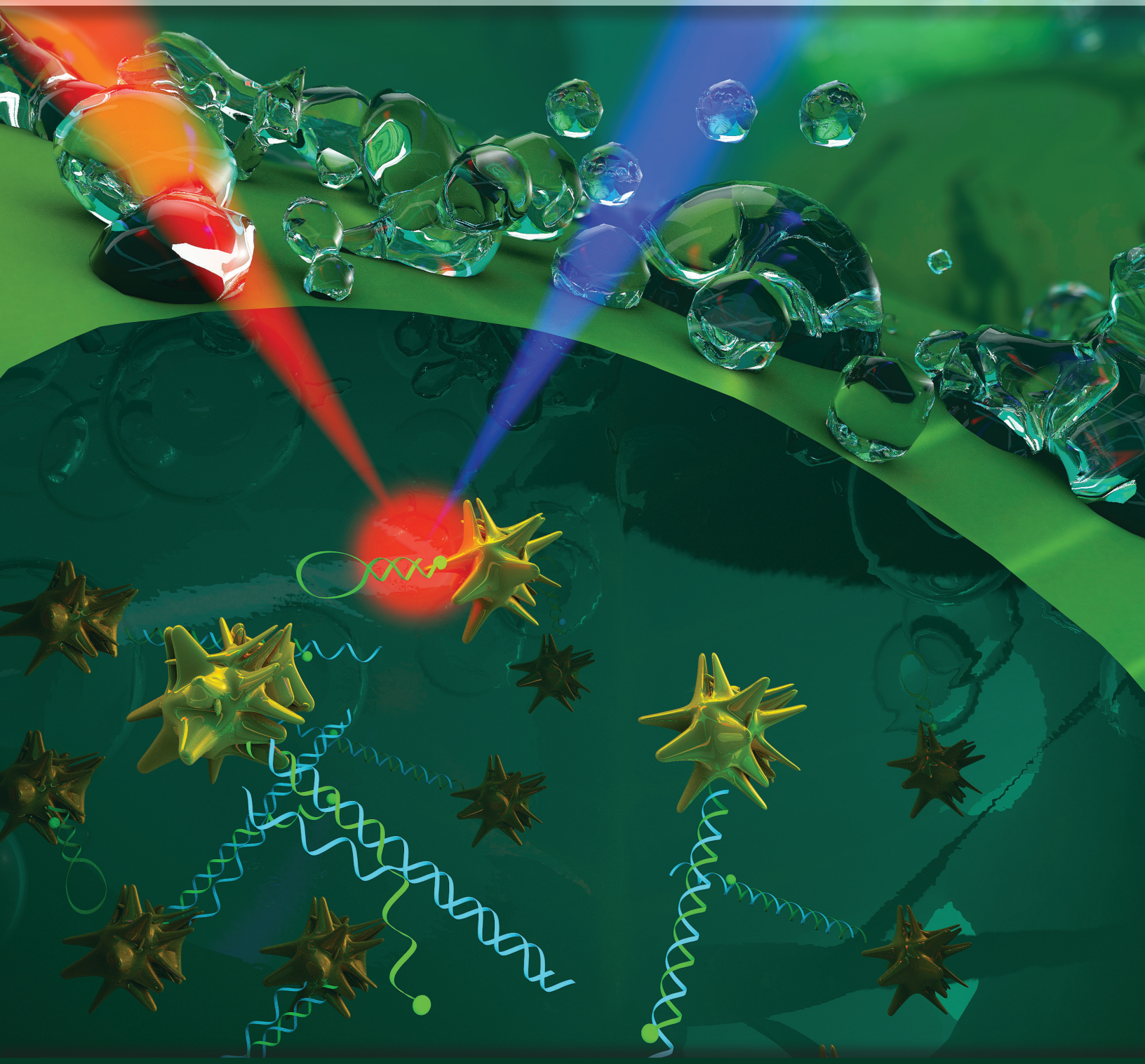
Bioimaging Science Program
2019 Principal Investigator Meeting Proceedings



U.S. DEPARTMENT OF ENERGY | Office of Science

Office of Biological and Environmental Research

Bioimaging Science Program

2019 Principal Investigator (PI) Meeting

February 27–28, 2019

Vienna, Virginia

Program Manager

Prem C. Srivastava
Office of Biological and Environmental Research
Office of Science
U.S. Department of Energy

Meeting Co-Chairs

Tuan Vo-Dinh
Duke University

Marit Nilsen-Hamilton
Iowa State University

Scott Lea
Pacific Northwest
National Laboratory

About BER

The Biological and Environmental Research program advances fundamental research and scientific user facilities to support Department of Energy missions in scientific discovery and innovation, energy security, and environmental responsibility. BER seeks to understand biological, biogeochemical, and physical principles needed to predict a continuum of processes occurring across scales, from molecular and genomics-controlled mechanisms to environmental and Earth system change. BER advances understanding of how Earth's dynamic, physical, and biogeochemical systems (atmosphere, land, oceans, sea ice, and subsurface) interact and affect future Earth system and environmental change. This research improves Earth system model predictions and provides valuable information for energy and resource planning.

Cover Illustration

Optical biosensing and bioimaging of molecular markers within whole plants for bioenergy research. Courtesy Tuan Vo-Dinh, Duke University.

Digital Download

These proceedings are available at science.energy.gov/ber/community-resources/2019_Bioimaging_Program_PI_Meeting.pdf.

Bioimaging Science Program
2019 Principal Investigator (PI) Meeting Proceedings

Published June 2019

Office of Biological and Environmental Research

Prepared for the
U.S. Department of Energy
Office of Science
Office of Biological and Environmental Research
Germantown, MD 20874-1290

Prepared by
Biological and Environmental Research Information System
Oak Ridge National Laboratory
Oak Ridge, TN 37830
Managed by UT-Battelle, LLC
For the U.S. Department of Energy
Under contract DE-AC05-00OR22725

Contents

Preface

The 2019 Office of Biological and Environmental Research (BER) Annual Bioimaging Science Program Principal Investigator (BSP-PI) Meeting was held February 27–28, 2019 at Sheraton Tysons Hotel, in Tysons, Virginia. The program's mission is to understand translation of genomic information into the mechanisms that power living cells, communities of cells, and whole organisms. The program's goal is to develop new imaging and measurement technologies to visualize the spatial and temporal relationships of key metabolic processes governing phenotypic expression in plants and microbes. Annual program PI meetings are intended to convene investigators contributing to the program and review progress and the current state-of-the-art in bioimaging research.

This year's BSP-PI meeting was held consecutively at the same venue with the 2019 BER Genomic Science Program PI meeting. Holding the two PI meetings consecutively provided a platform for networking, stimulating discussions, exchanging ideas, and forging new collaborative alliances among the investigators from the two sister programs. Important highlights of the meeting were two key note presentations by Dr. Ming Tien, Penn State University, on the topic of "Single Molecule Studies: New Tools to Study Interfacial Processes Involving Enzymatic Deconstruction of the Plant Cell Wall," and by Dr. Gokul (Srigokul) Upadhyayula, Harvard Medical School (now at UC-Berkeley), on the topic of "Imaging of Subcellular Dynamics in Multicellular Organisms." This year's BSP-PI meeting concluded with a brainstorming session and a discussion on Quantum Enabled Sensors and Technologies (QUEST) for Bioimaging (report attached, p. 19). The meeting's proceedings provide an outline of the program's current projects and discussion of potential future directions and opportunities for the science.

Prem C. Srivastava, Ph.D.
Program Manager
Biological Systems Science Division
Office of Biological and Environmental Research
Office of Science
U.S. Department of Energy
301-903-4071; prem.srivastava@science.doe.gov

List of Funded Projects

Universities

Plasmonics-Enhanced Optical Imaging Systems for Bioenergy Research
Tuan Vo-Dinh, Duke University

Real-Time Imaging and Quantification of Plant Cell Wall Constituents Using Cavity-Dumped Stimulated Raman Scattering (cdSRS) Microscopy
Shi-You Ding, Michigan State University

Single-Molecule Imaging of Lignocellulose Deconstruction by SCATTIRSTORM Microscopy
William Hancock, Pennsylvania State University

Time-Resolved 3D Multi-Resolution Microscopy for Real-Time Cellulase Actions *In situ*
Haw Yang, Princeton University

***In planta* Multimodal Single-Molecule Imaging to Study Real-Time Turnover Dynamics of Polysaccharides and Associated Carbohydrate Metabolites**
Sang-Hyuk Lee, Rutgers University

Development of Broadband Infrared Nano-Spectroscopy of Biological Materials in Fluid
Tina Jeoh, University of California, Davis

Understanding Plant Signaling via Innovations in Probe Delivery and Imaging
Jean T. Greenberg, University of Chicago

Spatiotemporal Dynamics of Photosynthetic Metabolism in Single Cells at Subcellular Resolution
Jeffrey Cameron, University of Colorado Boulder

Correlative Imaging of Enzyme and Metabolome Dynamics for Yield and Titer Co-Optimization in Biofuel-Producing Microorganisms
Andreas E. Vasdekis, University of Idaho

Hyperspectral Light Sheet Raman Imaging of Leaf Metabolism
Keith Lidke, David Hanson, Jerilyn Ann Timlin, and Jamey Young; University of New Mexico

Development and Implementation of an *In situ* High-Resolution Isotopic Microscope for Measuring Metabolic Interactions in Soil Mesocosms
Elizabeth A. Shank, University of North Carolina at Chapel Hill

Metaoptics Enabled Multifunctional Imaging
Paul Bohn, University of Notre Dame

Multiparametric Optical Label-Free Imaging to Analyze Plant Cell Wall Assembly and Metabolism
Marisa S. Otegui and Kevin W. Eliceiri, University of Wisconsin–Madison

National Laboratories

Detecting Chemical Signals in the Soil with 4DMAPS, an Integrated Aptasensor Assembly
Marit Nilsen-Hamilton, Ames Laboratory

FFFI: Development of a Full-Field X-Ray Fluorescence Imaging System for Near Real-Time Trace Element Microanalysis of Complex Biological Systems
Ryan Tappero, Brookhaven National Laboratory

Illuminating the Rhizosphere: Developing an Adaptive Optics, Multiphoton Microscope for 3D Label-Free Live Imaging of Microbes and Organic Matter in Soil and Roots
Peter K. Weber, Lawrence Livermore National Laboratory

Intrinsically Co-Registered Chemical Imaging of Living Plant and Microbial Systems via 3D Nonlinear Optical Mapping and *In situ*-Liquid Extraction–Mass Spectrometry
John F. Cahill, Oak Ridge National Laboratory

Multimodal Chemical Imaging Across Scales to Visualize Metabolic Pathways in Live Plants and Microbial Systems
Scott Lea, Pacific Northwest National Laboratory

Executive Summary

The Office of Biological and Environmental Research (BER) of the U.S. Department of Energy (DOE) Office of Science sponsors research at national laboratories and universities within its Bioimaging Science Program (BSP). BER BSP supports fundamental research towards enabling new bioimaging capabilities for the study of biological function of whole plant and microbial systems relevant to bioenergy research. New technologies are designed to solve specific problems in plant biology, biofuels, and biogeochemistry as well as in broad-based systems and design-based technologies that can lead to new discoveries. New and improved instruments are being designed and constructed from the ground up or are being adapted by merging new, innovative, and transformational improvements to existing capabilities, thereby creating a comprehensive and versatile toolbox for real-time dynamic imaging of metabolic pathways and the transport of materials within and among cellular organelles, including plant-root and organismal interactions, enzyme function, and cellular structures.

A wide variety of technologies spanning molecular, optical, fluorescence, Raman and nonlinear optical techniques (SERS, CARS, SRS, hsSRS hyperspectral SRS, TERS) and X-ray microscopy and spectroscopy is being developed to image dynamic events *in situ*. Whole plants or fixed samples are imaged by both nondestructive and destructive approaches. Due to their noninvasive nature, optical modalities ranging from infrared/UV absorption and adaptive optics multiphoton microscopy to fluorescence and Raman techniques (conventional, nonlinear, surface-enhanced) have been important topics for the BSP. These spectroscopic modalities are complemented by the research and development at DOE-sponsored user facilities involving innovative applications of ion microscopy as well as a full-field X-ray fluorescence imaging and crystallography.

Imaging mass spectrometry coregistered with spectrochemical imaging capabilities are also being further enhanced to yield highly selective, sensitive, and quantitative chemical maps that identify intra- and extracellular gradients and the distributions, abundance, and fates of stable isotopes, natural elements, and metabolites. This work supports simultaneous observation using conventional microscopies for correlated structural and chemical imaging and the interpretation of biological function of living plants and microbial systems.

Both label-based and label-free imaging approaches are being developed to monitor and image complex pathway dynamics occurring in living microbial and plant systems while also detecting chemical signatures and biomolecular and genomic markers. The performance of sensing and imaging technologies is enhanced through development of unique probes equipped with various bioreceptors (antibodies, aptamers, gene probes) for specific detection of important biomarkers (metabolites, proteins, genomic markers) related to specific processes and metabolic pathways of interest in microbial and plant systems. The development of unique probes and sensors is expanding the impact of the new instrumentation by allowing dynamic tracking of targeted cells, organelles, enzymes, biomarkers, and small molecules.

Recommendation

In addition to developing and improving each of these techniques (e.g., optical, X-ray, mass-spectrometric, and ion-based approaches), a major focus of the program moving forward should be to continue to further integrate these different and complementary approaches either in hybrid all-in-one instrumentation or by connecting the resulting data from multiple technologies and approaches. Cross-platform bioimaging systems will permit indexing and registration of images (e.g., multifunctional tracers, probes, and sensors to serve as cross-platform fiducial markers). Such data integration and fusion systems are needed to meaningfully coreference and coregister disparate datasets for the same sample that are of different formats, magnifications, or resolutions. With the diverse set of bioimaging data to be created by all the technologies described above, integrated data processing algorithms, visualization, and modeling are recognized as key components for properly interpreting and connecting the imaging data to omics-based models of the organisms, pore-scale lattice Boltzmann models, and root-scale water flux models.

