58. Correlated Molecular Imaging Applied to Chemically Communicating Microbial Communities of Relevance to the Rhizosphere

Sneha Polisetti[1], Nameera Baig[1], Jennifer Morrell-Falvey[2], Mitchel Doktycz[2], Sage Dunham[3], Nydia Morales-Soto[4], Joshua Shrout[4], Jonathan Sweedler[3], and Paul Bohn[1]

[1] Department of Chemistry and Biochemistry and Department of Chemical and Biomolecular Engineering, University of Notre Dame, Notre Dame, IN 46556
[2] BioSciences Division, Oak Ridge National Laboratory, Oak Ridge, TN 37831
[3] Department of Chemistry and Beckman Institute for Advanced Science and Technology, University of Illinois at Urbana-Champaign, Urbana, IL 61801
[4] Department of Civil and Environmental Engineering and Earth Sciences and Department of Biological Sciences, University of Notre Dame, Notre Dame, IN 46556

Project Goal: Our goal is to develop a multi-modal analysis program by integrating confocal Raman microscopy (CRM) and mass spectrometry imaging (MSI – both by SIMS and MALDI) and apply them to a system that closely mimics the rhizosphere. Specifically we are developing a three-component system comprised of two microorganisms and a plant root. We seek to understand the biological interactions, processes, and communication events that occur at the intra and inter species level between the microorganisms as well as the interactions occurring between the plant root and the individual microorganisms by following the spatial and temporal characteristics of the various biomolecules within the system.

http://www.bohnresearchgroup.com

Correlating image information across orthogonal platforms, such as CRM and MSI can provide insights that are superior to those obtained from either modality alone and uniquely positions our team to: (1) develop selective, non-perturbative probes to measure the spatial and temporal concentration profiles of nutrients, metabolites, signaling molecules, extracellular matrices and other biomolecules; (2) develop instrumentation to enable simultaneous observation of different intracellular processes with high spatial and quantitative resolution; and (3) investigate the spatial relationships, physical connections, and chemical dynamics that are responsible for the flow of information and materials at the interface between organisms and their environment.

Correlated CRM-MSI imaging has already uncovered new principles by which low-level signaling molecules, specifically the quinolones, govern the spatiotemporal organization of microbial communities of Pseudomonas aeruginosa. These new chemical imaging tools fundamentally change the nature of the questions that can be posed regarding microbial communities, permitting us to move beyond a taxonomic approach – cataloguing observed compounds and how they relate to gene expression – to an approach that addresses the temporal and structural features of community organization, especially in biologically relevant multi- organismal co-culture systems. Furthermore, correlated CRM-MSI imaging produces information across multiple dimensions, since molecular identities acquired from MSI data are combined with high spatial resolution chemical class information available from CRM. Multiplexing of these two platforms has now reached a stage of maturity where correlated CRM-MSI can be applied to multi-organismal microbe-plant communities under more complex and realistic conditions. We are currently extending the chemical imaging capabilities in two important ways – by enhancing the technological capabilities of correlated imaging and by applying the resulting capabilities to develop critical understanding of spatiotemporal organization in co-cultured models of the rhizosphere.

We have recently extended our correlated CRM-MSI work to the challenging problem of bacterial

biofilms. First, planktonic P. aeruginosa was compared to biofilms using CRM and metal-assisted LDI. Planktonic cells produced CRM spectra dominated by DNA and RNA scattering, while biofilms expressed significant quantities of rhamnolipids, polysaccharides and proteins. Corresponding LDI analysis provided MS/MS identification and spatial mapping for six rhamnolipids, including both Rha-C10 and Rha-C10-C10, which are critical surfactants for biomolecule distribution during biofilm development. Comparing a quorum-sensing deficient mutant to the wild-type strain showed dramatic differences, with the mutant producing little or no rhamnolipid and other extracellular polymeric substances (EPS) under conditions designed to elicit biofilm growth.1 The spatial registry problem was solved for these relatively featureless bacterial biofilms, by printing a nanoparticle fiducial array on the dried biofilm prior to analysis, viz. Figure 1, providing a general scheme for co-localizing spectral features across multiple platforms implementing image acquisitions under disparate conditions.

The authors acknowledge funding from the Department of Energy Office of Science (BER) through grant DE-SC0006642 and the Genomic Science Program, Plant-Microbe Interfaces Project (ORNL).

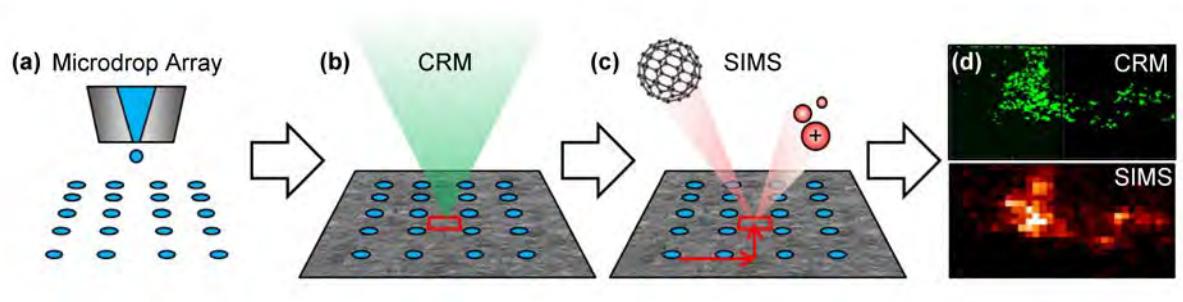

Figure 1. CRM-SIMS correlated imaging workflow. (a) A microdroplet array is applied to the dried biofilm. (b) CRM is performed to locate ROIs and array coordinates are recorded. (c) The sample is transferred to the SIMS instrument and the array is used to navigate back to the ROIs. (d) The CRM and SIMS data are correlated, using the array for alignment.

1. Lanni, E.J.; Masyuko, R.N.; Driscoll, C.M.; Aerts, J.T.; Shrout, J.D.; Bohn, P.W.; Sweedler, J.V., Correlated Imaging with C60-SIMS and Confocal Raman Microscopy: Visualization of Cell-Scale Molecular Distributions in a Bacterial Biofilm. Analyt. Chem. 2014, 85, 10885-10891.