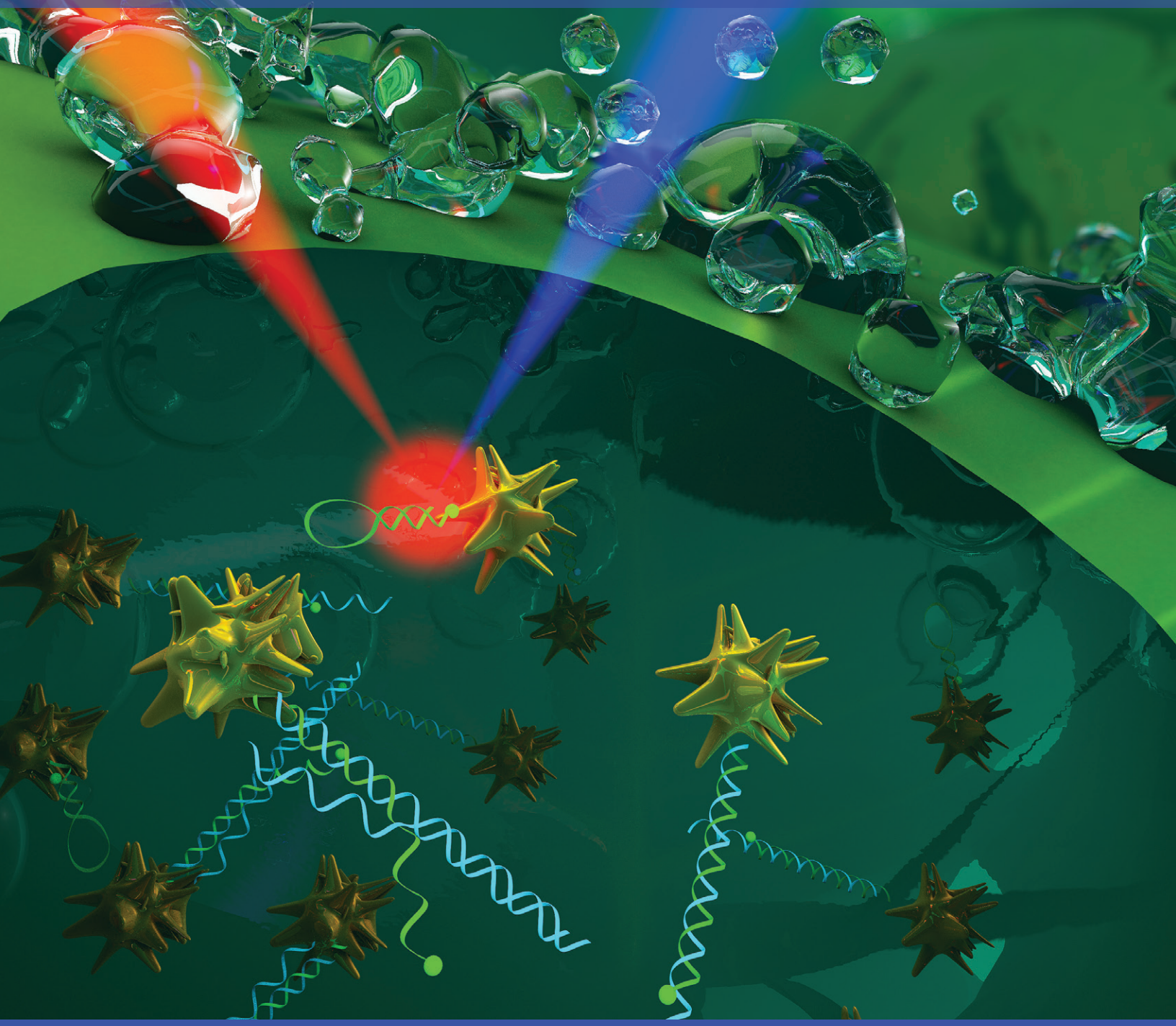
Biomolecular Characterization and Imaging Science

Bioimaging Science Program
2020 Principal Investigator Meeting Proceedings



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Office of Biological and Environmental Research

Biomolecular Characterization and Imaging Science

Bioimaging Science Program
2020 Principal Investigator Meeting

February 26–27, 2020

Washington, D.C.

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About BER

The Biological and Environmental Research (BER) 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.

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Preface

The 2020 Biomolecular Characterization and Imaging Science (BCIS) – Bioimaging Science Program (BSP) Annual Principal Investigator (PI) Meeting was held February 26–27 at the Washington Hilton in Washington, D.C.

The program's mission is to understand the translation of genomic information into the mechanisms that power living cells, communities of cells, and whole organisms. The goal of BSP 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.

BSP convenes annual PI meetings to bring together its contributing investigators to review progress and current state-of-the-art bioimaging research. This year's meeting was held consecutively at the same venue with the Genomic Science program PI meeting. Holding the two PI meetings conjointly provided a platform for networking and exchange of ideas, helping to forge new multidisciplinary collaborations among investigators from the two sister programs.

Important highlights of the BSP meeting included two keynote presentations: "*Imaging Cellular Structure and Dynamics from Molecules to Organisms,*" by Dr. Eric Betzig, University of California, Berkeley, and "*Entangled Photon Spectroscopy and Imaging in Biological Systems,*" by Dr. Theodore Goodson III, University of Michigan, Ann Arbor. All the BSP PIs made presentations describing their research focus and progress, and these were followed by roundtable discussions of each project. The meeting's proceedings provide an outline of the program's current state and potential future directions and opportunities.

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List of Funded Projects

Universities

Multimodal Single-Cell/Particle Imaging and Engineering for Energy Conversion in Bacteria
Peng Chen, Cornell University

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 Nanospectroscopy of Biological Materials in Fluid
Tina Jeoh, University of California, Davis

Inorganic Voltage Nanosensors as Tools for Bioelectricity Studies in DOE-Relevant Bacteria and Their Communities
Shimon Weiss, University of California, Los Angeles

Tracking Lignocellulosic Breakdown by Anaerobic Fungi and Fungal Cellulosomes
Michelle O'Malley, University of California, Santa Barbara

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

Quantum Dot Toolkit for Multimodal Hyperspectral Bioimaging
Prashant Nagpal, University of Colorado Boulder

Live-Cell, Quantum Dot–Based Tracking of Plant and Microbial Extracellular Vesicles
Jeffrey L. Caplan, University of Delaware

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

Development and Implementation of an *In Situ* High-Resolution Isotopic Microscope for Measuring Metabolic Interactions in Soil Mesocosms
Elizabeth A. Shank, University of Massachusetts Medical School

Expanding the Utility and Range of Quantum and Polymer Dots for Multiplexed Super-Resolution Fluorescence Imaging in Plants
Gary Stacey, University of Missouri, Columbia

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

Metaoptics-Enabled Multifunctional Imaging
Paul Bohn, Anthony Hoffman, and Joshua Shrout
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

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 U.S. Department of Energy's (DOE) Biomolecular Characterization and Imaging Science – Bioimaging Science program (BSP) supports fundamental research to develop and apply new and enhanced bioimaging and measurement capabilities that enable scientists to study the biological functions of plant and microbial systems relevant to bioenergy research. The program—within the DOE Office of Science's Office of Biological and Environmental Research (BER)—currently sponsors research at 5 national laboratories and 19 universities, with the mission of understanding the mechanisms that power living cells, communities of cells, and whole organisms. The novel and improved technologies emanating from BSP research are designed to solve specific challenges in plant biology, biofuels, and biogeochemistry across a range of scales, from single molecules to small unicellular organisms to complex microbial and fungal community interactions with plants. Beyond these specific targets, the program's innovative broad-based methodologies, analytical systems, and problem-based technologies will lead to new discoveries. Instruments and systems are being designed and constructed from the ground up or adapted by merging new and transformational improvements to existing capabilities. Together, these technologies will provide an impressive and versatile toolbox for real-time imaging of biological dynamics, chemistries, and structures, including metabolic pathways, the transport of materials within and among cellular organelles, plant-root and organism interactions, enzyme functions, and cellular structures.

Bioimaging Science Program Annual Meeting

Each year, BSP principal investigators (PIs) gather to present research progress, exchange ideas, and discuss potential program opportunities and directions. The annual PI meeting provides an important and useful mechanism to increase the cross-platform, cross-disciplinary, and cross-scale synergies needed to achieve BSP goals. Scheduling this meeting proximal to the DOE Genomic Science program's annual PI meeting creates invaluable opportunities for synergistic interactions with that program's research community. The BSP meeting also includes imaging experts external to the program who inject new perspectives and approaches into discussions. This document summarizes discussions from the meeting and also provides abstracts from each BSP-funded project.