Biological imaging is inherently transdisciplinary and successful teams should continue to reflect this approach by intermingling researchers in imaging, technology development, nanoscience, computer science, structural biology, genomic science, ecology, and biogeochemistry as a critical step toward the translation of laboratory-developed technologies into the natural environment. This process will address the need to dynamically image complex (and often unknown) native microbial populations, investigate community organization, and understand how multiple metabolisms and processes coexist in space and time.

Program Summary

With its mission to understand the mechanisms that power living cells, communities of cells, and whole organisms, particularly as it is relevant to bioenergy and biofuels, the Bioimaging Science Program supports the development of new imaging and measurement technologies. Initiated in 2015, the program has evolved and expanded to new instrumentation and technologies, currently sponsoring research at five national laboratories and 13 universities. These new projects have substantially broadened the program's portfolio with an impressive range of new instrumentation and measurement technologies for visualizing the spatial and

temporal relationships of key metabolic and molecular processes associated with phenotypic and genomic expression in plants and microbes in support of developing new knowledge for bioenergy and environmental needs.

Together, the instruments being created under BSP sponsorship will provide a versatile and powerful toolbox for imaging biological dynamics and chemistries occurring across length scales ranging from single molecules to small unicellular organisms to complex microbial and fungal community interactions with plants. Much of the new instrumentation under development is multimodal with the result that each instrument combination will provide high resolution at a range of length scales. The number of modalities included in these new instruments is dazzling. A wide variety of microscopy approaches ranges from optical methods such as luminescence, confocal, adaptive optics multiphoton, fluorescence scattering, reflected/transmitted light extinction spectroscopy, total internal reflection fluorescence (TIRF) to full-field X-ray fluorescence, imaging, polarimetry, and novel single-molecule sensing methods including stochastic optical reconstruction microscopy (STORM) and photo activated localization microscopy (PALM). These imaging modalities will also be complemented by advanced technologies such as high-speed atomic force microscopy (AFM), interferometric scattering microscopy, infrared (IR) vibrational sum frequency generation. A variety of forms of Raman spectroscopy, including spontaneous, far-field subdiffraction, tip-enhanced, coherent anti-Stokes, surface-enhanced Raman scattering (SERS), spatially offset Raman spectroscopy (SORS), shifted-excitation Raman difference spectroscopy (SERDS) and cavity-dumped SERS are also part of the portfolio. Added to these imaging modalities will be the capability of capturing samples to profile metabolites by several forms of mass spectrometry including laser ablation electrospray ionization mass spectrometry (LAESI-MS) and LAESI- Fourier transform ion cyclotron resonance mass spectrometry (FTICR-MS) using a 21 Tesla magnet. Nonlinear optical mapping and *in situ* liquid extraction-mass spectrometry are being developed to provide 3D spatiotemporal chemical information in the bulk and at the interface in biological systems. In a different approach, electrochemical impedance spectroscopy with nucleic acid aptamer sensors will be developed to profile larger molecules, such as proteins. The depth of analysis with this array of instruments will provide researchers with the necessary tools to visualize the complex pathway dynamics of living biosystems with the resolution to detect specific chemical signatures and molecular structures and to investigate interactions between organisms in mixed communities.

Participants in this program are focusing on a variety of biological systems that need to be understood for better control of plant health and growth to improve bioenergy resources. The subject organisms include plants, bacteria, fungi, and their combinations. Some projects are developing sophisticated instruments to image metabolism in a single organism and others to image gene expression and regulatory molecules (e.g., microRNAs) in whole plants as well as interactions and communications between organisms. The ability to understand mechanisms of metabolic flow within the plant, in microbial communities that support plant growth, or between plants and microbes has been limited due to the lack of instrumentation that can track molecules in intact organisms and between organisms. Other limitations for current instrumentation include the ability to image molecules in intact plants and in the soil where plants and microbes interact in the rhizosphere. Instruments being developed under BSP sponsorship will address this critical need and enable a future when molecular signatures can be tracked in real time over time periods consistent with the processes being studied. Some of these instruments will include the capability of visualizing biosystems as they respond to induced external stressors and perturbations such as nutrient starvation and chemical exchanges. Synthetic rhizosphere microhabitats, transparent soil microcosms, and versatile nanofluidic and microfluidic imaging and sampling devices are being employed to permit simultaneous cultivation and analysis of biosystems from single cells to complex communities.

The molecular signatures being studied by the sponsored research groups are highly diverse; cover a large length scale; and include atomic isotopes, metabolites, plant hormones, silica, trace elements, redox metabolism, cellulose and lignin synthesis and degradation, microRNAs that regulate lignification, enzymes, and other proteins secreted by plants and quorum-sensing molecules. By supporting the development of technologies to capture this broad range of molecular signatures, the program is developing a valuable array of imaging technologies.

The functional dimensional scales in biological systems are vast, reaching from the molecular to multiorganismal systems. Due to its hierarchical order, activities on longer length scales are built on activities and structures on smaller length scales. Therefore, processes must be fully explained at the molecular level to be fully understood at the organismal or multiorganismal level. Recognizing this need, the program is supporting some innovative single-molecule imaging approaches that include plasmonic nanoprobes to track single molecules, 3D tracking with high-speed AFM and optical tweezers to control molecules or microbes, enable force measurements, or to track molecules like cellulose synthase as it moves along the membrane or cellulase as it moves along cell walls. These studies will answer important questions relating to the mechanisms of cellulose synthesis by the cellulose synthase complex and cellulose degradation by cellulase. Understanding these mechanisms will enable the development of a biomass that can more readily and rapidly be converted to biofuels.

While focusing on high resolution imaging, some projects supported by the Bioimaging Science Program are well positioned to be applied to more complex biological systems of relevance to

bioenergy and the environment such as understanding quorum sensing, improving lipid feedstock yields, enhancing lignocellulosic deconstruction, or boosting feedstock sustainability and plant drought tolerance. Subjects of study in the BSP include living plants (*Arabidopsis thaliana, Medicago truncatula, Brachypodium distachyon, Populus* spp., *Pinus taeda, Zea mays, Panicum virgatum*, and *Avena barbata*), microbial chemotrophs (*Bacillus subtilis, Yarrowia lipolytica, Pantoea* sp.), microbial phototrophs (*Cyanothece, Rhodopseudomonas palustris, Ostreococcus tauri, Chlamydomonas reinhardtii, Pseudomonas aeruginosa*, and *Synechococcus* sp.), systems for studying plant-microbe interactions (Arbuscular mycorrhizal symbioses with *Rhizophagus irregularis*, *Glycine max* with *Bradyrhizobium japonicum*, and *Suillus brevipes* with *Pinus taeda*), viruses (*Pseudomonas* phage), and natural soil. With new instrumentation developed under BSP sponsorship, these investigations are expected to result in a better understanding of spatial and temporal metabolite distributions associated with growing microbial and plant systems and to reveal new insight into the fundamental biology of many macro events such as nutrient utilization and community and ecosystem interactions that include soil water retention due to the presence or absence of particular organisms or biomass.

This comprehensive portfolio, which includes the development of complex multimodal instrumentation to image key metabolites and molecular biomarkers across the length scales and hierarchies of biological systems, will enable the development of a better understanding of the molecular underpinnings of a diverse array of biological and environmental processes.

Recommendations and Future Directions

Biological imaging is fundamentally cross-disciplinary, and the research and development process required to advance DOE programmatic goals for BSP needs to continue to reflect this approach. Advances in biological imaging require the incorporation of recent developments in technology, nanoscience, and computation—demanding the integration of expertise within these communities with imaging scientists. Imaging scientists also need to reach across biological disciplines to structural biologists and genomic scientists to connect phenotyping and genotyping and to integrate imaging results with corresponding genomic, proteomic, lipidomic, and metabolomic changes within cells to facilitate the deciphering of biological complexity and heterogeneity. Equally important is the coordination with ecologists, soil scientists, and biogeochemists to better replicate the innate multiphase complexity of soils in laboratory experiments as scientists move toward deploying imaging approaches in complex natural environments.

The Bioimaging Science Program spans a broad spectrum of imaging approaches, including complementary targeted and untargeted, destructive and nondestructive imaging modalities (e.g., optical, scanning probe, X-ray, and ion-based approaches) that cover a wide range of spatial and temporal scales. In addition to pursuing advances within each of these respective techniques, a major focus of the program moving forward should center on the integration of these approaches. Some integration of these different imaging modalities is currently being implemented within the existing program, but additional opportunities to generate complementary data through a multipronged approach will present themselves. Linkages of the resulting complementary data will create a more holistic picture of the biological systems being imaged. There is also a critical need to develop selective probes that allow identification, sensing, and functional imaging of various targets ranging from key metabolites to molecular and genomic biotargets (e.g., mRNA and miRNA) in complex biological systems. Relevant key advances would include the simultaneous marking, spatially resolved tracking, and sensing of multiple players (e.g., elements, isotopes, enzymes, metabolites, and other molecular biomarkers) in a given biological system. Compiling these capabilities will also provide the essential flexibility to broaden the scope of investigations, opening new possibilities to discover yet unknown key biomarkers or intermediates. Probing a sample inherently perturbs it, yet methods based on selective probe-induced perturbations of key biotargets or metabolic pathways of particular organisms could provide opportunities to investigate and understand biological processes that would otherwise be difficult to decipher.

A substantial near-term challenge is the extension of laboratory-based approaches into applications for whole organisms and plants in their natural environment and under field settings. This process will involve the need to incorporate the dynamics of microbially driven biogeochemistry (e.g., within the rhizosphere, biofilms, and other key biological interfaces). The ability to image complex native microbial communities will enable deciphering of their organization and multiple metabolic processes co-occurring in space and time. Essential components include the development of approaches to probe inherent signals within nontractable microbes in the environment and the creation of pathways to enable microbial synthesis of probes for assaying function and activity. Furthermore, in addition to sophisticated and lab-based analytical methods, portable instrumentation and practical techniques will allow detection of weak optical signals of whole organisms and plants extracted from strongly interfering background signals such as fluorescence, ambient light, and fixed pattern noise under field conditions.

The long-term goal of such an approach is to generate spatially and time-resolved snapshots of relevant cellular metabolism, including both primary and secondary metabolites, as well as internal and secreted compounds. The real-time collection and interpretation of these integrated data will constitute a major advance in bioimaging technology—one that will provide new understanding for monitoring and understanding phenotyping in the laboratory and in complex natural environments. This advancement will require new approaches and algorithms to handle increasingly challenging volumes of data and auto-

mated means to sift through the data to identify biologically and environmentally meaningful signals. Also needed are new models capable of integrating multimodal data from across a range of spatial and temporal scales to most effectively extract causality from observations and understand the emergence of complex phenomena.

To reach these goals, advances in several areas are needed, including these key developments:

1. Combination of bioimaging techniques with advanced probes and delivery mechanisms that expand the monitoring capability for important biotargets, ranging from key metabolites to molecular and genomic biomarkers.

2. New or improved imaging technologies capable of observing systems evolving in their natural state or responding to environmental perturbations and stressors, while acquiring real-time data across the full spectrum of relevant spatial scales.

3. Cross-platform protocols for sample preparation; indexing and spatial registration; and cross-platform calibration, data verification, and correlation to increase the suite of complementary analyses that can be conducted on a given sample or suite of samples.

Along with these developments, improvements are needed in data storage, processing, and visualization to enable effective extraction of critical biological and environmental information from the experimental data. Of interest is a central clearinghouse for archiving experimental and simulation data that incorporates a standardized output and imaging framework for different and potentially widely adoptable analytical modalities. Such a data repository could be independent or integrated with the DOE Knowledgebase (KBase) system and could take advantage of advances in artificial intelligence to extract patterns from raw data for improved organization, interpretation, and representation.

With the advancement of these new technologies and approaches, it is important that they be developed in ways that ultimately enable researchers to use them. Deployment to DOE user facilities of some of the imaging technologies being developed within the Bioimaging Science Program would provide the broader research community access to these technologies thereby increasing their impact. Such an approach would also facilitate continued technological developments through synergistic interactions between imaging scientists and the facility in addition to expanding the scope of research being conducted with these new capabilities.

The BER Bioimaging Science Program's annual principal investigator (PI) meeting provides a useful platform for the program to increase the cross-platform and cross-scale synergies needed to achieve its goals. Scheduling this meeting proximal to the DOE Genomic Science Program (GSP) annual PI meeting creates invaluable opportunities for synergistic interactions with the GSP research community. The inclusion of imaging experts external to the Bioimaging Science program in the PI meeting enables injection of novel perspectives and approaches into discussions. Additional cross-team interactions (e.g., through teleconferencing or web conferencing) could help to maintain this interactive momentum and catalyze new directions of investigation. Moreover, the creation of a Bioimaging Capability portal, detailing the diverse technological approaches and highlighting the applications for which they are best suited, would enable the program to impact a wider community of scientists who could make use of new bioimaging approaches for their research.

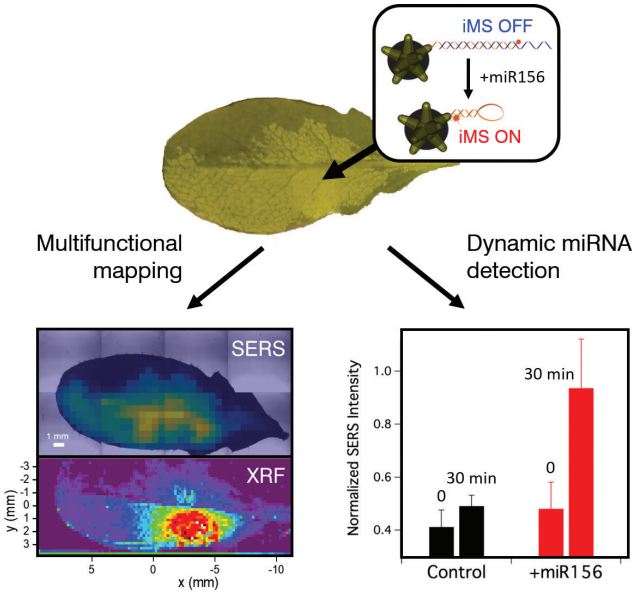
Abstracts

Plasmonics-Enhanced Optical Imaging Systems for Bioenergy Research

Principal Investigators: Tuan Vo-Dinh[1] (PI), Tai-Ping Sun,[1] and Kenneth Kemner[2]
Organizations: [1]Duke University and [2]Argonne National Laboratory
Email: tuan.vodinh@duke.edu

Project Summary: The goal of this project is aimed at addressing the DOE FOA need to develop innovative and improved imaging instrumentation that can enable visualization and quantitative characterization of nanoprobes for biomarkers and their dynamic role in metabolic processes and cellular functions in living plants relevant to DOE bioenergy programs.

