply them to model systems that closely mimic the rhizosphere. The biological system we are developing involves two microbes and a plant root. We seek to understand the biological processes occurring at the single cell and interactions and communication events among multiple cells at the molecular level. By spatially mapping the distribution of various molecular constituents within the model system using both MS and CRM imaging, we are able to acquire complementary information that gives great insights on: (1) the function of different molecular messengers found within microbial communities, (2) key molecular changes that occur as microbial species transition from single cells to communities, and (3) plant-microbe interactions.

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Multiplex, label-free, and non-targeted chemical mapping of sample surfaces can be performed with mass spectrometric imaging techniques such as matrix-assisted laser desorption ionization and secondary ion mass spectrometry (MALDI and SIMS) as well as confocal Raman microscopy (CRM). CRM provides 3D-resolved chemical information based on the molecular vibrations of the components, while MSI yields highly specific chemical maps of the sample surface by direct detection of ionized molecules. Correlated imaging with MSI and CRM – applying these methods to the same sample and combining the resulting data with high spatial fidelity – has the potential to reveal information about molecular composition, distributions, and temporal changes that is not available from either method used alone.

Correlated imaging offers great potential for elucidating the behavior of bacteria. Many bacteria naturally aggregate and adhere to surfaces forming biofilms – multicellular communities held together by a self-produced extracellular matrix. Bacteria within biofilms live in a complex microbial community that exhibits primitive homeostasis, a circulatory system, and metabolic cooperativity, and imparts multiple benefits such as increased antibiotic resistance and enhanced virulence.[1] Corresponding to differences in phenotype, bacteria growing in a biofilm show distinct gene expression profiles relative to planktonic cells[2,3] and also respond differently to environmental perturbations.

Spatially and temporally mapping the chemical composition of bacterial biofilms can provide valuable insight into the functions of molecular constituents, how they respond to their immediate microenvironment, the mechanisms of biofilm formation, and ultimately how they may be controlled or engineered to be useful.

Initial efforts in this project focused on the development of a correlated MS/CRM imaging platform. We have addressed the technical challenges associated with correlating information from two different spectral imaging techniques. Specifically, we have (1) developed sample preparation protocols that are amenable to both MSI and CRM experiments, (2) implemented a workflow that preserves the sample integrity for both analyses, and (3) developed a spatial registry method that is compatible with both CRM and MSI and precisely demarcates microscopic regions of interest as well as the sample orientation.

The new technologies for correlated MS and CRM imaging described above have been applied to study the biofilm formation process in the bacterium *Pseudomonas aeruginosa*. By characterizing planktonic cells and biofilms using CRM and MSI we are able to compare their molecular compositions and observe the major changes in molecular composition associated with the biofilm formation process. Of special interest are quinolone signalling molecules involved in biofilm production as well as multi- functional secreted glycolipid (rhamnolipid) compounds which act as surfactants, growth cues, and virulence factors.[4] CRM of *P. aeruginosa* biofilm revealed bands at 1030 cm^{-1} and 1068 cm^{-1} that are not present in the spectra of planktonic cells and indicate the presence of glycolipids, likely rhamnolipids. MSI analysis of the same sample confirmed the presence of rhamnolipids, identifying multiple rhamnolipid congeners. Furthermore, unique distributions of the rhamnolipid congeners were observed by MSI, which may indicate differing functions during biofilm growth.[5]

Our current and ongoing work focuses on extending the capabilities of correlated MS and CRM imaging to study microbial attachment on plant roots, as well as visualizing additional compounds of interest within bacterial biofilms.[6]

References
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