Summary of Current BSP Research

Expansion of New and Existing Techniques

BSP has significantly expanded its portfolio since its inception in 2015. An impressive range of novel instrumentation and measurement technologies supports advancement of new knowledge for bioenergy and environmental applications by enabling researchers to visualize the spatial and temporal relationships of key metabolic and molecular processes associated with phenotypic and genomic expression in plants and microbes. The program now includes a wide variety of new instrumentation and cutting-edge sensing approaches such as super-resolution microscopy, light-sheet microscopy, adaptive optics, code-aperture methods, and quantitative phase imaging. These new additions greatly complement currently developed technologies that involve (1) molecular, optical, fluorescence, and nonlinear optical techniques; (2) several types of Raman spectroscopy, such as surface-enhanced (SERS), stimulated (SRS), hyperspectral stimulated (hsSRS), and tip-enhanced (TERS); (3) and nano-Fourier transform infrared (FTIR) and X-ray (XRM) microscopies.

While spectroscopic techniques are being developed to image dynamic events and molecular processes *in situ*, various combinations of nondestructive and destructive approaches are being used to image laboratory-prepared or fixed samples. Due to their noninvasive nature, optical modalities have played an important role in BSP, ranging from infrared (IR) or ultraviolet (UV) absorption and adaptive optics multiphoton microscopy to fluorescence and Raman techniques (i.e., conventional, nonlinear, and plasmonics-enhanced). These spectroscopic modalities are complemented by research and development at DOE-sponsored user facilities, which are building and applying various technologies such as ion microscopy and full-field X-ray fluorescence imaging (FFFI).

BSP researchers are further enhancing co-registered mass spectrometry and spectrochemical imaging capabilities to yield highly selective, sensitive, and quantitative chemical maps that identify intra- and extracellular molecular 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.

The performance and impact of label-based and label-free sensing and imaging technologies are significantly expanded through the development of unique probes equipped with various bioreceptors (e.g., antibodies, aptamers, and gene probes). These probes are enabling specific detection of important biomarkers (e.g., metabolites, proteins, and genomic markers) related to particular processes and metabolic pathways in microbial

and plant systems relevant to bioenergy research. The development of these probes and sensors is expanding the applicability of the new instrumentation by allowing dynamic tracking of targeted cells, organelles, enzymes, biomarkers, and small molecules.

Multimodal Capabilities

Much of the new instrumentation under development is multimodal, resulting in each instrument combination providing high resolution at a range of length scales. An extraordinary number of modalities are included in these new instruments. The wide variety of microscopy approaches range from optical methods—such as luminescence, confocal, adaptive optics multiphoton, fluorescence scattering, reflected/transmitted light extinction spectroscopy, and total internal reflection fluorescence (TIRF)—to FFFI, imaging, polarimetry, and novel single-molecule sensing methods such as stochastic optical reconstruction microscopy (STORM) and photoactivated localization microscopy (PALM). Quantitative phase imaging has been combined with light-sheet fluorescence microscopy–based optical quasi-lattice to achieve increased imaging throughput rates and resolution of single cells. Dark-field fluorescence-based hyperspectral imaging enables the collection of high signal-to-noise images and will allow multiplex collections of multifluorophore images. In addition, these imaging modalities will be complemented by advanced technologies such as high-speed atomic force microscopy (AFM), interferometric scattering microscopy, IR, and vibrational sum frequency generation. To enable an understanding of the mechanisms of cellulose hydrolysis, the combination of plasmonic IR nanofocusing gratings with microfluidics is being applied to nanoscale mapping of cellulose surface fibrils undergoing hydrolysis by cellulase.

Various forms of Raman spectroscopy are important parts of the portfolio; these include spontaneous, far-field subdiffraction, TERS, coherent anti-Stokes (CARS), SRS, SERS, spatially offset (SORS), shifted-excitation Raman difference (SERDS), and cavity-dumped SERS. The program has developed a multimodal microscope integrating CARS, SRS, and two-photon excitation systems with adaptive optics. The combination of SERDS with hyperspectral Raman imaging (HSRI) demonstrated the possibility of directly imaging microRNA (miRNA) biotargets in intact living plants under ambient light conditions. 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 21Tesla magnet. To provide three-dimensional (3D) spatiotemporal chemical information in bulk and at the interface in biological systems, BSP researchers have developed nonlinear optical mapping and *in situ* liquid extraction–mass spectrometry (LE-MS) using a porous membrane microfluidic surface in combination with a continuous LE sampling probe. A wide-field CARS microscopy was developed for rapid and simultaneous acquisition of CARS images across an entire field of view. In a different approach, researchers are developing electrochemical impedance spectroscopy with nucleic acid aptamer sensors to study interactions between plants and microbes that involve communicating chemicals traveling through the rhizosphere. The capability also will enable monitoring of nutrient transformations and rhizosphere microbial metabolic activities that contribute to the growth of healthy, productive plants.

Multi- and Cross-Scale Analysis of Complex Plant, Microbial, and Fungal Systems

BSP researchers 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 are plants, bacteria, fungi, and combinations of them. Approaches include developing sophisticated instruments to image metabolism in a single organism or to image gene expression and regulatory molecules (e.g., miRNAs) in intact plants, as well as interactions and communications between organisms. Furthermore, the program capitalizes on the advantageous spectroscopic properties offered by semiconductor and polymer quantum dots. The ability to understand mechanisms of metabolic flow within a plant, in microbial communities that support plant growth, or between plants and microbes has been limited by the lack of instrumentation and optical probes that can track or image molecules in intact organisms, between organisms, and in the soil where plants and microbes interact in the rhizosphere. The instruments being developed under BSP sponsorship will address this critical need and enable a future when molecular signatures can be tracked in real time and over periods consistent with the biological processes being studied. Some of these developments will include the capability to visualize 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 BSP research teams are highly diverse and cover a large length scale. They include atomic isotopes, metabolites, plant hormones, silica, trace elements, redox metabolism, microbial electron transfer, membrane potential, intercellular trafficking, cellulose and lignin synthesis and degradation, miRNAs that regulate lignification, enzymes, and

other proteins secreted by plants and quorum sensing molecules. By supporting the development of technologies that will 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, spanning molecules to multiorganism systems. Due to their hierarchical order, activities on longer length and time scales are built on activities and structures on shorter length and time scales. Therefore, processes must be fully explained at the molecular level to be completely understood at the organismal or multiorganismal level. Recognizing this need, BSP supports some innovative cross-scale imaging approaches that include plasmonic nanoprobes to track single molecules. Also supported is 3D tracking with high-speed AFM and optical tweezers to control molecules or microbes, enable force measurements, or track molecules such as cellulose synthase as it moves along the membrane or cellulase as it moves along cell walls. These studies will answer important questions related to the mechanisms of cellulose synthesis and degradation. Understanding such mechanisms will enable the development of biomass that can be converted more readily and rapidly into biofuels and bioproducts. While focusing on high-resolution imaging, some BSP projects are well positioned for applications to more complex biological systems relevant 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. Organisms under study include:

- Living plants — *Arabidopsis thaliana*, *Medicago truncatula*, *Brachypodium distachyon*, *Populus* sp., *Pinus taeda*, and *Zea mays*.
- Microbial chemotrophs — *Bacillus subtilis*, *Yarrowia lipolytica*, and *Pantoea* sp.
- Microbial phototrophs — *Cyanothece*, *Rhodopseudomonas palustris*, *Ostreococcus tauri*, *Chlamydomonas reinhardtii*, and *Synechococcus* sp.
- Systems for studying plant-microbe interactions — Arbuscular mycorrhizal symbioses, *Glycine max* with *Bradyrhizobium japonicum*, and *Suillus brevipes* with *P. taeda*.

With the new instrumentation and optical probes 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. Also anticipated are new insights into the fundamental biology of many macro events such as nutrient use and community and ecosystem interactions that include soil water retention caused by the presence or absence of particular organisms or biomass.

The comprehensive BSP portfolio, which includes the development of complex multimodal instrumentation and novel probes 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.

Research Challenges and Future Opportunities

Roundtable discussions at the PI meeting highlighted several advances for moving bioimaging science forward and expanding BSP impact (see sidebar, Key Needed Developments, which are described in more detail in this section).

Continued Development of Multimodal Technologies and Approaches

The BSP research portfolio is progressing significantly and now includes a broad spectrum of complementary techniques that cover a wide span of spatial and temporal scales. These technologies include imaging approaches ranging from labeled and label-free techniques to destructive and nondestructive imaging modalities (e.g., optical, scanning probe, X-ray, mass-spectrometric, and ion-based approaches). The recent addition of cutting-edge laboratory-based imaging methods (e.g., super-resolution techniques such as STORM and PALM) strongly

Key Needed Developments

- Combinations 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.
- New or improved conventional or quantum-enabled imaging technologies capable of monitoring biological systems in their natural state or as they respond to environmental perturbations and stressors.
- New or improved approaches that allow real-time data collection across the full spectrum of relevant spatial scales.
- 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.
- Methods to increase throughput for more mature yet novel imaging technologies.

complements the sensing and imaging approaches more suitable for field use. In addition to advances pursued within each of these respective techniques, a major BSP focus moving forward should be to continue integrating these different approaches into hybrid all-in-one instruments. Multimodal spectral imaging in a single and easy-to-use setup across nano-, micro-, meso-, and macroscopic spatial domains will be a useful and versatile tool for future users.