Research Plans and Progress, Including Objectives and Goals for the Project Period: Monitoring gene expression in whole plants is a key requirement in many important fields, ranging from fundamental plant biology to biofuel development. However, current methods to monitor gene expression in plants require sample extraction and cannot be performed directly *in vivo*. To overcome these limitations, we have developed *in vivo* imaging and biosensing of microRNA (miRNA) biotargets using plasmonic nanoprobes referred to as inversed Molecular Sentinels (iMS) that can be monitored using surface-enhanced Raman scattering (SERS). We reported the application of iMS to dynamically track the presence of exogenous miRNA targets *in vivo* within *Arabidopsis* leaves. Furthermore, we demonstrated accurate mapping of miRNA targets within leaves using the combination of SERS-mapping with complementary techniques, such as SERS and X-ray fluorescence (XRF) imaging (see figure). Our results demonstrate the possibility of SERS monitoring and imaging and represent a major innovation that has a strong transformative potential in monitoring genomic targets in whole plant leaves without sample extraction.



(Top) Delivery of iMS nanoprobes to *Arabidopsis* leaf. (Bottom Left) SERS and XRF mapping of nanoprobes in whole leaf. (Bottom Right) SERS detection of nanoprobes for miR156 targets in a control and infiltrated leaf. Reprinted with permis sion from Crawford, B. M., et al. 2019. "Plasmonic Nanoprobes for *in Vivo* Multimodal Sensing and Bioimaging of MicroRNA within Plants, *Applied Materials & Interfaces* 11 (8), 7743–54. Copyright American Chemical Society.

Current and/or Anticipated Accomplishments and Deliverables: To further the research of the previous project focused mainly on nanoprobe development (Multi-functional Plasmonics Nanoprobes for Cellular Sensing and Imaging), we are currently developing innovative imaging technologies for visualization and quantitative characterization of biomarkers related to molecular processes and cellular function within living plants. We develop an advanced Multimodal Optical Sensing And Imaging Combinatory (MOSAIC) System that integrates plasmonics-enhanced spectrochemical techniques including SERS, plasmonics-enhanced two-photon luminescence (PE-TPL) imaging for use in imaging and tracking important miRNA biomarkers associated with bioenergy applications. We also develop multimodal XRF-SERS-TPL imaging for spatial co-registration of nanoprobes in living plant systems. The advanced MOSAIC system will provide the much-needed tools for biofuel research such as elucidating the regulation of the pathway to synthesize photosynthetic terpenes more efficiently for biofuel production, and tracking pathways of carbon fixation in plant systems.

Potential Benefits and Applications: This project will be applied to research on next-generation biofuels, which aim to use nonfood biomass, such as lignocellulose (woody parts) in plant wastes or hydrocarbon produced by photosynthesis (e.g., terpenes and fatty acids) in plants and certain microbes. Current production of cellulosic and hydrocarbon biofuels is far from optimal and requires further research to improve the efficiency and reduce costs. The development of new imaging methods for RNAs will allow for successful monitoring of the abundance and subcellular localization of these factors within living plants. These novel tools will greatly facilitate studies on the regulatory mechanism for photosynthetic terpene production in plants.

Real-Time Imaging and Quantification of Plant Cell Wall Constituents Using Cavity-Dumped Stimulated Raman Scattering (cdSRS) Microscopy

Principal Investigator: Shi-You Ding
Organization: Michigan State University
Email: sding@msu.edu

Research Plans and Progress: The project leverages our previously developed custom-built imaging systems, with a particular focus on the development of cavity-dumped stimulated Raman scattering (cdSRS) technique that will significantly improve the chemical sensitivity to allow us to detect physicochemical features of cellulose and lignins in the cell wall during biosynthesis in living plants and during enzymatic deconstruction in biomass at low concentrations that have not been achieved previously. These physicochemical data will be analyzed in combination with atomic force microscopy (AFM) imaging at the subnanometer scale to provide in-depth understanding of biomass deconstruction processes. We will use the extensively studied maize (*Zea mays*) as a living plant and as corn stover for deconstruction. Previously, we have demonstrated that the cell wall structure changes during the course of enzymatic hydrolysis, and that the efficiency of biomass deconstruction is negatively correlated with the overall lignin content and the localization in the cell walls at the molecular and cellular levels.

Current and/or Anticipated Accomplishments and Deliverables: This project is driving innovations in (1) developing a new generation of cdSRS imaging tools to quantitatively analyze cellulose and lignins with high sensitivity. We propose to leverage the SRS instrumentation funded by a previous BER lignocellulose imaging program with new development of cdSRS that has potentially one order of magnitude of improvement in signal-to-noise ratio; (2) imaging the co-localization of plant cell wall polymers (e.g., cellulose and lignins) and their changes *in situ* during plant development and biomass deconstruction; (3) enabling the overlay of the physicochemical properties identified by *in situ* cdSRS with the nanoscale structure imaged by real-time AFM; and (4) elucidating the correlation between the structural/chemical characteristics of biomass and the efficiency of deconstruction.

Potential Benefits and Applications: Once developed, the new imaging tool will synergistically interact with projects in the Great Lakes Bioenergy Research Center (GLBRC). The method will provide the required spatial and temporal (as well as structural) information to augment the detailed structural information that is available from other analytical approaches, but that suffers from only delineating bulk structure (i.e., from materials that have been finely ground and thus lose all ultrastructural information). The research will significantly enhance our understanding of cellulose structure and lignification *in planta* and their roles in biomass deconstruction processes. This technique will also have broad application in systems biology, such as in the tracking of metabolism within a single cell, and co-localization of chemical transformation processes in the inter- or intracellular spaces. The *in situ* and *in vivo* chemical informtion could be further correlated with single-cell genomics in future development.

Single-Molecule Imaging of Lignocellulose Deconstruction by SCATTIRSTORM Microscopy

Principal Investigator: William Hancock
Organization: Pennsylvania State University
Email: wohbio@engr.psu.edu

Research Plans and Progress: The goal of this project is to build a multimodal optical microscope to measure the binding, processive degradation, and pausing behaviors of cellulases as they interact with and degrade both synthetic and naturally occurring lignocellulosic walls. To achieve this, we will use high spatiotemporal single-molecule imaging to track cellulases, while visualizing specific molecular components of cellulose, lignin, and hemicellulose that make up their lignocellulose substrate. The microscope will combine Interferometric Scattering (iSCAT), which provides unprecedented spatiotemporal resolution; Total Internal Reflection Fluorescence (TIRF), which provides single-molecule resolution of multiple fluorophore-labeled molecules; and Stochastic Reconstruction (STORM), which allows for 3D super-resolution imaging of intact plant cell walls during degradation. Initial studies will investigate cellulase dynamics on *in vitro*–assembled cell wall analogs, and later work will progress to using native plant cell walls.

Current and Anticipated Accomplishments and Deliverables: Since the grant started on September 1, 2018, we have designed and are in the process of building our microscope, and we are carrying out preliminary single-molecule experiments on our existing microscopes. We have been successful in fluorescently labeling the cellulase TrCel7A, and have visualized single cellulase molecules by TIRF microscopy. We are able to visualize both purified cellulose nanocrystals and nanofibers; these will serve as the simple cellulose substrates for our experiments. We are using the same dye-labeled TrCel7A on isolated cell walls and working to visualize single-molecule interactions with these complex cellulose substrates.

Potential Benefits and Applications for DOE and Other Research: There is scant single-molecule data of cellulases degrading cellulose substrates, and we believe that increasing the temporal and spatial resolution of cellulase dynamics will greatly enhance our understanding of the basic mechanism of enzymatic cellulose degradation. Our microscope will apply cutting-edge microscopy tools developed in the motor protein and related fields to the plant biology and bioenergy communities.

Time-Resolved 3D Multi-Resolution Microscopy for Real-Time Cellulase Actions *In situ*

Principal Investigators: Haw Yang[1] (PI) and Ming Tien[2] (Co-PI)
Organizations: [1]Princeton University; [2]Pennsylvania State University (PSU)
Emails: hawyang@princeton.edu, mxt3@psu.edu

Research Plans and Progress: The project is currently at its first-year funding since it was officially launched on October 1, 2018. The Year-1/Q1 objective was to develop cellulase and substrate for 3D multi-resolution microscopy and imaging (3DMM), as well as the time-gated real-time 3D single-particle tracking technology. These two tasks were to be carried out by two postdocs, one in each lab. Currently, the Yang lab at Princeton is still in the process of hiring a suitable postdoc for this task. The Tien lab at PSU has successfully secured a postdoc who will hopefully start in April 2019. Personnel staffing aside, on the other hand, tasks within the "3DMRM optimization and assay" goal (Year-2/Q2) have been progressing nicely with a manuscript currently in preparation. The manuscript reports the use of unsupervised learning technique to enhance low-resolution/low signal-to-noise curvilinear objects in an image. This new development will allow us to enhance the resolution from conventional two-photon microscopy to match that of 3D single-particle tracking (10 nm XYZ). This way, we will be able to quantitatively integrate the dynamics of the cellulase with the cellulose images (i.e., lines with mild curvatures) obtained from two-photon microscopy.

Current and/or Anticipated Accomplishments and Deliverables: The expected deliverable for Year-1/Q1 is the time-gated 3D single-particle tracking technology. The Tien lab hopes to generate mutant and tagged forms of Cel7A from *Trichoderma reesei* such that single-molecule visualization will be enabled. As mentioned in the previous section, this is predicated on finding a suitable postdoc. While ads for such a position have been in place since summer 2018, no suitable candidates have emerged, unfortunately. Additional personal letters have been sent out to specific labs for such a candidate.

Potential Benefits and Applications: In addition to facilitate achieving the DOE-BER bioimaging goals, more broadly, the new instrumentation and technologies will help to fundamentally understand the molecular actions in such complicated environments as in the plant cell wall, which is composed for a mixture of at least three different polymers in addition to cellulose. For example, the initial 3DMM technology, which was made possible through a prior DOE funding, already has been used to study how virus-like nanoparticles (Yang lab, published work) and viruses may enter living cells (Professor Kevin Welsher at Duke University). For broad dissemination, commercialization is another effective means in addition to academic publications. To that end, the 3DMM technology already has been awarded patents in the United States, Japan, and China with European Union patent application pending. The new advances outlined in this project, namely adding time-resolved capability to the instrument, will help to make the technology more attractive to the private sector. The scope of fundamental questions in basic sciences that it can address also will be even greater, hence empowering the broader scientific community.

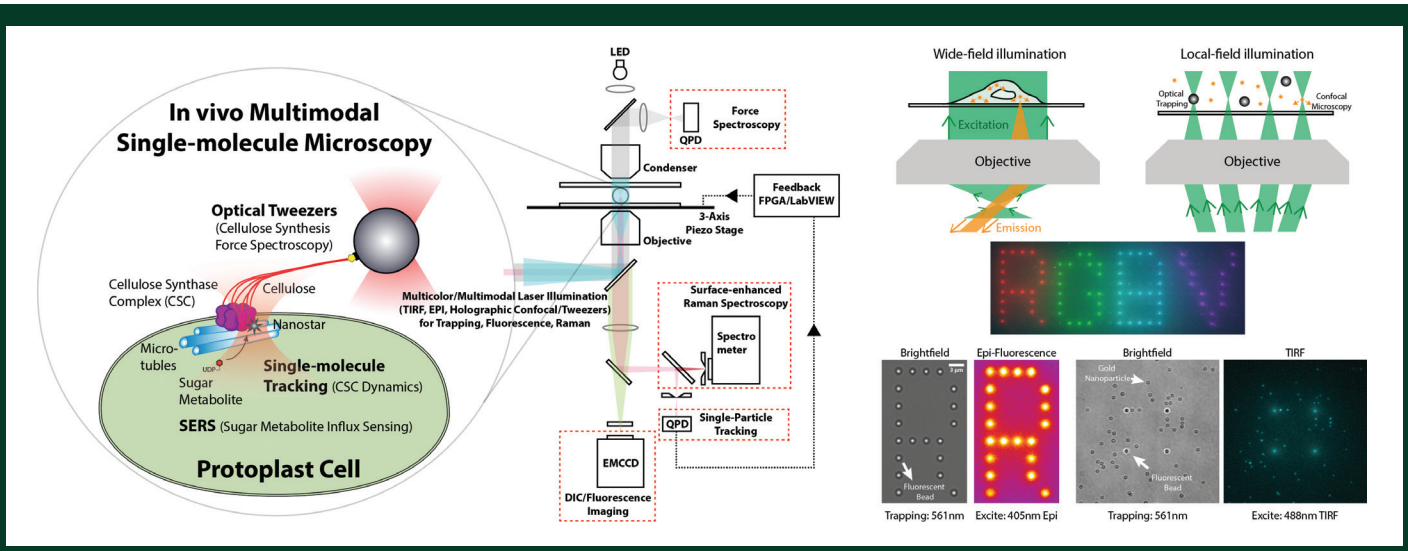
In planta Multimodal Single-Molecule Imaging to Study Real-Time Turnover Dynamics of Polysaccharides and Associated Carbohydrate Metabolites

Principal Investigators: Sang-Hyuk Lee[1] (PI); Co-PIs: Shishir Chundawat,[1] Eric Lam,[1] and Matthew Lang[2]
Collaborators: Wellington Muchero,[3] Sai Venkatesh Pingali,[3] and Laura Fabris[1]
Organizations: [1]Rutgers University, [2]Vanderbilt University, and [3]Oak Ridge National Laboratory
Email: shlee@physics.rutgers.edu

Research Plans and Progress: This project aims to study plant cell wall polysaccharide synthesis using *in vivo* multimodal single-molecule manipulation/imaging techniques. We plan to simultaneously conduct: (1) optical tweezers-based force spectroscopy on the nascent polysaccharide chain extruding out of cellulose synthase complex (CSC) on protoplast membrane; (2) real-time single-particle tracking of CSC; and (3) SERS-sensing of sugar metabolites using plasmonic gold nanostars attached to CSC (see figure). Three specific objectives are to develop (1) a single-molecule instrument that integrates holographic optical tweezers, single-particle tracking, and SERS; (2) *in vitro* multmodal single-molecule assays on the cellulose synthesis machinery reconstituted with purified CSC; and (3) *in vivo* multimodal single-molecule assays on the cell wall regeneration process using *Arabidopsis*/poplar protoplasts. Progress is currently being made on the instrumentation development in parallel with generation of reconstituted CSC and engineered plant protoplasts for *in vitro* and *in vivo* work, respectively.