It is also important to realize the benefits of combining additional approaches that may be destructive or only applicable to frozen or fixed samples typically outside the scope of the BSP portfolio. Many current BSP capabilities based on optical approaches empower real-time or *in situ* observations of living systems but do not provide a complete picture of the sample or a whole-cell context. Some science questions require more holistic imaging and analysis to decipher complex associations within biological cells. Combining current BSP approaches with sequential downstream frozen (i.e., cryo-electron microscopy) or fixed-sample correlative imaging (i.e., nano-secondary ion mass spectrometry) can provide the additional spatial, ultrastructural, or chemical context needed for critical scientific breakthroughs related to cellular sensing and metabolite response, flow, and fate.

Cross-Platform Data Fusion and Integration

Another important program focus should be data fusion (i.e., linking the data from multiple technologies and approaches). These fusion efforts will result in a better and more holistic understanding of the imaged biological systems. The important tasks of indexing and registering images (such as multifunctional tracers, probes, or sensors to serve as cross-platform fiducial markers) will facilitate multimodal bioimaging systems. To meaningfully co-reference and co-register a sample's disparate datasets—which could all have different formats, magnifications, or resolutions—BSP should emphasize the need for data integration and fusion in future systems. Integrated data processing algorithms, visualization, and modeling are key components for properly interpreting the diverse sets of imaging data to enhance knowledge and results from omics-based models of organisms and other BER genomics-based programs.

Multidisciplinary Research Teams

Biological imaging is inherently interdisciplinary, and successful teams need to continue to reflect this approach by intermingling researchers with expertise in a variety of fields. For example, the integration of experts in conventional and quantum-enabled technology, nanoscience, computation, and imaging will lead to breakthrough advances. Imaging scientists also need to reach across biological disciplines, engaging structural biologists and genomic researchers to connect phenotypes with genotypes. Together, the researchers can integrate imaging results with the corresponding genomic, proteomic, lipidomic, and metabolomic changes within cells to facilitate the understanding of biological complexity and heterogeneity. Also important is close coordination with ecologists, soil scientists, and biogeochemists to better replicate the innate multiphase complexity of soils in laboratory experiments as investigators move toward deploying imaging methods and instruments in complex natural environments.

New Probes and Quantum-Enabled Techniques to Expand Investigations

In parallel with the instrumentation development efforts, there is also a critical need for probe development that enables 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. BSP's wide range of biosensing and imaging capabilities are expected to 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 specific organisms could provide opportunities to investigate and understand biological processes that otherwise would be difficult to unveil. Another approach to minimize perturbation might be to incorporate quantum-enabled science and technologies. The potential of using "ghost" imaging for bioimaging applications is intriguing because this approach can image a sample by detecting a photon that never interacted with the sample. Furthermore, the capability for quantum-entangled two-photon imaging to provide higher detection efficiency and decrease the total photon flux needed to observe a high-contrast image permits very low dose imaging that minimizes photodamage effects, facilitating longer-term, time-resolved imaging of biosystems. Integration of these and other quantum-enabled imaging technologies or sensors into the BSP portfolio could significantly expand the range of scientific questions addressable by the program.

Field-Deployable Capabilities for Whole Organisms and Complex Communities

Another important challenge for the near term is the extension of laboratory-based approaches into applications for whole organisms and plants in their natural environments and under field settings. This extension will require techniques that address the dynamics of microbially driven biogeochemistry (e.g., within the rhizosphere, biofilms, and other key biological interfaces). Although there is progress in imaging genomic biotargets in living plants, the research community still needs the ability to image complex native microbial communities to decipher their organization and the multiple metabolic processes occurring simultaneously in space and time. Also needed is development of capabilities to probe inherent signals within nontractable microbes in the environment and to create pathways that enable microbial synthesis of probes for assaying function and activity. Furthermore, in addition to sophisticated and laboratory-based analytical methods, portable instrumentation and practical techniques will allow the detection of weak optical signals from whole organisms and plants extracted from strongly interfering background signals such as fluorescence, ambient light, and fixed-pattern noise encountered 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, genomic biomarkers, and internal and secreted compounds. Achieving real-time collection and interpretation of these integrated data will lead to a major advance in bioimaging technology—one that will provide new understanding for monitoring and understanding phenotyping as well as molecular and genomic pathways in the laboratory and in complex natural environments. This advancement will require new approaches and algorithms to handle increasingly challenging volumes of data, permit automated analysis, and employ machine-learning approaches to rapidly process and interpret the massive amounts of data needed 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.

Advances in Data Management and Analytics

To enable effective extraction of critical biological and environmental information from the experimental data, major advances are needed in data storage, processing, and visualization. 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. This type of data repository could be independent or integrated with the DOE Systems Biology Knowledgebase (KBase) and take advantage of advances in artificial intelligence to extract patterns from raw data for improved organization, interpretation, and representation. Another opportunity for improving data interpretability is to leverage computer science graduate programs to help accelerate image processing or data analytic pipelines for the large datasets already being collected within BSP. Many computer science graduate programs require students to gain access to and experience with real-world data by building new software or algorithms for more effective analytics. Using the plethora of BSP data, the program's PIs could potentially sponsor graduate students in these computer science programs to develop the next frontier of bioimaging analytic tools.

Community Access to BSP-Developed Technologies Through User Facilities

User accessibility to the new technologies and approaches emanating from BSP will also be a key factor in the program's success and longevity. Deploying some BSP imaging technologies to DOE user facilities would provide the broader research community with access to these technologies, thereby increasing their impact. Such an approach would also facilitate continued technological developments through the important user-developer feedback loop and the synergistic interactions between imaging scientists and facilities, in addition to expanding the scope of research being conducted with these new capabilities.

Additional PI Interactions and Community Engagement

In addition to BSP's annual PI meeting, regular cross-team interactions (e.g., through teleconferencing or web conferencing) could help to maintain the interactive momentum of the annual PI meeting and catalyze new directions of investigation. Moreover, BSP's impact on a wider community of scientists who could use program capabilities would be enhanced by the creation of a Bioimaging Capability portal detailing BSP's diverse technological approaches and the applications for which they are best suited.

Abstracts

Multimodal Single-Cell/Particle Imaging and Engineering for Energy Conversion in Bacteria

Principal Investigators: Peng Chen[1] (PI), Tobias Hanrath,[2] and Buz Barstow[3]
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Research Plans and Progress: This project's research aims to combine quantum materials synthesis, bacterial synthetic biology, and multimodal single-entity imaging to quantitatively study how hybrid quantum dot (QD)–bacteria systems convert light to valuable chemicals at the single- to subcell level, with the ultimate goal of gaining insights to guide the engineering of QDs and bacterial genetics for more efficient bioenergy conversion.

On quantum materials synthesis, the project aims to understand and control foundational thermodynamic and kinetic aspects of charge transport from QDs to bacteria. The research is exploring the size-tunable energy of QDs to systematically adjust the energy level offset governing charge injection from QDs to bacteria. The first-step efforts toward that goal focus on establishing a QD surface chemistry that ensures chemical stability of the QD in the aqueous electrolyte environment while at the same time enabling efficient transport of photogenerated transport across the QD/bacteria microbe. The CdSe QDs at the focus of the project's initial experiments were selected by virtue of their tunable LUMO energy level alignment enabling either direct electron injection into the bacteria or hydrogen evolution as an intermediate energy carrier. *On bacterial synthetic biology*, the team is genetically tagging enzymes and proteins that are important for QD-to-cell charge transport with photoconvertible fluorescent proteins for single-molecule super-resolution imaging of single bacterial cells. The team is also generating gene knockout strain collections to probe the function of individual genes in the charge transport chains. *On multimodal single-entity imaging*, the team is measuring the photoelectrochemical current generated by single bacterial cells that are interfacing semiconductor particles, as well as mapping at the single-molecule level and nanometer spatial resolution the copy number, spatial distribution, and diffusion dynamics of proteins in single bacterial cells that are essential for charge transport into the cell.

Current and/or Anticipated Accomplishments and Deliverables: The project anticipates the following accomplishments in the first year of the project: (1) Surface chemistry that ensures chemical stability of CdS or CdSe QDs in aqueous electrolyte environment to enable photogenerated transport across the QD/bacteria microbe. (2) Tagging with photoconvertible fluorescent proteins a few components of the membrane hydrogenase in *Ralstonia eutropha* for single-molecule/single-cell imaging. (3) Creation of corresponding deletion strains of the tagged proteins. (4) Update of Knockout Sudoku algorithm to improve the accuracy of location prediction of transposon mutants of microbes with high GC–content genomes (e.g., *Ralstonia eutropha*). (5) Completions of a comprehensive molecule-to-reactor model of electrosynthesis, which shows that the upper limit efficiencies of electrosynthesis are higher than even the most efficient form of photosynthesis and that it could achieve a much higher fraction of this than photosynthesis in the real world. (6) Demonstration of measuring photoelectrochemical current from a *Ralstonia* cell interfacing with semiconductor particles, down to the subcell level. (7) Quantification of membrane hydrogenase concentration in individual *Ralstonia* cells. (8) Mapping of spatial distribution of membrane hydrogenase in single *Ralstonia* cells.