Current and/or Anticipated Accomplishments and Deliverables: We have developed a versatile microscope platform that accomplishes a multimodal operation of the same laser for both wide-field illumination and holographic focused beam illumination either interchangeably or simultaneously in a way scalable to multiple lasers (see figure). We are currently working to integrate a force measurement module, feedback-looped single-particle tracking system, and Raman spectroscopy module into our existing microscope. This new instrument is anticipated to allow multiplexed molecular force measurement and subnanometer motion detection in the moving frame of an enzyme complex along with label-free chemical sensing of local metabolite influx.

Potential Benefits and Applications: Our research will reveal *in vivo* plant cell wall polysaccharide synthesis processes with unprecedented molecular-level detail through the concurrent characterization of dynamics and function of a single enzyme complex as well as intracellular metabolite flux. The results from this project will greatly advance the mechanistic and holistic understanding of *in vivo* cell wall synthesis, which will accelerate the development of better transgenic crops for bioenergy-related applications. Moreover, the new toolbox, combining powerful advanced microscopy assays with cell/protein engineering, will have broader impacts on molecular and cellular biology fields by paving the way for multimodal single-molecule studies in native cellular environments.



(Left) The grand scheme of multimodal single-molecule assay to study plant cell wall synthesis *in vivo*. (Right) Currently established instrument that integrates holographic optical tweezers and wide-field fluorescence for multiple lasers. Courtesy Sang-Hyuk Lee.

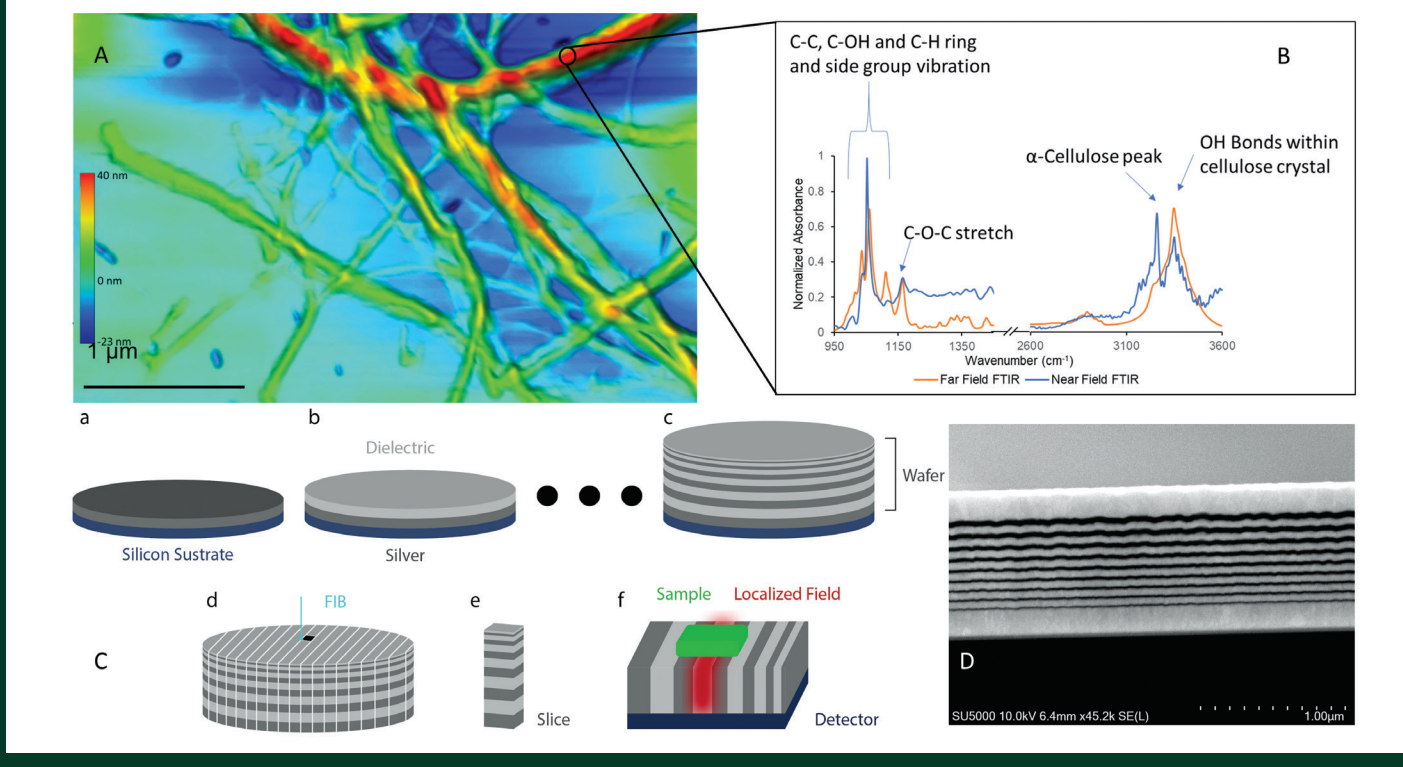
Development of Broadband Infrared Nano-Spectroscopy of Biological Materials in Fluid

Principal Investigators: Tina Jeoh[1] (PI) and Hoi-Ying Holman[2] (Co-PI)
Organizations: [1]University of California, Davis, and [2]Lawrence Berkeley National Laboratory
Email: tjeoh@ucdavis.edu

Research Plans: This research aims to develop a label-free, nanometer scale and time-resolved imaging technique to study surface reactions in aqueous biological reactions. State-of-art nanoscale imaging [i.e. scattering–scanning near-field infrared microscopy (s-SNIM)] can map topography and chemical composition at nanometer spatial resolution but is limited to dry samples. We are overcoming limitations of near-field energy delivery and extensive background scattering in aqueous samples by developing plasmonic infrared nanofocusing probes and a peptoid-matrix digital microfluidics device. This technique will be applied for nanoscale mapping of cellulose surface fibrils undergoing cellulase hydrolysis towards solving the mechanisms of cellulose hydrolysis.

Accomplishments and Deliverables: (1) Towards designing infared radiation (IR) wave guiding plasmonic gratings, we have demonstrated a novel layer-by-layer, high-yield fabrication approach for the production of plasmonic gratings. Using radio frequency (RF) magnetron sputter deposition, alternating thin film layers of silver and an appropriate dielectric are deposited on a silicon substrate. The resulting wafer is sliced using focused ion-beam (FIB) milling to appropriate dimensions. Further development is ongoing to fabricate gratings on the side of an atomic force microscopy (AFM) tip. (2) The development of the peptoid-matrix microfluidic device in now underway with the onboarding of a new postdoctoral researcher. (3) Bulk measurements relating hydrolysis rates to the availability of productive cellulase (Cel7A) binding sites on cellulose have been completed for celluloses of varying source and processing histories. s-SNIM measurements of these samples are ongoing.

Potential Benefits and Applications: Due to the large size of the grating wafer (~15 cm diameter) and small size of the gratings (~10 µm x 10 µm), millions of devices can be obtained from a single wafer. Furthermore, the ultrasmooth deposition ensures highly consistent layer thickness profiles across the devices. Fabrication techniques being developed as part of this project will enable low-cost mass production of plasmonic gratings. Preliminary s-SNIM data of cellulose fibrils show good agreement between near- and far-field FTIR spectra; cellulose crystalline morphology (e.g., cellulose Iα peaks) can be resolved even at the nanoscale.



Nanoscale mapping of surface topography and chemistry of cellulosic substrates to study the spatial distribution of productive binding sites where enzymes can bind and hydrolyze cellulose. (a) s-SNIM height image of 30% hydrolyzed bacterial cellulose. Circle indicates region where FTIR spectra are shown. (b) Near- and far-field FTIR spectra of cellulose in the circular region in (a). Fabrica tion of plasmonic nanofocusing probes for use in suppressing scattering and localizing IR light at probe tip. (c) Fabrication process of gratings. (d) Cross-section SEM image of a wafer. Courtesy Jennifer Nill, Arthur Montazeri, Hoi-Ying Holman and Tina Jeoh.

Understanding Plant Signaling via Innovations in Probe Delivery and Imaging

Principal Investigator: Jean T. Greenberg
Organization: University of Chicago
Email: jgreenbe@uchicago.edu

Research Plans and Progress, Including Objectives and Goals: We are (1) optimizing nanospikes to deliver nonpermeable signaling probes/biomolecules to plant cells and (2) building a robotic fiber optic microscope and image analysis platform that enables iterative, nondestructive measurements to be made and compared. These tools are being developed in conjunction with research aimed at understanding receptor-mediated peptide trafficking and responses relevant to plant cell growth and longevity. Goals for the project period are (a) to design and build the microscope in a manual mode; (b) to design and begin testing different nanospike designs for delivering probes to plants, including the feasibility of delivering DNA constructs for expression studies; (c) to develop fluorescent peptide probes (active and inactive versions of a secreted receptor ligand called phytosulfokine, or PSK) and perform initial mobility tests after application to plants using microscopy; (d) to construct transgenic plants in the appropriate genetic backgrounds to facilitate PSK trafficking and response mechanisms (includes making new recombinant DNA constructs); and (e) to determine the transcriptional changes due to PSK-induced signaling related to growth versus longevity.

Current and/or Anticipated Accomplishments and Deliverables:

Microsope: We designed the first version of the fiber optic microscope (manual mode) and expect to build and begin testing it within this project period.

Nanospikes: We have used one type of nanochip design (previously published by members of our team) to successfully deliver a nonpermeable fluorescent probe to *Arabidopsis* (one of the models we are using). We made a successful application to use the Oak Ridge National Laboratory nanophase material science facility and will fabricate and test an optimized carbon chip. We also designed a new type of chip that deploys silicon nanowires to determine if there are improved delivery properties. We expect to have a first feasibility study to know what types of biomolecules are amenable for delivery into plants using these materials.

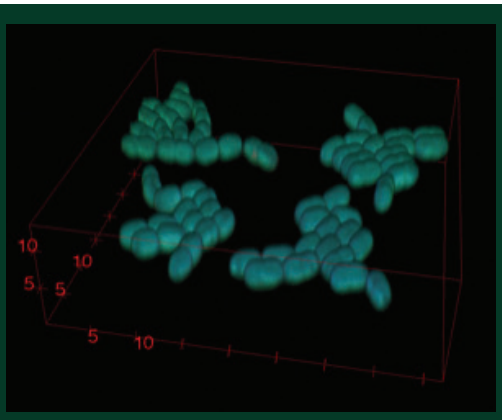
Biological materials/deliverables: We designed the active and inactive fluorescent versions of PSK and expect to have the first data on the potential internalization into cells of these in different plant genotypes this year. We performed needed crosses of receptor-GFP plants into different backgrounds (mutants lacking receptors or the ability to produce active PSK) to construct appropriate plant genotypes for trafficking studies. We expect to treat the relevant plant genotypes with PSK for the transcriptional study. We designed and are building several recombinant DNA constructs for whole plant mapping of PSK sites of action that we expect will be ready to transform into plants within this project period.

Potential Benefits and Applications: (1) A major advance will be iterative, nondestructive imaging of peptide signaling responses in plants that are highly relevant to improving traits for energy applications. This includes documenting changes in growth parameters and cell longevity and the accompanying signaling events, imaging of probes within plants and the ability to track their movement nondestructively. (2) Nanospikes for introducing nonpermeable probes and biomolecules into plant cells will permit researchers to accelerate the discovery of plant signaling response components in many plant species in response to many stimuli/environmental conditions. These spikes serve the dual goal of providing fiducial markers for the iterative imaging developed.

Spatiotemporal Dynamics of Photosynthetic Metabolism in Single Cells at Subcellular Resolution

Principal Investigators: Jeffrey Cameron (PI) and Ivan Smalyukh (Co-PI)
Organization: University of Colorado Boulder
Emails: jeffrey.c.cameron@colorado.edu; ivan.smalyukh@colorado.edu

Research Plans and Progress: The overall objective of the proposal is to design and build a multimode computational/optical nanoscopy system to generate adaptive 3D images with high-resolution, real-time, dynamic label-free chemical imaging of metabolic processes in photosynthetic organisms. The proposed system will overcome limits in resolution in addition to providing quantitative phase, amplitude, spectral, polarization information such that each biological feature will be associated with quantitative information in 3D space. Coherent anti-Stokes Raman scattering spectroscopy subsystem will be used for chemically specific 3D imaging of various endogenous chemicals and storage granules without the need for dyes or sensors. Optical tweezers will enable direct manipulation of cells and subcellular processes during imaging and also will be used to reduce sample motion for improved resolution. Various strains of cyanobacteria will be utilized for system benchmarking and calibrating of resolution and sensitivity. We will then apply the system to generate a dynamic spatiotemporal map of photosynthetic metabolism, with a focus on tracking carbon from CO_2 fixation to storage in the form of glycogen.



3D reconstructions of intact 16-cell micro colonies using laser-scanning confocal microscopy. The spatial location pigment-pro tein complexes containing chlorophyll (blue) and phycobilisome antennae (red) are shown. Courtesy Colin Gates and Jeffrey Cameron.

Current and Anticipated Accomplishments and Deliverables: We are currently in the design/build phase of the project and are testing individual imaging modalities to determine the best way to integrate them into a single multimodal imaging platform. We have developed a robust sample preparation pipeline that enables the reproducible growth of single–cell derived microcolonies followed by spectrally resolved 3D confocal microscopy to map the spatial and temporal dynamics of pigment-protein complexes at subcellular resolution *in situ*.

Potential Benefits and Applications: The multifunctional nanoscope developed in this proposal will be the first integration of these capabilities in a single setup and will enable an entirely new class of experiments that take advantage of high-resolution and optical nanomanipulation while studying DOE-relevant biological systems. This study will provide mechanistic insights on the subcellular location and regulation of photosynthetic pathways and identify potential opportunities to engineer and improve these pathways for the production of food, fuel, and other high-value chemicals that will benefit society and the environment.

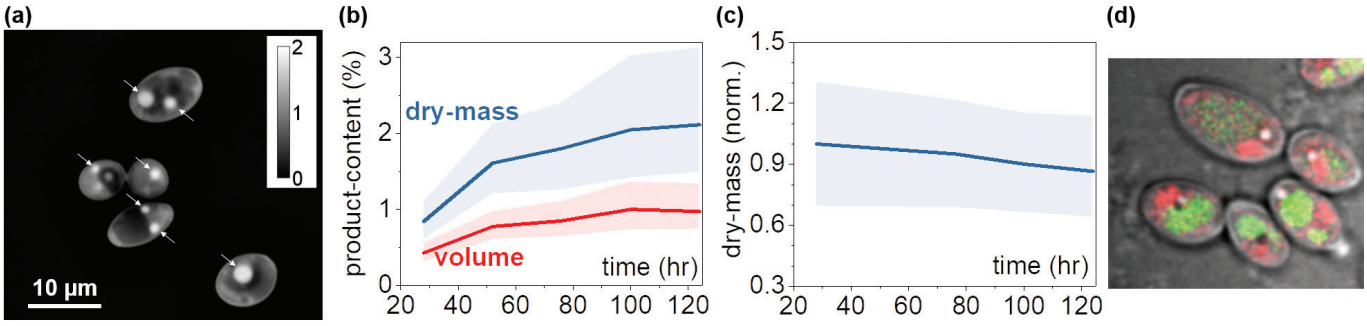
Correlative Imaging of Enzyme and Metabolome Dynamics for Yield and Titer Co-Optimization in Biofuel-Producing Microorganisms

Principal Investigators: Andreas E. Vasdekis[1] (PI); Co-PIs: Scott E. Baker,[2] Armando McDonald,[1] and Lucas Sheneman[1]
Organizations: [1]University of Idaho and [2]Pacific Northwest National Laboratory
Email: andreasv@uidaho.edu

Research Plans: Efficient biofuel production comes with a major challenge, namely to predict and execute metabolic engineering strategies that optimize productivity without disrupting cellular growth. The goal of this project is to develop an optical imager to quantify growth-productivity trade-offs and their dependence on metabolic compartmentalization and systems-level, cellular noise. To meet this goal, the project sets out to fuse and evaluate three distinct imaging modalities into a single device: (1) Quantitative-phase imaging for high-throughput metabolic trade-off phenotyping; (2) Light-sheet (LS) fluorescence imaging of metabolites and enzymes via gene-encoded biomarkers; and (3) LS Raman imaging of nutrient fate.

Current and Anticipated Accomplishments: We have applied quantitative-phase imaging to determine the growth-production metabolic trade-offs in the oleaginous yeast *Yarrowia lipolytica* (Figure a). With the aid of nanoscale secondary ion mass spectrometry, we converted the optical phase delay through the cell cytosol to the dry-mass of individual cytosolic compartments, demonstrating more than 55% higher precision in metabolic trade-off phenotyping than conventional, volumetric microscopy, such as fluorescence (Figure b). Further, our quantitative-phase imaging strategy determined for the first time the autophagy-based protein and fatty-acid recycling with subcellular resolution in a label-free fashion (Figure c). This led us to the discovery that systems-level cellular noise impacts not only growth and productivity, but also autophagy-based nutrient recycling under starvation. Importantly, these autophagy-related findings were impossible to collect with conventional, volumetric bioimaging methods. Our immediate next steps in the project are to: (1) fuse quantitative-phase imaging with lattice LS fluorescent imaging; (2) complete the expansion of our gene-encoded biomarker palette to include double-tagged strains for tracking two enzymes simultaneously (Figure d); (3) assess the signal strength and metabolic compatibility of both carbon and nitrogen-based Raman tags; and (4) evaluate different supervised machine learning methods for rapid and label-free organelle recognition from quantitative-phase images.

Benefits and Applications: This project reduces phototoxicity and maximizes temporal resolution in linking molecular information with growth and productivity performance at the single-cell level. Further, high-throughput and subcellular resolution unmask the impacts of system-level cellular noise and the cytosolic compartmentalization of metabolic reactions. As such, the project will improve our fundamental understanding and enable important engineering insight in optimizing biofuel production. The integration of quantitative-phase with LS imaging will be executed in conventional microscope frames, thus making our technology accessible to the broader scientific community, including nonspecialists.



(a) Quantitative-phase image of *Y. lipolytica* in radians; white arrows denote the triacylglyceride (TAG) packed lipid droplets. (b) Precision improvement in single cell TAG content by quantitative-mass versus volumetric imaging; line and shaded area denote the median and the interquartile range (IQR) of ~2,000 single cell observations per timepoint, respectively. (c) Evolution of single cell dry-mass with the decreasing line (median) and shaded area (IQR) indicating the onset of autophagy-based catabolism of cytosolic macromolecules. (d) Dual-tagged *Y. lipolytica* co expressing carboxypeptidase (green) and arginase (red). Panels (a), (b), and (c) courtesy Andreas E. Vasdekis, University of Idaho. Panel (d) courtesy Erin L. Bredeweg, Environmental Molecular Sciences Laboratory at Pacific Northwest National Laboratory.

Hyperspectral Light Sheet Raman Imaging of Leaf Metabolism

Principal Investigators: Keith Lidke, David Hanson, Jerilyn Ann Timlin, and Jamey Young
Organization: University of New Mexico (UNM)
Email: klidke@unm.edu

Research Plans and Progress:

Hire a 1.0 FTE postdoc for the project in biology (Hanson): Dr. Roxana Khoshravesh joined the UNM team. Her past work includes using light and electron microscopy for studying photorespiration.

Identify plants for the study: We have focused on bioenergy crops, model species, and close relatives with expected leaf thickness within the range of the microscope's scanned distance (200-300 µm). We are acquiring seeds for all species (sources have been identified).

Test detection of labeled metabolites in live tissues to determine signal strength using a separate Raman detector: We have ordered key substrates and metabolites including $^{13}CO_2$, $^{18}O_2$, $H^{13}CO_3^-$, glycine (1-13C), L-serine (1-^{13}C), L-aspartic acid (3-^{13}C), and glycine (^{15}N). These substrates and metabolites, native and ^{13}C, will be tested with a Raman spectrometer at Sandia National Laboratories (Timlin).

Instrument design and construction: We are currently identifying and accumulating the critical hardware components needed for the microscope. We have selected and ordered the excitation source. Detection sensitively is critical for our project. We have researched and compared the best available sCMOS scientific cameras in the market and will purchase the Hamamatsu Orca Fusion sCMOS. We have performed theoretical calculations for the spectrometer design for optimum spectral resolution without compromising on the image quality. The parameters for the design of the spectrometer will be finalized taking input from the Raman frequency shift characteristics of the leaf and metabolite samples to be studied as part of the above goal.

Current and/or Anticipated Accomplishments and Deliverables: By the end of the first project period, we anticipate that the design and construction of the hyperspectral light sheet Raman microscope will be completed, the imaging performance characterized, and the system ready to proceed with plant leaf imaging. We will also have the full range of selected plants available with a clear expectation of 13C Raman spectral shifts of the key metabolites and substrates.

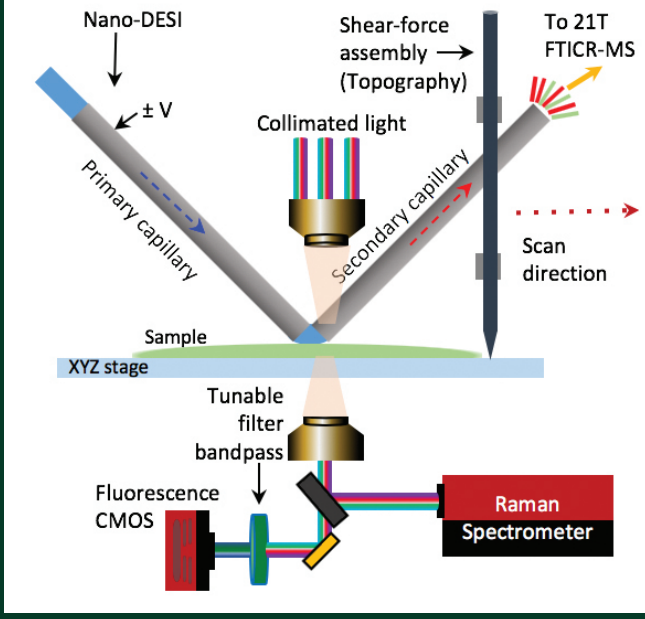
Potential Benefits and Applications: Major efforts are underway to improve energy and food crop productivity through engineering photosynthesis. These include replicating C4 and related pathways in crops and other ways to reduce photorespiration. Our imaging system and the group of species to be examined will demonstrate the power of our technology for assessing and understanding the effectiveness of these attempts to reengineer photosynthesis.

Development and Implementation of an *In situ* High-Resolution Isotopic Microscope for Measuring Metabolic Interactions in Soil Mesocosms

Principal Investigators: Elizabeth A. Shank[1] (PI); Co-PIs: Christopher R. Anderton,[2] Venkateshkumar Prabhakaran,[3] David Berry,[4] and Carol Arnosti[1]
Organizations: [1]University of North Carolina at Chapel Hill, [2]Environmental Molecular Sciences Laboratory (EMSL), [3]Pacific Northwest National Laboratory, and [4]University of Vienna
Emails: eshank@unc.edu, christopher.anderton@pnnl.gov, venky@pnnl.gov, berry@microbialecology.net, and arnosti@email.unc.edu

Research Plans and Progress: Many of our planet's ecosystems rely on the activities of soil microbes. However, our ability to directly observe the enzymatic and metabolic activities of microbes within soil currently is critically limited, both by the complexity of soil microbial communities and the lack of experimental tools to study them and their molecular interactions *in situ*. Our goal is to create an integrated platform that combines multiple disparate imaging modalities [fluorescence microscopy, Raman microspectroscopy, and nanodesorption electrospray ionization (nano-DESI) mass spectrometry (MS); see figure] to overcome these challenges. Using this instrument, we can exploit the use of both fluorescent- and stable-isotope labels to directly investigate microbial activities and molecular transformations occurring in soil and soil-like model environments. Specifically, our integrated instrument will use: (a) fluorescence detection to map microbial gene activity and uptake of polysaccharides by bacteria, (b) Raman detection to visualize incorporation of these carbon species into microbial cells, and (c) ultrahigh-resolution nano-DESI mass spectrometry imaging to detect liberated carbon and other metabolites. The integration of these imaging capabilities will thus enable us to visualize how individual members of microbial communities interact with one another and with the organic carbon species that they degrade in soil. We will use this multimodal imaging platform to visualize, *in situ*, the interactions between bacteria, fungal biomass, and soil carbon in a series of mesocosms of increasing complexity to address fundamental questions in soil ecology about the microbial decomposition of complex polysaccharides. Our aims are: (1) develop a tractable test system for instrument validation; (2) construct a high-resolution isotopic microscope that integrates Raman, nano-DESI-MS, and fluorescence imaging for *in situ* measurements of carbon transformation; and (3) interrogate polysaccharide decomposition in model and native soils.

Current and Anticipated Accomplishments: Construction of this high-resolution multimodal instrument is underway. To begin, we have developed a simplified agar-based model to spatially interrogate the degradation of xylan by the model bacterium *Bacillus subtilis*. Using isotopically unlabeled and labeled xylan and wild-type and xylanase-minus mutant strains of *B. subtilis*, we will benchmark our instrument against data collected from established dual-modality (fluorescence-MS and fluorescence-Raman) microscopes.



Design of high-resolution isotopic microscope integrating fluorescence, Raman, and nano-DESI modalities to measure microbial metabolic activities and their resulting molecular transformations in soil microenvironments. Courtesy Chris Anderton and Venkateshkumar Prabhakaran.

Potential Benefits and Applications: The capacities provided by this potentially transformational technology will enhance our understanding of the microbial and metabolic interactions occurring within soil communities that are relevant to carbon degradation. We will develop this instrument at EMSL, where it will then be available to the entire EMSL User Base, enabling a variety of related DOE-relevant systems to be interrogated in the future.