Potential Benefits and Applications: The team expects that research will provide quantitative knowledge to understand the basic materials and biological factors as well as the guiding principle to engineer and improve such systems. If successful, this research will transform the study of hybrid inorganic-bacteria systems for energy and chemical conversions. The proposed experiments should break new scientific grounds and open unforeseen opportunities.

Plasmonics-Enhanced Optical Imaging Systems for Bioenergy Research

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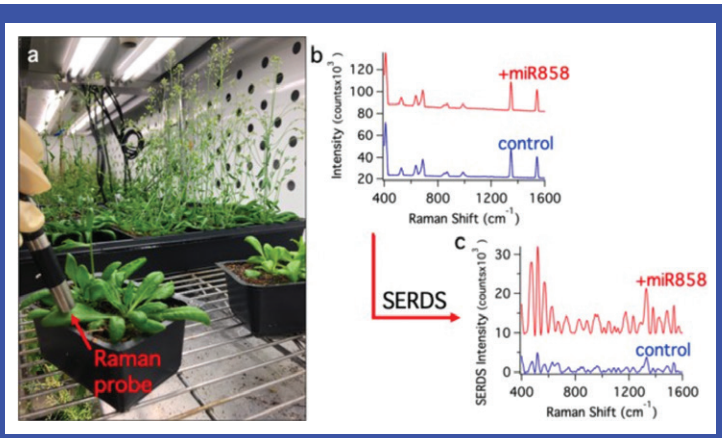
The goal of this project is aimed at addressing the DOE Funding Opportunity Announcement need to develop innovative and improved imaging instrumentation that can enable visualization and quantitative characterization of biomarkers and their dynamic role in cellular functions in living plants relevant 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, this project has developed *in vivo* imaging and biosensing of microRNA (miRNA) biotargets using plasmonic nanoprobes referred to as inverse Molecular Sentinels (iMS) that can be monitored using surface-enhanced Raman scattering (SERS). The team is currently developing innovative imaging technologies for visualization and quantitative characterization of biomarkers related to molecular processes and cellular function within living plants, namely *Multimodal Optical Sensing And Imaging Combinatory (MOSAIC) System*. 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.

Current and/or Anticipated Accomplishments/Deliverables for the Project Period: The project demonstrated the first combination of the iMS technology with shifted-excitation Raman spectroscopy (SERDS; shifted-excitation Raman difference spectroscopy), a unique spectroscopic technique that uses a subnanometer shift in the excitation wavelength to extract intrinsically weak Raman signals in complex background conditions. This advancement permits the use of SERS and sensing iMS in real-world applications, such as the detection of miRNA in plants under ambient light conditions for continuous monitoring during growth. The team also developed a new imaging technique, called HSERI (hyperspectral surface-enhanced Raman imaging), which exploits the mechanism of SERDS for accurate hyperspectral Raman imaging. HSERI allows imaging SERS nanoprobes with high spatial and temporal resolution, compared with traditional Raman scanning systems. The use of HSERI in combination with the iMS sensing enables direct imaging of miRNA biotargets. The project is currently working on strategies to enhance the delivery of iMS in plant cells and to prove iMS sensing in single cells. To detect the presence of the particles in single plant cells, the team will take advantage of the novel 3D X-ray fluorescence (XRF) microscopy capabilities at Argonne National Laboratory.

Potential Benefits/Applications of DOE-Funded Research for DOE and Dissemination and Deployment of Bioimaging Technology to Public and Private Sector for Generic Biological Imaging Use by the Broader Scientific Community: 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 efficiency and reduce costs. The project's novel tools will greatly facilitate studies on the regulatory mechanism for photosynthetic terpene production in plants.



Demonstration of the capability of SERDS technology to detect miRNAs in whole plants under ambient conditions. **(a)** Photograph of a leaf being measured with a Raman probe in the growth chamber under ambient light conditions. **(b)** Untreated SERS spectra. **(c)** SERDS-reconstructed spectra of a leaf infiltrated with iMS solution + miR858 and control solution measured inside a growth chamber. The peaks observed in the untreated spectra originate from the lights used in the growth chamber. Reprinted by permission from Springer Nature from Strobbia, P., et al. 2020. "Direct SERDS Sensing off Molecular Biomarkers in Plants Under Field Conditions," *Analytical and Bioanalytical Chemistry* **412**, 3457–66. DOI: 10.1007/s00216-020-02544-5. © 2020

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

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Research Plans and Progress: This project leverages its previously developed custom-built imaging systems, with a particular focus on the development of cavity-dumped stimulated Raman scattering (cdSRS) technique. This technique will significantly improve chemical sensitivity to allow detection of 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. The team will use the extensively studied maize (*Zea mays*) as a living plant and as corn stover biomass for deconstruction. Previously, the project demonstrated that cell wall structure changes during the course of enzymatic hydrolysis, and that the efficiency of biomass deconstruction is negatively correlated with overall lignin content and localization in the cell walls at the molecular and cellular levels.

Current and/or Anticipated Accomplishments and Deliverables: In year 1, the project finished installation and setup of a picosecond stimulated Raman system based on an Advanced Physics and Electronics (APE) picoEmerald laser. The new system features a laser pulse duration of 2 ps and spectral width of ~0.5 nm. This allows the team to identify and image Raman features that were not possible previously. Coupled with the project's femtosecond SRS with spectral focusing and fast wavenumber scanning, the team is able to track physicochemical changes with hyperspectral SRS while pinpointing the target of interest. Using the new setup, the team has been imaging genetically modified plant cell walls with incorporation of novel lignin structure and the gamma-Valerolactone (GVL) pretreatment on biomass carried out by the DOE Great Lakes Bioenergy Research Center (GLBRC). The project is able to identify different lignin linkages, such as new *p*-coumarate conjugates in the cell wall layers. In addition, during biomass pretreatment the mixture of GVL and diluted acid works together to solubilize the plant cell wall including lignin. It has been hypothesized that ions in diluted acid serve as an electron donator that attacks the electrophile carbon on lignin backbone. This also contributes to the lowered efficiency of dilated sulfuric acid compared to hydrochloric acid. The sulfate ion is less electronegative and more restricted and thus less effective in destabilizing the lignin carbon. Although it is hard to visualize the intermediate process, with the improved sensitivity, the team can directly focus its attention on the reaction surface and collect evidence related to the reaction mechanism. This approach helps elucidate the correlation between the structural/chemical characteristics of biomass and the efficiency of deconstruction.

Potential Benefits and Applications: With availability of the high sensitivity and resolution, this project will be able to build a multichannel stimulated Raman scattering microscopy system with much enhanced capability. The system 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 that suffer from only delineating bulk structure (i.e., from materials that have been finely ground and thus lose all ultrastructural information). The new imaging tool will synergistically interact with projects in GLBRC. The research will significantly enhance the understanding of cellulose structure and lignification *in planta* and their roles in biomass deconstruction processes. This technique will also have broad potential application in other biological systems, such as in the tracking of metabolism within a single cell and the co-localization of chemical transformation processes in inter- or intracellular spaces. The *in situ* and *in vivo* chemical information could be further correlated with single-cell genomics in future development.

Single-Molecule Imaging of Lignocellulose Deconstruction by SCATTIRSTORM Microscopy

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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, the project 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 optical reconstruction microscopy (STORM), which allows for three-dimensional 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/Anticipated Accomplishments and Deliverables for Project Period: Over the last year, the project has built the microscope and is currently optimizing the instrument. The microscope uses two approaches to total internal reflection excitation, which will be used for fluorescence (TIRF) and dark-field scattering (TIRDF) microscopy. The first approach employs micromirrors in the back focal plane of the objective and uses the total internally reflected beam that returns through the objective for autofocus. This micromirror illumination will be employed most frequently since it allows easy multilaser excitation, it provides the brightest illumination, and it is optimal for co-localization. The second illumination uses a standard dichroic mirror and has the advantage that it can be easily switched from TIRF to small-angle (dirtyTIRF) illumination to standard epifluorescence illumination. Movies are collected on a Photometrics Prime 95B sCMOS camera. The team is currently working on an illumination pathway for iSCAT imaging.