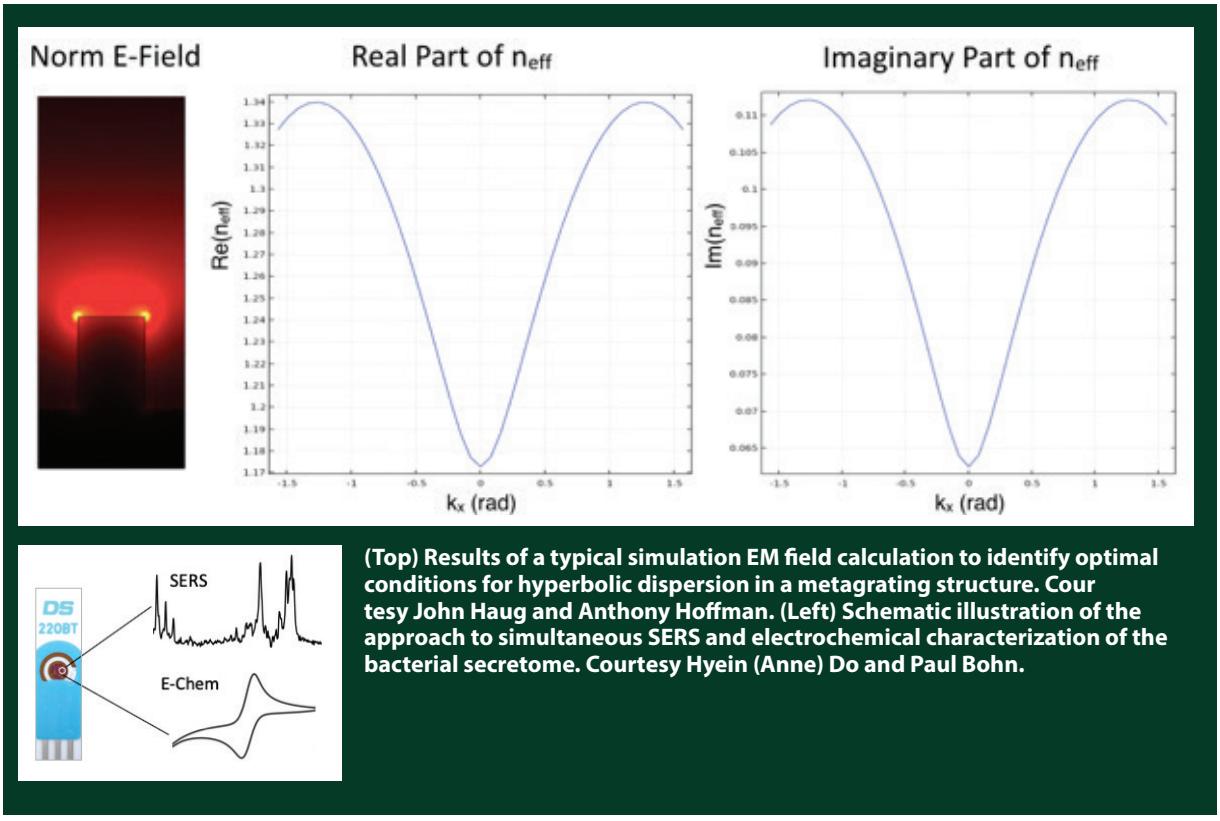
Metaoptics Enabled Multifunctional Imaging

Principal Investigator: Paul Bohn
Organization: University of Notre Dame
Email: pbohn@nd.edu

Research Objectives: This project is developing enhanced imaging tools by pursuing two overarching technical goals: (1) the development of new metaoptics-enabled approaches to imaging and spectroscopic characterization, and (2) the development of tools to control the chemical environment of a microbial sample with nanometer-scale precision.

Current and/or Anticipated Accomplishments and Deliverables: Work in this initial project period has focused on two principal objectives: (1) identifying the conditions under which hyperbolic dispersion can be obtained in gold (Au) metasurfaces, and (2) developing spectroelectrochemical tools and applying them to characteristic members of the bacterial secretome. (1) The unique dispersion of hyperbolic metamaterials supports high spatial frequency waves that do not propagate in conventional optical material. The propagation of these so-called high-*k* waves is the basis for super-resolution imaging using hyperbolic metamaterials. The high spatial frequency interference patterns formed from confined high-*k* hyperbolic plasmon polaritons on patterned 2D metasurfaces will enable imaging at the tens-of-nanometers scale required to resolve intracellular events. In pursuit of this objective, we have carried out numerous modeling calculations targeting the behavior of Au nanoridge structures (see top figure). (2) Pyocyanin (PYO) is a well-known virulence factor, which is both redox active and Raman active, so we have sought to simultaneously acquire both spectroscopic and redox state information regarding its behavior (see bottom figure). These data can provide insights into the molecular redox behavior of PYO while controlling its surface-enhanced Raman spectroscopy (SERS) and electrochemical (EC) response with external stimuli, such as pH and applied potential.

Potential Benefits and Applications: The imaging strategies being developed in this project combine metaoptical architectures with active *in situ* nanoscale control of the chemical environment and apply them to a microbial system, *Myxococcus xanthus*, with particular relevance to the DOE BER mission. The combination of metaoptical architectures and nanoscale control over the molecular environment enables: (a) precise control over the spatiotemporal distribution of the electromagnetic (EM) field at subwavelength scales; (b) control of the interaction of the EM field with critical molecular systems in DOE-relevant microbes; (c) control over the chemical environment—especially the presence and quantity of ROS that can affect redox homeostasis; and (d) the ability to ask new kinds of questions not accessible to "omics" approaches or standard methods of biological imaging. These capabilities are applicable to detailed studies of metabolic pathways in microbes and to lignocellulosic biomass deconstruction.



(Top) Results of a typical simulation EM field calculation to identify optimal conditions for hyperbolic dispersion in a metagrating structure. Courtesy John Haug and Anthony Hoffman. (Left) Schematic illustration of the approach to simultaneous SERS and electrochemical characterization of the bacterial secretome. Courtesy Hyein (Anne) Do and Paul Bohn.

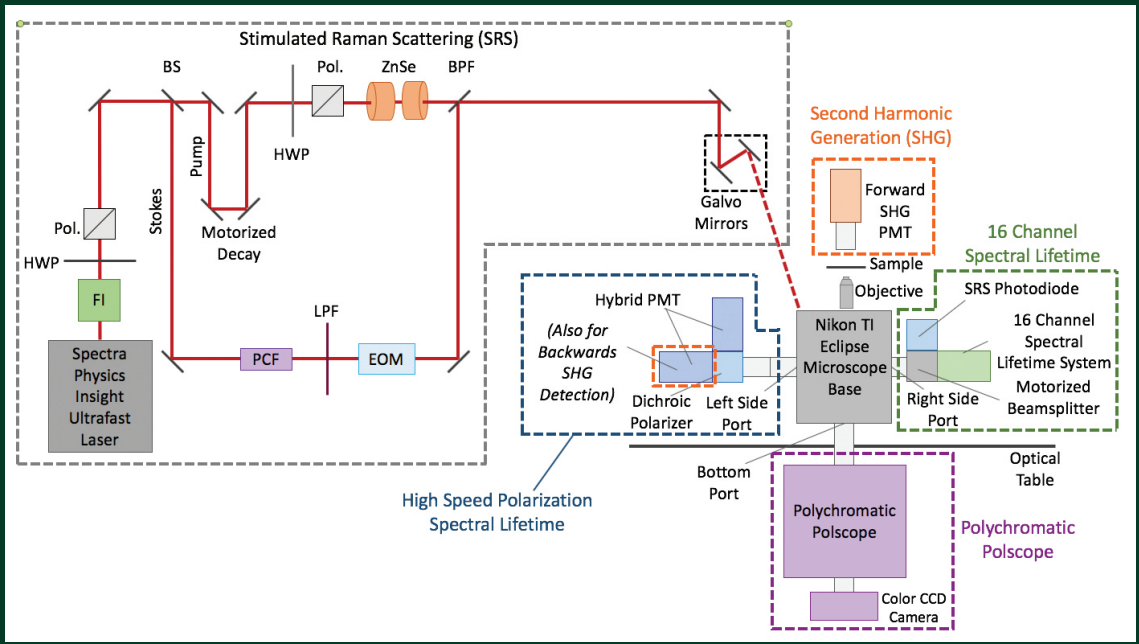
Multiparametric Optical Label-Free Imaging to Analyze Plant Cell Wall Assembly and Metabolism

Principal Investigators: Marisa S. Otegui and Kevin W. Eliceiri
Organization: University of Wisconsin–Madison
Email: otegui@wisc.edu

Research Plans and Progress: Plant tissues often are considered not ideal for fluorescence imaging because of the pervasive intrinsic fluorescence of many plant metabolites and the intricate interactions with light of the many semicrystalline polymers at the cell wall. This proposal aims to take advantage of this observed shortcoming to develop a label-free, optical microscopy platform for characterizing multiple fingerprints of important cell wall components and stress-related conditions, at subcellular scale resolution. The new device will collect fingerprints from both emitted and scattered light that can inform on the chemical nature, subcellular distribution, anisotropy, and molecular environment of multiple cell wall components in intact plant tissues. We will combine these imaging capabilities with computational tools that enable correlated registration, integration, and analysis. This fully integrated, multiparametric optical system would be the first of its kind. We will use it to address biological problems connected to cell wall assembly in grasses. Most specifically, we will focus on developmental and environmental variation of cell wall impregnation with silica, lignin, suberin, and cutin in different tissues and cell types. The research plan comprises three main goals: (1) To develop an accessible imaging platform and associated open-source software able to extract and integrate fingerprints from fluorescence-associated (multispectral emission, lifetime, and polarization), wide-field polarimetry, second harmonic generation (SHG), and stimulated Raman scattering signals (SRS). (2) To determine unique combination of fingerprints for various cell wall components and selected metabolites. (3) To analyze the process of cell wall silicification in grasses and determine how silicification affects cell wall properties and lignin, cutin, and suberin deposition in other cell types under differing stress conditions.

Current Accomplishments and Deliverables: (1) Development of a fully functional open-source multiphoton scanning system; (2) implementation of a fast two-channel time domain based FLIM system with polarization control; (3) development of a novel fiber-based spectral detector; and (4) development of a novel scheme to detect forward and backwards second harmonic generation.

Potential Benefits and Applications: Understanding the assembly and deconstruction of cell walls in grasses is very important for bioenergy-related purposes. Grass cell walls have many chemical singularities, including high content of silica. The extent of cell wall silicification is inversely correlated to lignin accumulation. However, how the two processes are coordinated is not known. As an example of what the new device will be able to accomplish, we will analyze patterns of cell wall silicification in maize and sorghum and determine how silicification affects cell wall properties and lignin and suberin deposition in other cell types under differing stress conditions.



Optical layout diagram depicting SRS, SHG, high-speed polarization, 16-channel spectral lifetime, and poly chromatic Polscope components. Key: FI, Faraday isolator; HWP, achromatic half wave plates; Pol., Glan-laser polarizers; BS, plate beam splitter; PCF, photonic crystal fiber; EOM, electro optical modulator. Courtesy Marisa S. Otegui and Kevin W. Eliceiri.

Detecting Chemical Signals in the Soil with 4DMAPS, an Integrated Aptasensor Assembly

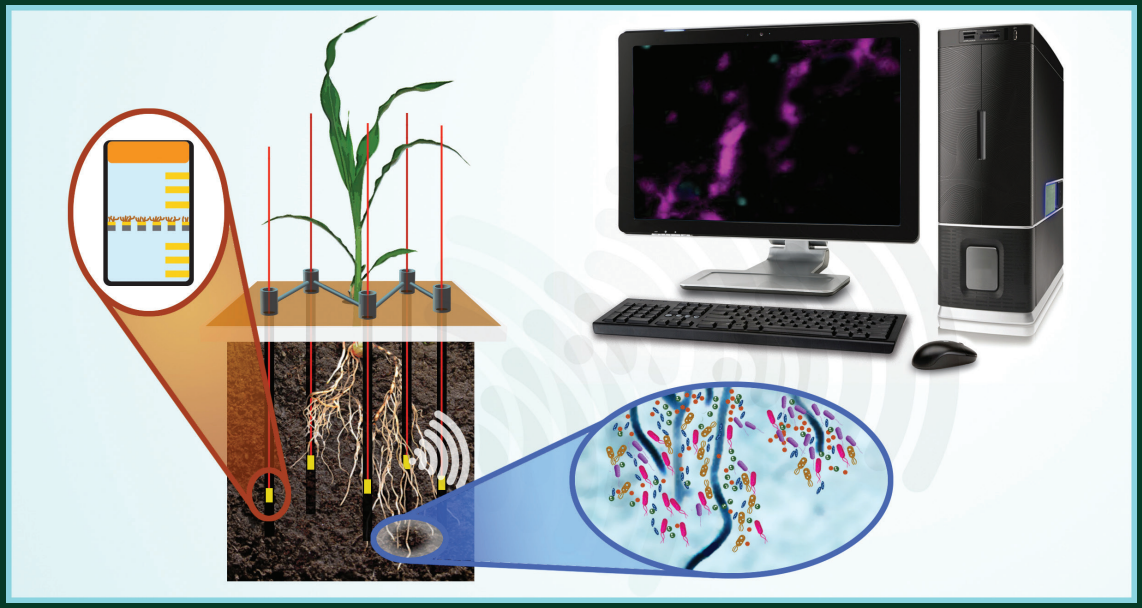
Principal Investigators: Marit Nilsen-Hamilton (PI); Co-PIs: Ludovico Cademartiri, Larry Halverson , George Kraus Pranav Shrotriya, and Olga Zabotina
Organizations: Ames Laboratory and Iowa State University
Emails: marit@iastate.edu, lcademar@iastate.edu, larryh@iastate.edu, gakraus@iastate.edu, shrotriy@iastate.edu, and zabotina@iastate.edu

Overview: We are developing an instrument, 4DMAPS (4D analysis of Molecules by APtamers in Soil), which will produce 3D images of biochemicals excreted by plants and microbes throughout the rhizosphere over time for the 4th dimension.

Research Goals and Progress: The 4DMAPS instrument will integrate input from a large number of electrochemical sensors that are distributed throughout the soil and vertically mobile. The molecular specificity of the sensors will be provided by aptamers, which will be selected to recognize specific molecules that are known to be secreted by plants or microbes in the rhizosphere. For these "proof of concept" studies, the analytes will include NH_3, chitinase, and autoinducer 2. The experiments will be performed in the laboratory with plants growing in a transparent soil mimic.

Current and/or Anticipated Accomplishments and Deliverables: Designs for prototypes of the portable sensing elements are underway that will include electrochemical cells and Arduino®-based impedance analyzers. Code is being drafted to integrate the sensor signals and to automate the imaging. A synthetic microbiome community in a "plug-and-play" format is under development to use in the synthetic soil system for monitoring microbial metabolic activities and signal production in the rhizosphere. For selecting the needed aptamers, target analytes are being synthesized chemically (autoinducer 2 and an adenyl-carboxy-pyrrole for trapping NH_3) or being cloned for expression of chitinase and gluconase. Protocols for aptamer selection and screening are underway.

Potential Benefits and Applications: The goal of this project is to develop an instrument that can image in real time the chemicals in the rhizosphere that govern the plant's nutrition and health and the metabolic activities and interspecies communication potential of microbes colonizing roots. Although this early period of development will create an instrument in a laboratory setting, the long-term goal is to create an instrument with the flexibility of being fitted with a variety of sensors that can be used in the field in experimental and in agricultural applications to monitor nutrient transformations and rhizosphere microbial metabolic activities that contribute to growth of healthy, productive plants.



4DMAPS aptasensors are attached to moveable rods in shafts around a plant root. The sensors report through wireless on the concentration of a specific molecular component to a receiver. The collected data are integrated to show where around the plant root a particular chemical compound is located and how this changes with time. Courtesy Ames Laboratory.

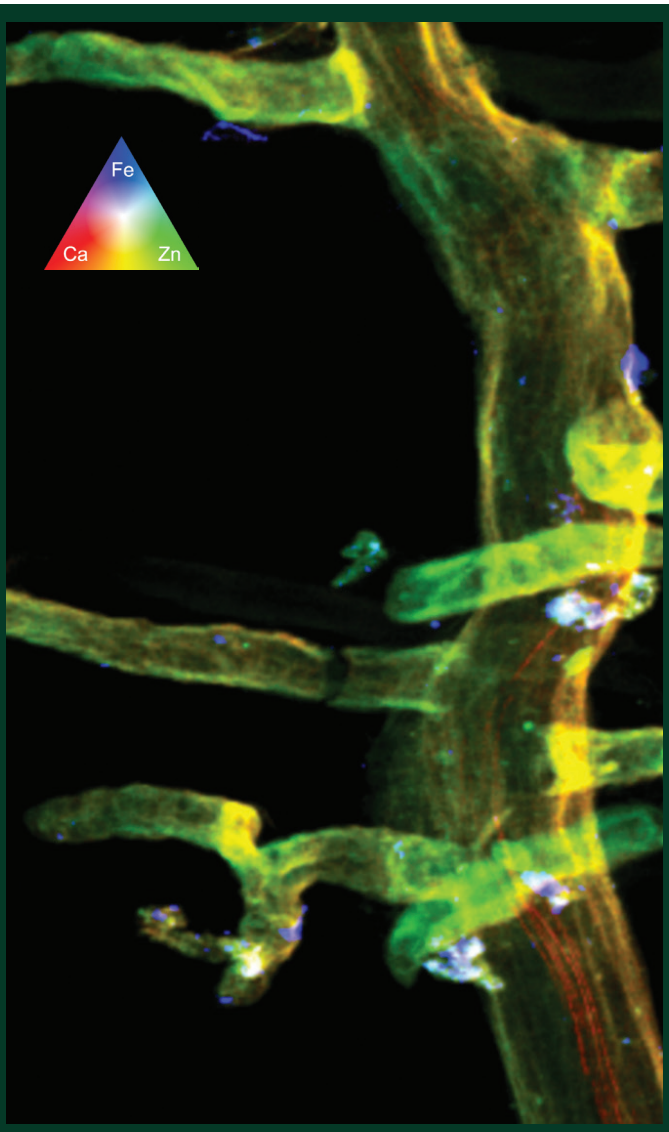
FFFI: Development of a Full-Field X-Ray Fluorescence Imaging System for Near Real-Time Trace Element Microanalysis of Complex Biological Systems

Principal Investigator: Ryan Tappero[1] (PI); Co-PIs: David (Peter) Siddons,[1] Shaorui Li,[1] Mourad Idir,[1] Jenny Bhatnagar,[2] Sunny Liao,[3] and Rytas Vilgalys[4]
Organizations: [1]Brookhaven National Laboratory, [2]Boston University, [3]University of Florida, and [4]Duke University
Email: rtappero@bnl.gov

Research Plans and Progress: To develop a full-field X-ray fluorescence imaging system (FFFI) for studying spatial and temporal dynamics of trace elements in complex biological systems. Conventional X-ray fluorescence (XRF) imaging is performed in a scanning probe modality. A limitation of scanning XRF is the time required to record images one pixel at a time. Here we propose a full field scheme for XRF imaging enabled by development of a new imaging detector capable of recording both a photon "hit" and its energy (i.e., spectrometer). Such a detector system will transform XRF imaging by enabling studies of dynamics, transport processes and studies requiring high-throughput for adequate statistics or replication (e.g., mutant screening to link genotype and phenotype). A scientific driver of understanding detailed interactions among synergistically functioning organisms, particularly fungi and roots, provides a focus for this Bioimaging technology development.

Current and/or Anticipated Accomplishments and Deliverables: Progress to date includes: (1) Kick-off meeting on January 22, 2019; (2) benchmark measurement of *Pinus-Sullius* model system; (3) purchase of a plasma oven for construction of laboratory-scale ecosystems from polydimethylsiloxane (PDMS); (4) recruitment of postdoc to assist with development of the optical imaging system and research associate to assist with conceptual design, testing, and evaluation of detector ASIC; (5) subcontracts to university co-PIs for experimental work and the development of rhizosphere microcosms.

Potential Benefits and Applications: A first-generation working prototype of FFFI detector system for *in situ* and near–real time monitoring of nutrients and trace elements in complex, heterogeneous materials such as soil and sediments. Subminute temporal resolution is expected for detection of the first row transition elements (e.g., Fe, Cu, Zn). Dynamics and transport processes on this time scale can be studied. Another expected outcome is a blueprint for future development of a second generation, sub-10 micron resolution version of the FFFI detector system that can be broadly deployed with benchtop X-ray sources.



Roots from *Pinus Sullius* model system in marginal soil imaged for trace elements—iron (Fe), zinc (Zn), and calcium (Ca)—using conventional scanning-probe XRF (ca. 4 hr) as benchmark measurement for signal intensity. Image from the XFM Beamline of the National Synchrotron Light Source II, a U.S. Department of Energy (DOE) Office of Science User Facility operated for the DOE Office of Science by Brookhaven National Laboratory under Contract No. DE-SC0012704.

Illuminating the Rhizosphere: Developing an Adaptive Optics, Multiphoton Microscope for 3D Label-Free Live Imaging of Microbes and Organic Matter in Soil and Roots

Principal Investigators: Peter K. Weber (PI); Co-PIs: Diana C. Chen, Sonny Ly, Ted A. Laurence, Lisa A. Poyneer, S. Mark Ammons, Erin E. Nuccio, and Jennifer Pett-Ridge
Organization: Lawrence Livermore National Laboratory (LLNL)
Email: weber21@llnl.gov

Goals and Objectives: The purpose of this research is to advance the ability to visualize plant-microbe-mineral interactions in the rhizosphere to study carbon cycling, sustainable food and fuel production, and environmental processes, including contaminant transport. We will use adaptive optics (AO) and label-free multiphoton microscopy and spectroscopy to develop a new microscope that overcomes the challenges of optical imaging in mineral and soil matrices. Our objectives are: (1) develop a label-free method of visualizing microbial cells and organic matter in mineral and soil matrices; (2) develop adaptive optics for optical imaging in mineral and soil matrices; (3) design and build an integrated AO-multiphoton microscope capable of working with plant-scale rhizospheres; and (4) apply our AO-multiphoton microscope to rhizosphere and soil pilot studies.

Anticipated Deliverables: In this first year, we will (1) perform proof-of-concept experiments to determine optimal label-free methods in mineral and soil matrices; (2) finalize our multiphoton microscope design; (3) purchase the major components for our multiphoton microscope and initiate construction; (4) obtain data on mineral matrix and soil wavefront characteristics for our adaptive optics modeling; (5) conduct AO modeling to define the relationship between matrix characteristics and AO corrections; and (6) determine the optimal AO design for our multiphoton microscope and initiate purchasing of AO components.

Potential Benefits Beyond DOE: Adaptive optics, multiphoton microscopy, and label-free imaging are active areas of biological imaging research. In recent years, these approaches have been brought together for numerous applications, including neuroscience, ophthalmology, and developmental biology. Our research will focus on extending these approaches to highly scattering materials. Therefore, our research has the potential to improve methods for imaging in and through skin, bone, and other highly scattering biological materials. We anticipate that the major contributions of this project to the general state-of-the-art of biological imaging in complex matrices will be in the areas of modeling and integration of adaptive optics with multiphoton microscopy.

In addition, agricultural, microbial, and environmental researchers are interested in the specific application that we are targeting—rhizosphere and soil processes. A major part of our research will be determining the optimal modes of imaging in these systems. Therefore, our AO, multiphoton microscope development has the potential to benefit these fields of research by directly enabling new modes of investigation. In the area of biosecurity, we at LLNL are interested in the capability this technology could bring to research into soil reservoirs of pathogens.

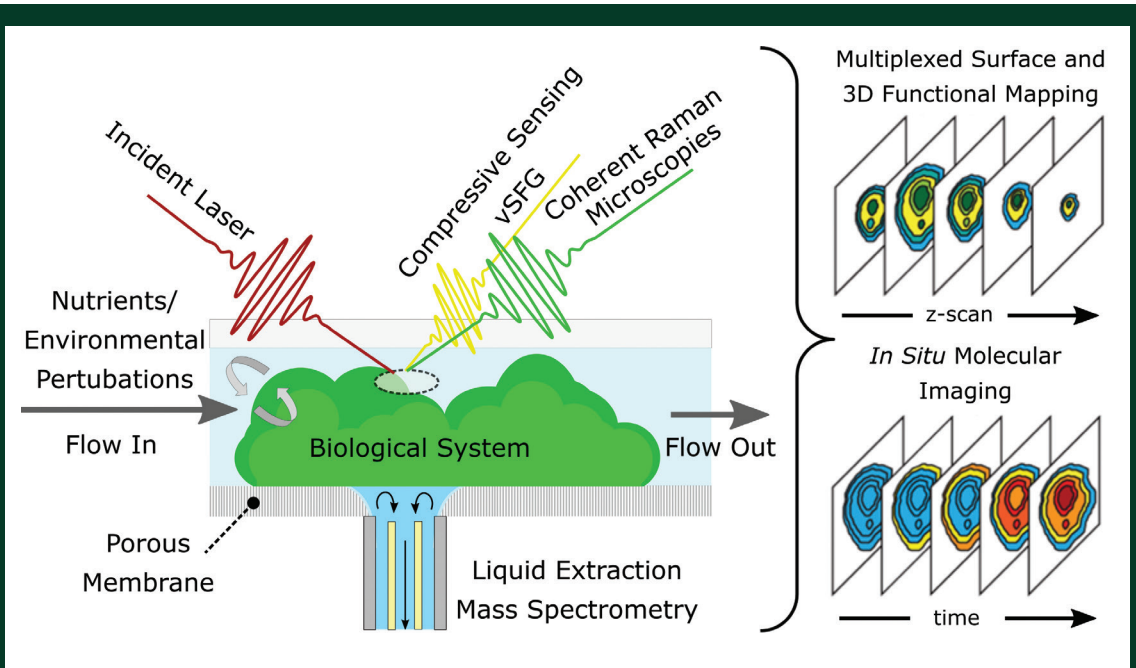
Intrinsically Co-Registered Chemical Imaging of Living Plant and Microbial Systems via 3D Nonlinear Optical Mapping and *In situ*-Liquid Extraction–Mass Spectrometry

Principal Investigator: John F. Cahill
Organization: Oak Ridge National Laboratory
Email: cahilljf@ornl.gov

Research Objectives: This project aims to develop a multimodal imaging platform integrating novel and existing analytical capabilities whose synergy yields unprecedented 3D spatiotemporal chemical information in the bulk and at the interface in biological systems *in situ*. Our approach uses a suite of novel and existing nonlinear optical spectroscopy and liquid extraction–mass spectrometry chemical imaging modalities, whose combination is used to overcome their inherent limitations, enabling the ability to broadly chemically characterize the spatiotemporal distribution of rhisospheric metabolites in 3D. We plan to achieve our goal through the combination of vibrational sum frequency generation (vSFG) microscopy, *in situ* liquid extraction–mass spectrometry (*in situ* LE–MS), and coherent anti-Stokes Raman scattering (CARS) imaging in 3D, to provide interface-selective molecular chemistry, bulk metabolomic information, and 3D chemical structure, respectively. Our objectives are to (1) develop new bioimaging modalities, vSFG microscopy and *in situ* LE–MS; (2) couple vSFG, CARS, and *in situ* LE–MS imaging modalities into a singular, co-registered, multimodal imaging system; and (3) measure the dynamic chemical environment of a living biofilm and through imaging of stress-induced rhizosphere dynamics occurring among plant roots, microbial colonies, and soil.

Accomplishments and Deliverables: We have constructed the liquid extraction 2D chemical imaging system onto a Thermo Q Exactive-HF-X mass spectrometer and have begun to validate the technique for *in situ* sampling of liquid through porous membrane containing flow cells. Several flow cell prototypes containing known physical and chemical features have been constructed for validation experiments. Design of the the integrated CARS and vSFG spectroscopy systems have been finalized, the major essential components have been procured, and development is underway.

Potential Benefits and Applications: This combined platform could enable spatiotemporal chemical mapping of living systems with unprecedented chemical coverage, from the bulk to the interface. The synergy of nonlinear optical spectroscopies, which can target both the bulk and the interface with high resolution, and liquid extraction–mass spectrometry, which can measure the chemistry of the system with high chemical specificity and coverage, would enable currently unobtainable insights into the chemical dynamics and localization in living systems. A benefit of the fusion of these data sources is to mitigate the limitations each imaging modality has alone. This approach would overcome the longstanding problem of relating chemical measurement of molecules in the bulk and at the interface throughout the entirety of a living biological system in space and in time. Applications of this technology are wide-ranging; but, for BER, this capability will enable *in situ* imaging of microbial communities, nutrient transport, and rhizosphere processes in 3D. This would enable answering questions such as how metabolite production throughout a biofilm impacts biofilm growth, or how environmental perturbations are reflected throughout the rhizosphere of plant-microbe systems in space and time.



Concept of an *in vivo* multimodal imaging system using 2D liquid extraction–mass spectrometry chemical and 3D spectroscopic imaging to create 3D spatiotemporal images of living biological systems. Courtesy John F. Cahill.