In parallel with instrument building, the project has been carrying out single-molecule experiments of fluorescently labeled TrCel7A cellulase on immobilized cellulose substrates using existing microscopes. Besides cellulose nanofibers and nanocrystals derived from poplar, the team is working with *Cladophora* and *acetobacter* cellulose. The team is able to visualize binding interactions and is measuring the on-rates, off-rates, and displacements using point-spread function fitting. In parallel, the team is measuring the enzymatic activity of the same enzymes on the same substrates so as to compare the single-molecule binding behavior to the bulk enzymatic behavior. This project has spent considerable effort in optimizing approaches for binding the cellulose to the glass coverslip at a proper concentration for single-molecule observations and with minimal nonspecific binding to the glass surface.

Potential Benefits/Applications for DOE and Other Research: There are scant single-molecule data of cellulases degrading cellulose substrates, and the team believes that increasing the temporal and spatial resolution of cellulase dynamics will greatly enhance the understanding of the basic mechanism of enzymatic cellulose degradation. This 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*

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Research Plans and Progress, Including Objectives and Goals for the Project Period: This project aims to build the time-resolved 3D multi-resolution microscopy (TR-3DMRM) to observe the real-time actions of cellulases to gain better insights of their processivity during catalytic activities. The plan involves two major components. One is to build the two-submodule microscope, a combination of a time-gated real-time 3D single-particle tracking (TG-RT3DSPT) and a two-photon laser-scanning microscope with the capability of a fluorescence-lifetime imaging microscope (2PLSM-FLIM). The other is to develop suitable protocols and assays for the application of 3DMRM on the cellulose-cellulase system. This involves cellulase and substrate development characterization, single-molecule assay for TR-3DMRM, and 3DMRM optimization and assay. Progress has been made on both the instrumentation and the assay development components. The Snee Group is developing new, faster syntheses for a spectrum of nonblinking bright quantum dots for this purpose. On the instrument side, the Yang Lab is close to finishing up the TG-RT3DSPT except for some further optimization on parameter settings of the hardware. After necessary evaluation of TG-RT3DSPT, the construction of 2PLSM-FLIM (Year-2/Q1) will be immediately carried out and integrated with TG-RT3DSPT (Year-2/Q2). On the assay side, the Tien Lab has generated dye-labeled cellulases from commercial sources and will continue to work out the details to produce cellulases in house so that the team has the capability to do genetic modifications. The Yang Lab has also made progress on 3DMRM optimization and assay development in that a method has been devised to extract subpixel information for low signal-to-noise ratios that enables video-rate super-line localization and resolution matching between two 3DMRM modules. This method is currently being upgraded for compatibilities on a 3D image stack with nonisotropic point-spread function and voxel shape, an anticipated situation in the coming experiments.

Current and/or Anticipated Accomplishments/Deliverables for the Project Period: Currently, the Snee Group has developed a new, faster synthesis of "giant" CdSe/CdS quantum dots and is currently developing similar chemistry for other systems. The Tien Lab has generated dye-labeled cellulases, and single-molecule assay development is carried out by both teams in a collaborative manner. Construction of the TG-RT3DSPT is in the final optimization phase, with a manuscript being prepared. The current result already showed a twofold increase in the signal-to-background ratio due to the additional time-gating capacity when observing the longer-lifetime giant quantum dots (gQD) diffusing in the shorter-lifetime 5-(4,6-dichlorotriazinyl) aminofluorescein (DTAF)–labeled cell lysate.

Potential Benefits/Applications of DOE-Funded Research for DOE and Dissemination and Deployment of Bioimaging Technology to Public and Private Sector for Generic Biological Imaging Use by the Broader Scientific Community. This project will reveal *in situ* single cellulase actions in real time and thus facilitate the basic scientific understanding of cellulose degradation. Such understanding will lead to a greater impact for biofuel and energy-related applications. In addition, the application scope of the new tool built for this study, the next-generation 3DMRM platform, can be extended to more complicated environments beyond the cellulose-cellulase system. The original 3D multi-resolution microscope (Yang Lab, Professor Kevin Welsher at Duke University, published work), a technique made possible through prior DOE funding, already enables more direct scientific discoveries by its powerful multimodel approach and high spatial and temporal resolutions. The current generation microscope, however, is also applicable to scenarios with high background, which is not uncommon for systems requiring dye labeling of the substrate or the environment.

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), Shishir Chundawat,[1] Eric Lam,[1] and Matthew Lang[2]
Collaborators: Wellington Muchero,[3] Sai Venkatesh Pingali,[3] and Laura Fabris[1]
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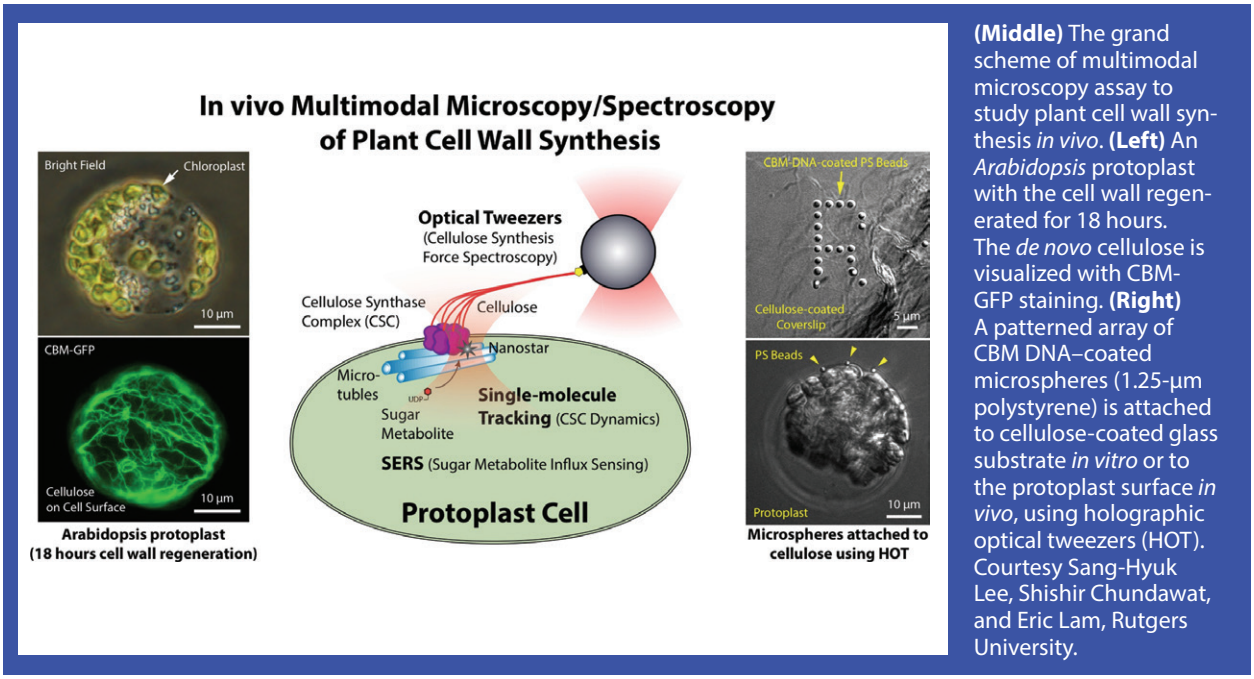
Research Plans and Progress: This project aims to study plant cell wall polysaccharide synthesis using *in vivo* multimodal single-molecule manipulation/imaging techniques. The team plans to simultaneously conduct (1) optical tweezers–based force spectroscopy on the nascent polysaccharide chain extruding out of the cellulose synthase complex (CSC) on a protoplast membrane; (2) real-time single-particle tracking of CSC; and (3) surface-enhanced Raman scattering (SERS) sensing of sugar metabolites using plasmonic gold nanostars attached to CSC (see figure).

Current and/or Anticipated Accomplishments/Deliverables: Progress in four directions has been made in parallel thus far. First, two model plant cellulose synthase proteins have been successfully expressed in *Pichia pastoris*, purified, and solubilized in detergent. Reconstitution of these proteins into active CSC complexes in liposomes and nanodiscs is currently underway for *in vitro* single-molecule imaging and force/Raman spectroscopy of cellulose synthesis. Second, the project has established the optimized workflow to isolate protoplasts from *Arabidopsis* and poplar leaf mesophyll cells and, subsequently, to initiate *Arabidopsis* cell wall regeneration. The team has successfully imaged the network of *de novo* cellulose fibers on protoplast membrane along the progression of plant cell wall regeneration via staining cellulose with either calcofluor dye or green fluorescent protein (GFP) fused to a cellulose-specific carbohydrate-binding module (CBM-GFP; see figure). Third, this team has succeeded in assembling DNA tethers between cellulose and polystyrene (PS) microbeads in a specific manner, using holographic optical tweezers (HOT) and CBM DNA–coated PS beads, both *in vitro* and *in vivo* (see figure). The project will track the cellulose-tethered PS beads, using them as motion/force transducers by which the activity of CSC and thus the mechanochemistry of cellulose synthesis can be studied in real time at the single-molecule level. Fourth, the team has been working to integrate Raman spectroscopy into its existing multimodal microscopy platform. With completion of the instrumentation in the near future, the team anticipates that it will be able to monitor the local chemical environment (e.g., sugar metabolite influx) near an active CSC while simultaneously investigating the mechanochemistry of cellulose synthesis with optical tweezers.