Multimodal Chemical Imaging Across Scales to Visualize Metabolic Pathways in Live Plants and Microbial Systems

Principal Investigators: Scott Lea[1] (PI), Co-PIs: Patrick El-Khoury,[1] Christer Jansson,[1] Victoria Orphan,[2] and Sam Hazen[3]
Organizations: [1]Pacific Northwest National Laboratory, [2]California Institute of Technology, and [3]University of Massachusetts-Amherst
Email: scott.lea@pnnl.gov

Research Plans: Through this project, we will develop a novel next-generation spectral imaging platform that may be used to image and identify biomolecules involved in both microbial and plant metabolic processes through different chemical contrasts and across multiple length and time scales. The spectroscopic methods of choice include a combination of Raman and fluorescence scattering, as well as reflected/transmitted light extinction spectroscopy in a single optical setup. Once developed, this technology will be initially tested using two model systems of relevance to BER's bioenergy and environmental microbiology research thrusts. Using the first system, we will explore the role of microbial communities in controlling carbon cycling. Through the second system, we strive to advance the existing knowledge of plant metabolic processes influencing cell wall composition, synthesis, function, and deconstruction for dedicated bioenergy biomass crop development. In both cases, we anticipate that our proposed first-of-its-kind BioImager will significantly advance the existing fundamental understanding of basic metabolic processes in live microbial and plant systems.

Anticipated Accomplishments: Our novel spectral imaging platform will provide significant advantages over the currently employed approaches to metabolic mapping, simultaneously achieving: (1) high spectral resolution on the order of 0.1 cm^{-1}, which is required for our Raman module; (2) fast acquisition speeds on the order of milliseconds to track, e.g., microbial motion and nutrient uptake in real time with different chemical contrasts; (3) high detection sensitivity to both facilitate high-speed acquisition and empower low–light level registration with adequate signal-to-noise ratios for an overall strictly noninvasive/nonperturbative optical imaging approach; and (4) ease of switching between nanoscopic and macroscopic probing volumes. These desirable properties will be realized through the development of novel spectroscopic grade hyperspectral/spectral imaging detectors coupled to a flexible custom-built optical platform that affords multimodal/multiscale spectral imaging in a single setup across the nano-micro-meso-macroscopic spatial domains.

Potential Benefits: We anticipate our platform will result in an unprecedented level of molecular level insight into metabolic pathways in microbial communities and plants. This imaging technology will be able to nondestructively image biomolecules in their native environments from the macroscale (cm) in plants down to nanoscale in microbes. Achieving this goal will provide technology critical to the understanding of the intra- and intercellular metabolic controls in plants and microbes relevant to carbon cycling, bioenergy production, and biogeochemical transformations and will allow researchers to accelerate engineering of plants and microbial communities for improved performance. By leveraging the capabilities of the BioImager, we would be able to build a knowledge base that puts us in position to deliver information for modeling biomass accumulation in plants under future climate scenarios and for genome-engineering approaches aimed at improving the yield and/or saccharification potential of lignocellulosic biomass.

Appendix: Quantum Enabled Sensors and Technologies (QUEST) Discussion Summary Report

February 28, 2019

Chair:
James Evans, Pacific Northwest National Laboratory

Co-chairs:
Jeffrey Cameron, University of Colorado Boulder
Haw Yang, Princeton University

Organizer:
Sujata R. Emani, U.S. Department of Energy
AAAS Science and Technology Policy Fellow

Quantum Enabled Sensors and Technologies (QUEST) Discussion Summary Report

A 3-hour roundtable discussion on Quantum Enabled Sensors and Technologies (QUEST) occurred at the end of the U.S. Department of Energy's (DOE) Office of Biological and Environmental Research's (BER) 2019 Bioimaging PI meeting on February 28, 2019. The intent was to bring together a group of bioimaging researchers to identify topical interest areas where advancements in quantum capabilities could impact BER science. The relatively short duration required a narrower focus compared to the broad field of quantum science, so the discussion largely highlighted quantum sensing and its applications.

Discussion Format

To help establish a framework, the meeting began with brief introductory comments by Dr. Prem Srivastava and Dr. Todd Anderson from BER, followed by an interactive quantum concepts primer session by Dr. James Evans (chair). The primer session utilized live polling and encouraged full audience participation with real-time feedback by the host. The primer session was designed to: 1) engage the group and address predispositions and misconceptions related to what the discussion was to cover (i.e. QUEST goes far beyond quantum dots) while also mentioning the overall categories of Quantum Computing, Quantum Detection, and Quantum Sensors; 2) remind the audience that the attendees are mostly nonexperts in QUEST and that the desired outcome was brainstorming of niche science areas and applications of interest to BER — a discussion in which everyone in the audience could participate; 3) introduce the difference between improvements made by employing quantum concepts versus other classical means like improving sampling rate; 4) clarify that while most current quantum sensing publications largely focus on applications for bioimaging using photons, other particles such as neutrinos, electrons, and even molecules can be entangled and should therefore be considered; and 5) highlight that quantum technologies can provide access to nonclassical bioimaging applications so considering ideas that currently seem impossible or futuristic is worthwhile. Following the primer session, Dr. Jeffrey Cameron (co-chair) provided a short summary presentation and overview of the recent DOE Office of Science (SC) Quantum Information Science (QIS) PI Meeting (www.orau.gov/qispi218/), as well as a few slides delving deeper into the current literature for quantum sensors and other quantum technologies and applications. The applications were focused on areas that could address current limitations in achieving high spatial and temporal resolution in areas of bioimaging relevant to BER. The combination of these introductory comments and presentations helped ensure participants had a common base-level understanding of quantum concepts pertinent to the QUEST discussion.

The bulk of the discussion centered around two separate breakout sessions, which were each further divided into two subsections to discuss ideas independently before reconvening for a summary report out and whole-group discussion. The first breakout session was dedicated to identifying potential BER science application areas that could be advanced with quantum sensing, while the second breakout session focused on the technological gaps and potential niche applications. Specific questions posed to the attendees included:

- What areas of bioimaging could be improved using quantum coherence and correlation to overcome resolution limits?
- What areas of bioimaging could be improved using quantum entanglement to improve the sensitivity of a measurement?
- What are the current limits or gaps in bioimaging that could be bridged by quantum technologies?
- What type of inherent biological signals can be exploited for direct quantum sensing in the niche areas?
- What type of custom signals could be designed to allow indirect reporting of bioprocesses via quantum sensing without significant perturbation to sample in the niche areas?

Following each breakout session, the groups reconvened to report on the ideas discussed in the smaller groups and to solicit comments and other ideas from the larger group. Both sets of breakouts generated new ideas as well as a lot of synergy and overlap, some of which is detailed below in bullet format.

Ideas Generated from the Discussion

1. ***Determining BER science and technology needs for QUEST***
    a. While super-resolution optical approaches have significantly impacted BER science and bioimaging in general by empowering incredible spatial resolu-

tion on the nanoscale, a gap still remains with the approach since it relies on fluorescent labels to detect cellular components. This label-based requirement can result in an incomplete understanding of whole-cell or dynamic context due to the inability to label all components of interest simultaneously. New QUEST capabilities could create novel ways of recording a more holistic snapshot of cellular structure, dynamics, and metabolic processes.

b. Another recognized gap relates to the fact that many current state-of-the-art bioimaging techniques have been widely proven on animal and mammalian cells, but their adaptation and application to BER-relevant organisms have been hindered by difficulties inherent to microbial cells and plants. Even though plant cells are similar in size to animal cells, the plant cell wall causes additional scattering (especially off the cortex) that degrades signal; while native plant architecture and overall dimensions can cause trade-offs of lower resolution when trying to image deep into plant tissues. Current approaches to bypass these issues are to compensate for using high power, which can cause damage artifacts, or to physically section the sample into thin segments, which prevents true *in situ* and non-perturbative live measurements. Besides their physical limitations, plants also exhibit chemical limitations (as do photosynthetic organisms in general) that arise from the presence of endogenous chlorophyll fluorescence, which can overwhelm detectors. This fluorescence has such broad emission and excitation profiles that use of other exogenous labeling fluorophores is prevented—effectively restricting the total number and types of labeling events that can be exploited for normal optical, Raman, or super-resolution applications. On the other end of the spectrum, the small size of microbes can hinder adaptation of some state-of-the-art methods if there is a disconnect between the achievable and required spatial resolution or chemical sensitivity to enable efficient or meaningful imaging.

c. The attainable depth-of-field for imaging complex environments (e.g. soil) presents another major challenge that could be overcome using quantum imaging technologies in combination with long-wavelength light for increased sample penetration.

d. One final identified gap relates to the need to enable high-resolution optical imaging of samples without suffering artifacts, such as localized heating or photobleaching, that limit time-course studies. This is a common problem shared across the biological sciences that could be overcome by QUEST developments.

2. *Exploiting QUEST to expand label-free imaging and molecular identification*

a. The possibility of entangling two photons at different wavelengths and using the longer wavelength photon to illuminate the sample while detecting the lower wavelength photon via ghost imaging was of considerable interest to the attendees. Specifically, there was excitement over the possibility of using a longer wavelength photon for all direct interactions with the sample to impart less heating or other photobleaching artifacts and to expand the range of available probes and tags that can be detected while still permitting high-resolution imaging.

b. A longer time-horizon idea was the possibility of using quantum technology to identify post-translational modifications on the single molecule level or to detect structural changes of individual molecules inside the cell *in situ*. Such an achievement would have applications toward revealing new molecular associations and protein interactions within the cellular environment as well as delving deeper into systems biology approaches to directly discern molecular changes in individual cells rather than relying on transcriptomics or metabolomics.

c. Exploiting quantum technology to provide new hybrid bioimaging instruments or methods for visualizing intracellular dynamics without labels is really appealing. Coherent, tip-enhanced, surface-enhanced, and spontaneous Raman are all important label-free bioimaging techniques. However, all of these approaches are limited either by their identification of whole classes of molecules (i.e. detect all molecules with a specific resonance so cannot tell difference easily between C16 and C18 lipid molecule), or have minimum detection limits that are still higher than physiological concentration levels, or are restricted to being localized within a few nanometers of the surface- or tip-enhanced probe. One exciting prospect for improving future label-free imaging approaches is the possibility that improved QUEST capabilities could reveal new molecular signatures that can be exploited to allow tracking of probes with more precision or even lower copy number.

3. *Harnessing new quantum sensors for a better view of cellular dynamics via hybrid detection schemes*

a. The potential for improving chemical mapping both in terms of spatial resolution but also in terms of chemical sensitivity and chemical contrast (or multivalence state and spin-state identification) is highly intriguing for a range of applications. For example,

improving the ability to detect sub-part-per-million ions and molecular species within individual cells, microbial communities, and plant microbe interactions would dramatically accelerate our ability to truly understand the flux and fate of material within biosystems. The group envisioned various scenarios combining QUEST with electron microscopy, X-ray diffraction, FRET, and plasmonics to achieve this goal.

b. Combining QUEST and nonionizing radiation to better understand nanoscale phenomena with intracellular imaging is a widespread need. Potential applications include capturing dynamic interactions between proteins and other proteins or lipids, solving the structure of protein complexes within the living cell *in situ*, or revealing unknown protein networks. The group recognized that cryo-electron tomography (and X-ray tomography) already exist and can reconstruct the whole cell context and even resolve individual protein structures with sub-tomogram averaging; however, this approach relies on ionizing radiation, which limits *in situ* and live-cell observations, especially as direct time-course datasets.

c. Several attendees could see clear application areas for QUEST to improve multimodal bioimaging of organic/inorganic interfaces. For example, the possibility of using quantum technologies to improve tunneling and plasmonics at interfaces while retaining the ability to also visualize the biological matter above and below the depth of focus is an exciting prospect.

d. One of the more future-focused topics of the discussion was the possibility of turning cellular components such as microtubules into local intercellular detectors. If realized, this approach could potentially permit directly sensing of changes in a local environment, all while using inherent features within the cells. This would likely require finding unique quantum signatures of the microtubules themselves that change depending on chemical or physical stimuli, but if the microtubules or other cellular features could somehow be used as quantum sensors or detectors, it could revolutionize our ability to link genotype to phenotype and response to environmental perturbation.

e. Another futuristic idea is related to the desire to possibly encode quantum information in terms of molecular context or environment, and whether it's possible to entangle multiple times? In particular, the question was raised as to whether one could entangle an electron that then generates an X-ray, which could maintain an entanglement with the original electron or that could be separately entangled with a second X-ray? If so, the discussion led to considerable excitement about the possibility of using both X-rays and electrons to probe different parts of a sample simultaneously or to perform two-color coherent X-ray studies.

f. Besides the topic of entangling the information carrier (electron, X-ray, photon, etc.) additional discussion centered on the intriguing question of how large a biosystem could one entangle? A published example demonstrating the wave-particle duality of Buckyballs was highlighted in the primer session and stimulated discussions about possible extension to other large molecules. While that example uses a homogenous sample geometry that is not at all similar to whole cells (where the internal morphology between two clonal cells can still vary), it was an interesting thought process to consider using cells as sensors themselves. Although likely impossible, a game changer would be the ability to entangle two cells together and put them into two different environments to see how perturbations to one cell couples through entanglement to the environment of the other cell. This would open up new ways to explore localized biosystem sense-and-response behaviors without perturbing the entire system.

Looking to the Future

The possibility of using quantum sensors to help improve the signal-to-noise ratio, the signal-to-background ratio, or the overall sensitivity of experiments is very intriguing, as is using quantum technologies to break the spatiotemporal limits across many modalities. Clearly this discussion was just the tip of the iceberg in terms of identifying areas of interest where quantum-enabled technologies can advance biological and environmental research. One key thing to remember is that this discussion group was formed from existing bioimaging researchers, and none of the people in attendance considered themselves experts in quantum sensing, quantum detection, or quantum computing. It will be important to involve experts in the quantum field and scientists funded by other SC offices during future discussions to fully engage the leading edge of quantum science and capabilities. Nonetheless, the discussion made it clear that there is a lot of excitement in the bioimaging community for the potential new science frontiers that could be realized by new imaging modalities enabled by quantum technologies. Two main areas were the possibility of new hybrid imaging modalities that permit probing two or more sample characteristics simultaneously and the promise of overcoming current gaps in spatiotemporal resolution and chemical sensitivity when imaging plants *in situ*.

June 2019