Potential Benefits/Applications: This 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, accelerating the development of better transgenic crops for bioenergy-related applications.



(Middle) The grand scheme of multimodal microscopy assay to study plant cell wall synthesis *in vivo*. **(Left)** An *Arabidopsis* protoplast with the cell wall regenerated for 18 hours. The *de novo* cellulose is visualized with CBM-GFP staining. **(Right)** A patterned array of CBM DNA–coated microspheres (1.25-µm polystyrene) is attached to cellulose-coated glass substrate *in vitro* or to the protoplast surface *in vivo*, using holographic optical tweezers (HOT). Courtesy Sang-Hyuk Lee, Shishir Chundawat, and Eric Lam, Rutgers University.

Development of Broadband Infrared Nanospectroscopy of Biological Materials in Fluid

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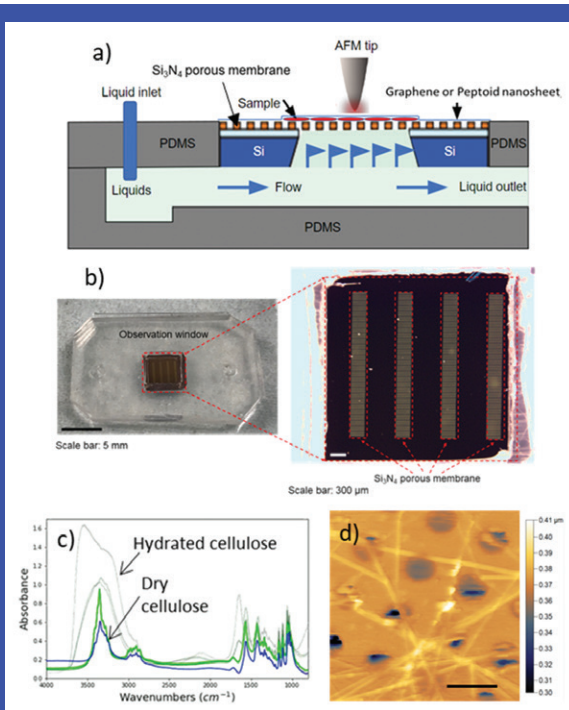
Research Plans and Progress: This research aims to develop a label-free, nanometer-scale, and time-resolved imaging technique to study surface reactions in aqueous biological reactions. State-of-the-art nanoscale imaging (i.e., nano-Fourier Transform Infrared; nano-FTIR) can map topography and chemical composition at nanometer spatial resolution but is limited to dry samples. The project is overcoming limitations of near-field energy delivery and extensive background scattering in aqueous samples by integrating plasmonic infrared nanofocusing gratings with microfluidics. This technique is being applied to nanoscale mapping of cellulose surface fibrils undergoing cellulase hydrolysis toward solving the mechanisms of cellulose hydrolysis.

Accomplishments/Deliverables: In the second year of this project, the team progressed in multiple but synergistic fronts toward an integrated technology for conducting nano-infrared in the presence of water. (1) Mid-infrared waveguiding plasmonic gratings have been manufactured with demonstrated enhancement of infrared signals of lipid membranes and carbohydrates. The gratings are currently undergoing detailed characterization and will be integrated with the microfluidic device (MFD) developed for this project. (2) The MFD with the ability to control sample hydration is compatible with both optical and nano-infrared (see figure panels a and b). The team has demonstrated the ability to monitor cellulose hydration/dehydration in this device by synchrotron-FTIR (SR-FTIR; see figure panel c). To image hydrated samples in the MFD with the nano-infrared system, the use of peptoid matrix and graphene monolayers is currently being optimized (e.g., see figure panel d). (3) The project has acquired and installed a Neaspec nano-FTIR at UC Davis. Nano-FTIR characterization of cellulose is ongoing at UC Davis and at the LBNL Advanced Light Source (ALS) on comparable nano-FTIR systems.



(a) Schematic of the microfluidic device (MFD) developed to enable nano-infrared of hydrated reactions; **(b)** a prototype of the MFD showing the arrangement of the silicon nitride (Si_3N_4) porous membranes; **(c)** SR-FTIR spectra of cellulose at varying states of hydration taken over a time course in the MFD; **(d)** atomic force microscope (AFM) image of cellulose microfibrils in the MFD covered by a graphene sheet. (scale bar = 1 µm; PDMS, silicon polymer polydimethylsiloxane) Courtesy Wujun Zhao and Jennifer Nill.

Potential Benefits/Applications: The microfluidic device developed in this project has scale versatility, as it is compatible with both conventional optical infrared and nano-infrared systems. While testing is ongoing, the novel membrane design (see figure panels a and b) appears promising for controlling and maintaining hydration of biological samples. Success of this prototype will enable time-resolved nano-infrared of hydrated biological samples.

Inorganic Voltage Nanosensors as Tools for Bioelectricity Studies in DOE-Relevant Bacteria and Their Communities

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Research Plans and Progress: In order to make headway in quantitatively understanding microbial metabolism and how cells communicate within communities, improved tools are needed that are capable of mapping cells' bioelectrical circuits. Existing tools for reading membrane resting potential (Δψ) in bacterial cells and bacterial communities are limited. This project has recently demonstrated that engineered quantum dots (QDs) and nanorods (NRs) display a large quantum-confined Stark effect (QCSE) at room temperature that is observable on the single-particle level. Such nanoparticles could be used as efficient nanoscale membrane potential nanosensors (MPNs) by monitoring changes in quantum yield, lifetime, and/or emission spectrum in response to Δψ changes. The team has also developed a novel functionalization approach for MPN self-insertion into cell membranes and demonstrated feasibility of voltage sensing in mammalian cells and cultured neurons.

The project will develop and optimize MPNs and novel electron-transfer dyes for simultaneous imaging and mapping of Δψ in bacterial films together with chemical signals and metabolic activities. The team will correlate these different spatiotemporal signals to gain a better understanding of Δψ significance in biofilm homeostasis and behavior.

The team synthesized, characterized, and optimized QDs and NRs with enhanced QCSE. An upper limit for the temporal response of individual ZnSe/CdS nanorods to voltage modulation was characterized by high-throughput, high temporal–resolution intensity measurements using a novel photon-counting camera. The measured 3.5-µs response time is limited by the voltage modulation electronics and represents ~30 times higher bandwidth than is needed for recording an action potential in a neuron. Using a single NR to record electric field modulation at a 1-kHz frame rate was also demonstrated. Lastly, the team developed peptide-based and lipid-based coatings for MPNs and demonstrated membrane insertion and Δψ recordings.

Current and/or Anticipated Accomplishments and Deliverables: The project will improve MPN synthesis and functionalization, methods for delivery, and targeting of bacterial membranes. The project will continue to develop detectors and methodology for wide-field imaging of Δψ in bacterial communities. The team will apply novel electron transfer dyes and a single-photon avalanche diode (SPAD) array phasor–fluorescence-lifetime imaging microscopy (FLIM) approach for imaging Δψ with absolute calibration.

Potential Benefits and Applications: Synthetic biology approaches are actively being employed to engineer microbes for useful applications in bioenergy and chemical production, carbon fixation, and bioremediation. Because the Δψ plays a central role in metabolism, quantitative measurements of its strength and polarity are needed to guide these studies. Maintaining Δψ homeostasis is critical for ensuring the normal function of a variety of cellular processes that include energy conservation, nutrient uptake, environmental sensing, cell mobility, and responses to cell stress. Understanding the electrical properties in individual microbial cells and in biofilms is needed to decipher and model their biology, such as anaerobic carbon cycling in freshwater and marine habitats. This knowledge is also needed for improving microbial processes involved in biotechnical applications, ranging from agriculture to waste recycling and remediation, to plant cell wall deconstruction, and biofuel production. In fact, being able to monitor the electrical potential in microbial cells and ensure that it is kept within acceptable levels to maintain metabolism rate might become a powerful tool to guarantee optimal productivity and viability. These tools could also be used to learn how bacteria interact with one another within DOE-relevant communities such as rhizospheres, soil, syntrophic assemblages, microbial fuels, and consortia that dismantle complex polysaccharides, among others.

Tracking Lignocellulosic Breakdown by Anaerobic Fungi and Fungal Cellulosomes

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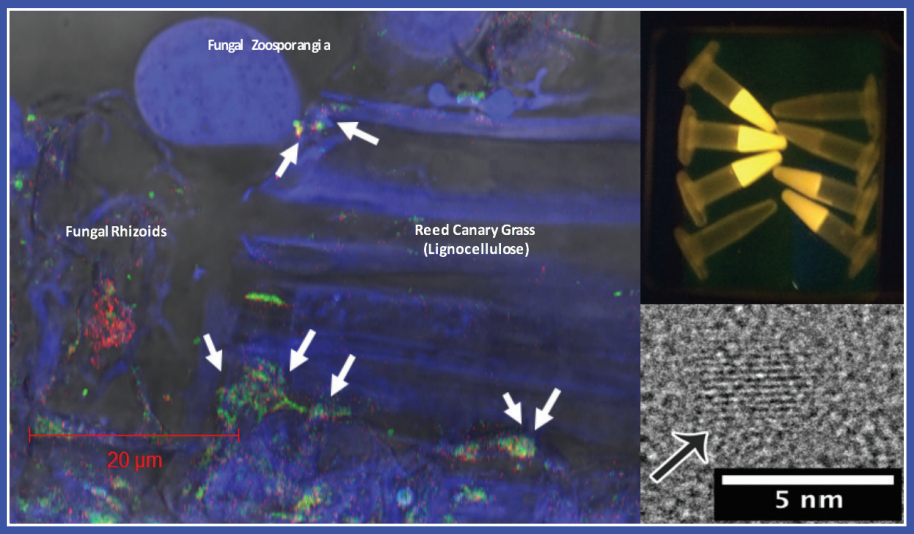
Research Plans and Progress: Anaerobic fungi degrade plant biomass through invasive, filamentous growth and the secretion of multiprotein biomass-degrading complexes called fungal cellulosomes. Despite the potential of these fungi for lignocellulosic bioprocessing, no nondestructive, real-time imaging tools exist to probe anaerobic fungi or the action of their cellulosomes across spatial and temporal scales. This project will develop new bioimaging approaches to learn how anaerobic fungi orchestrate biomass degradation through their unique multiprotein cellulosomes—and how these fungi are able to access carbohydrate biopolymers encased in lignin. Multiplexed imaging tools based on the synthesis of novel quantum dot (QD) nanobody fusions targeted at different components of fungal cellulosomes will reveal where cellulosome components are localized within the fungal ultrastructure and at the interface with lignocellulose. This approach benefits from a suite of new genomic, transcriptomic, and proteomic data obtained for multiple strains of anaerobic fungi, which enables the synthesis of custom QD and nanobody probes to localize cellulosomes and track their dynamics without suffering photobleaching effects. The team will also leverage a cell-free production pipeline to reconstitute QD-tagged fungal cellulosomes *in vitro* and characterize enzyme rearrangement, kinetics, substrate breakdown, and high-resolution structure via cryo-electron microscopy (cryo-EM). As a complementary approach, the project will advance genetic tools for the anaerobic fungi to conjugate QD probes onto cellulosome components *in vivo*, a capability which further enables hypothesis testing of protein function in genetically recalcitrant anaerobic systems.

Current and/or Anticipated Accomplishments/Deliverables for the Project Period: The project is early in its first year of funding. However, for the current project period, the team has already begun work on isolating the cellulosomes, performed an initial screening for transmission electron microscopy (TEM) compatibility, and made contact with various vendors to determine the optimal avenue for generating the nanobodies specific to the natively isolated cellulosomes. The team has also begun initial efforts toward cell-free expression of the various individual protein components of the larger fungal cellulosome complex.

Potential Benefits/Applications of DOE-Funded Research: Overall, this project will establish new state-of-the-art bioimaging capabilities to observe cellulosome dynamics and localization *in situ* with label-based and label-free approaches and will reveal critical attributes of fungal cellulosomes that can be engineered and exploited for biobased fuel and chemical production. Ultimately, the bioimaging capabilities will fold into the Environmental Molecular Sciences Laboratory (EMSL) user program and be accessible to the general research community with applications well beyond fungal cellulosome dynamics.



(Left) Immunofluorescence of the anaerobic fungus *Piromyces finnis* colonizing reed canary grass, where blue indicates DNA staining, red indicates staining of an anti-ScaA (scaffoldin) antibody, and green indicates staining of a dockerin-containing anti-GH48 antibody. Co-localization of the scaffoldin and dockerin (arrows) is seen near the grass, indicating fungal cellulosome degradation. Scale bar = 20 µm. **(Top right)** High-resolution TEM (HRTEM) image of a functionalized gold nmQD grown in the Evans Lab showing an overall core diameter of around 3 nm. **(Bottom right)** Example series showing the effect of various functionalizations on overall nmQD emission intensity following exposure to 405-nm light. Courtesy James Evans, Will Chrisler, and Stephen Lillington.

Understanding Plant Signaling via Innovations in Probe Delivery and Imaging

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This project is (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 together with research aimed at understanding receptor-mediated peptide trafficking and responses relevant to plant cell growth and longevity. The goals for the project period are (a) to further optimize the microscope; (b) to improve and test different nanospike designs that the team has implemented for delivering probes to plants, including the feasibility of delivering DNA constructs to deeper cell layers for expression studies; (c) to use validated fluorescent peptide probes (i.e., active and inactive versions of a secreted receptor ligand called phytosulfokine or PSK) in uptake and 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 and validate the transcriptional changes due to PSK-induced signaling related to growth versus longevity.

Current and/or Anticipated Accomplishments/ Deliverables for the Project Period:

Microsope: The team built the first version of the fiber optic microscope (manual mode) and expects to optimize plant stabilization and imaging within this project period. The microscope includes two LED light sources and interchangeable fiber optic lenses with different magnifications. The project has already imaged a cell-impermeable fluorescent dye introduced to a *Populus deltoides* leaf using the nanospike arrays. Ongoing efforts in image processing should improve the quality of microscope images. These initial experiments demonstrate the ability to introduce a cell-impermeable signal to a live plant and image the signal nondestructively using this microscope. This research sets the stage for imaging biologically relevant biomolecules.

Nanospikes: The project used vertically aligned carbon nanofiber arrays with lateral pitches (spacing between each of the fibers) of 10 µm and 35 µm to successfully deliver nonpermeable fluorescent probes to *Arabidopsis* (leaves, roots, etiolated hypocotyl, stem, and cotyledon), tomato fruit, switchgrass, and *Populus* leaves. The project successfully delivered and got expression of DNA constructs with reporter fusions in *Arabidopsis* roots, leaves, and cotyledons and tomato fruit. The team successfully renewed the application to use the Oak Ridge National Laboratory (ORNL) Center for Nanophase Materials Sciences and will fabricate and test optimized carbon chips.

Biological materials/deliverables: The project (1) validated that its designed active and inactive fluorescent versions of PSK work as expected; (2) collected positive preliminary data on the internalization into cells of these in different plant genotypes; (3) constructed the needed crosses of receptor–green fluorescent protein (GFP) plants into different backgrounds (mutants lacking receptors or the ability to produce active PSK) for trafficking studies; (4) currently is setting up to treat the relevant plant genotypes with PSK for the transcriptional study; and (5) designed and built several recombinant DNA constructs for whole-plant mapping of PSK sites of action that the team will transform into plants within this project period.

Potential Benefits/Applications of DOE-Funded Research for DOE and Dissemination and Deployment of Bioimaging Technology to Public and Private Sector for Generic Biological Imaging Use by the Broader Scientific Community:

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

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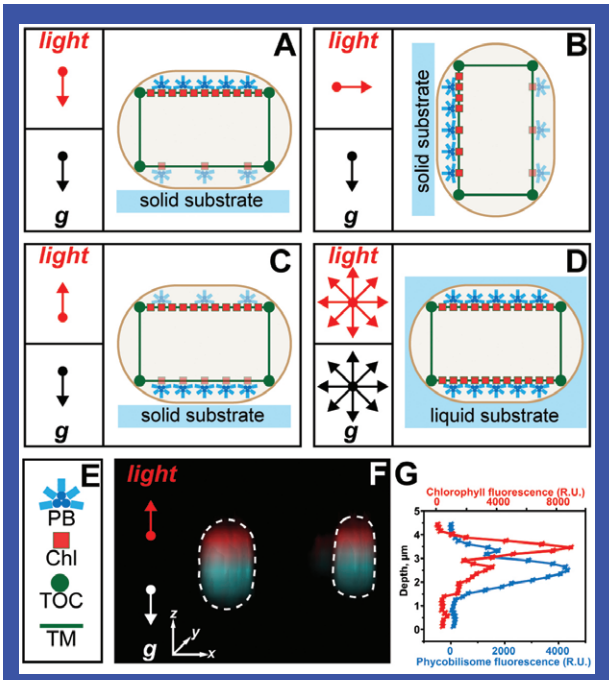
Research Plans and Progress: The overall objective of the proposal is to design and build a multimode computational/optical nanoscopy system to generate adaptive 3D images with high-resolution, real-time, dynamic label-free chemical imaging of metabolic processes in photosynthetic organisms. Various strains of cyanobacteria will be utilized for system benchmarking and calibrating of resolution and sensitivity. This project will then apply the system to generate a dynamic spatiotemporal map of photosynthetic metabolism, with a focus on tracking carbon from carbon dioxide (CO_2) fixation to storage in the form of glycogen. The team is currently on track to complete each of the project milestones.

Current and Anticipated Accomplishments and Deliverables: The team has completed the initial design/build phase of the project and is now integrating imaging modalities into a single multimodal imaging platform. The team has 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/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.



Effect of gravity and light on the cyanobacterium *Synechococcus* sp. PCC 7002. Models represent a summary of actual data from PCC 7002 grown with gravity and light **(A)** in the same direction, **(B)** at perpendicular angles, **(C)** opposite directions, or **(D)** random directions. **(E)** Legend showing phycobilisomes (PB), chlorophyll (Chl), organization centers (TOC), and thylakoid membranes (TM). **(F)** Spectrally resolved 3D confocal image showing spatial separation of phycobilisome and chlorophyll domains (corresponds to model in C). Red, Chl; cyan, PB. Solid substrate parallel to xy plane. **(G)** Line profile plot through representative cell in C and F showing chlorophyll (red) and phycobilisome (cyan) distributions. Courtesy Colin Gates and Jeffrey Cameron.

Quantum Dot Toolkit for Multimodal Hyperspectral Bioimaging

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A long-standing goal toward advanced understanding of plant and microbial systems for bioenergy applications involves characterization and quantification of multiple complex biological processes *in vivo*. Such quantification includes determining the specific copy number and function of enzymes, tracking metabolic pathways, and specifically activating the selected pathways with desired flux. While conducting these studies, it is also desirable to monitor the associated transport of materials (metabolites) within cells or across cellular membranes and the microbe-microbe interactions. Such an ambitious and expansive effort in uncovering molecular biology of interest using *in vivo* high-throughput, nondestructive, real-time tracking of subcellular components in living cells requires multiple simultaneous modes (multimodal) for imaging using quantum probes and sensors.

This project has developed design rules for creating targeted atomic and molecular clusters to study such real-time molecular phenomena using designed quantum probes, and even to control cell biology at a single-molecule level using light. There are three paradigms/functionalities developed by the team so far: (1) active atomic cluster and passive molecules on the surface (to ensure efficient cellular uptake); (2) passive atomic cluster (to aid transport/imaging) and designed sequence of biomolecules to track and modulate cell biology; and (3) active atomic and molecular components leading to creation of new nano-organism (nanorgs) with desired biological function and tunable material functionality, so cell biology can be controlled externally using light. The team has prepared an extensive quantum dot (QD) toolkit to study these three regimes, using >100 different colored QDs through multicolor or hyperspectral imaging, and is using five different simultaneous imaging modalities [super-resolution, hyperspectral, Föerster resonance energy transfer (FRET), cryogenic transmission electron microscopy, and pump-prove visible-terahertz] to achieve the desired high imaging resolution. The high resolution being developed includes high spatial (ångström- to nanometer-scale), spectral (~1- to 5-nanometer), and temporal (few-millisecond) resolutions, using the extensive QD toolkit with designed atomic and molecular clusters. The QD probes with unique chemical structures are developed for selective targeting of subcellular components with high precision to uncover their structure, function, and location. Imaging studies of these subcellular components, such as macromolecules that catalyze metabolic and/or transport reactions, and specific biochemical substrates and metabolites will provide a better understanding of the metabolic processes (anabolic and catabolic) in biological systems. This work represents a paradigm shift in bioimaging, uncovering metabolic pathways, and mapping the spatiotemporal dynamics in living biological systems in real time. The QD toolkit and multimodal imaging can have applications in the study of a range of microbial and plant systems for bioenergy production.

Live-Cell, Quantum Dot–Based Tracking of Plant and Microbial Extracellular Vesicles

Principal Investigators: Jeffrey L. Caplan[1] (PI), Roger W. Innes,[2] and B. C. Meyers[3]
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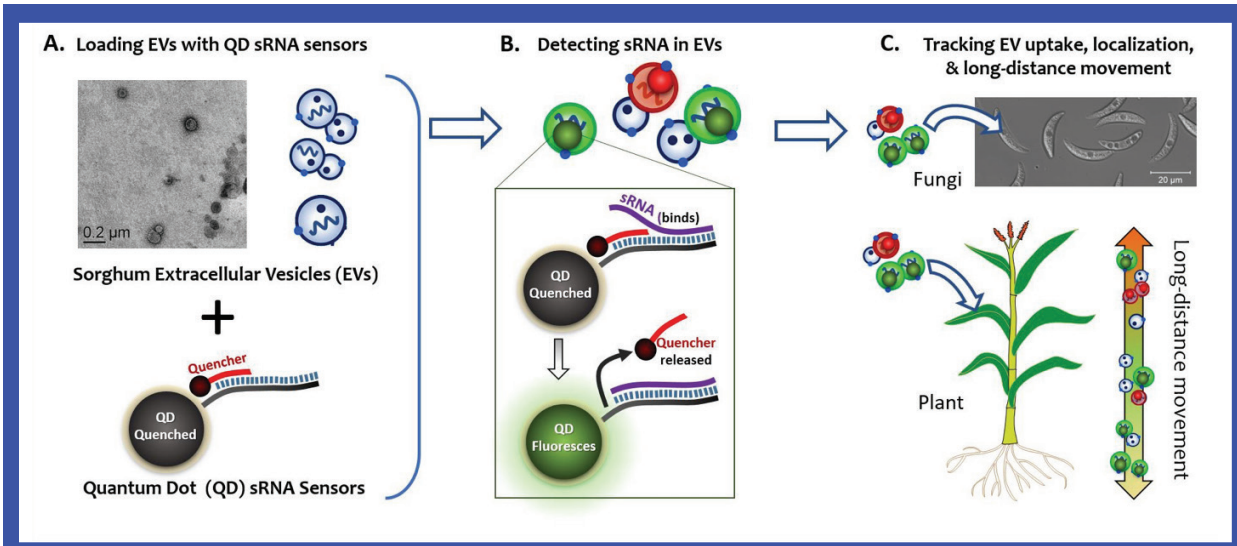
Research Plans and Progress: This project focuses on the role of extracellular vesicles (EVs) in the plant immune system, using *Sorghum bicolor* as the host plant and *Colletotrichum sublineolum* as the fungal pathogen. The project's aim is to develop new quantum dot (QD)–enabled small RNA (sRNA) sensors by making molecular beacons (see figure panels a and b) or by using QDs as scaffolds for fluorescent aptamer sRNA sensors. The goal is first to use these new tools to detect and quantify different types of sRNA in plant EVs and then to track the uptake, localization, and long-distance movement of EVs in both sorghum and *C. sublineolum* (see figure panel c). The team will use the superior properties of QDs to multiplex its sRNA sensors and for correlative light and electron microscopy to determine the subcellular localization of EVs after uptake. This approach will be complemented by fluorescent protein fusions to examine the live-cell interactions and exchange of EVs at the extrahaustorial interface between sorghum and *C. sublineolum*. A critical first step of this project is to identify the sRNAs and proteins in EVs isolated from sorghum by sequencing and mass spectrometry. These data will guide which sRNA to target with the QD sRNA sensors, and they will be the first characterization sorghum EVs.

Current and/or Anticipated Accomplishments and Deliverables: The team has acquired the biological materials necessary to reach project aims. The project has the reference strains of sorghum, BTx623, and *C. sublineolum*. All three labs have been using the exact BTx623 and *C. sublineolum* strains. The team has developed a protocol for EV isolation from sorghum (see figure panel a) and expects a protocol for sorghum EV isolation to be delivered in this project period.

The project is in the process of choosing the best QD for its molecular beacon design (see figure panels a and b). The team has characterized CdSe, InP, CuInS, and ZnSe QDs (all have ZnS shells) by transmission electron microscopy, single-molecule fluorescence, and spectral characterization. Currently, the CuInS QDs have the best characteristics. The team has successfully conjugated the sensor oligos to CuInS QDs with a 590-nm emission peak. The design was modified with Alexa Fluor 405 dye conjugated oligos to make the sensor ratiometric and, thus, more quantitative. The team expects to have a CuInS QD molecular beacon characterized in this project period.

Potential Benefits and Applications: These tools will be used to study the role of EVs during the plant-fungal interactions of sorghum and *C. sublineola*. Multimodal, correlative microscopy approaches will be used to uncover the mechanisms of long-distance EV movement and the modes of EV uptake in plants and fungi. EVs have been implicated in both cell wall modifications and pathogen defense, and this study may lead to new insights into improving the biofuel crop, sorghum.



(A) Extracellular vesicles (EVs) were isolated from sorghum and detected by transmission electron microscopy. Quantum dots (QDs) are used to make molecular beacons, a type of sRNA sensor. QDs are conjugated to oligonucleotides (oligo) that are complementary to a specific EV sRNA. A quencher oligo hybridizes to this oligo, which functions to quench QD fluorescence. This sensor is loaded into EVs by electroporation. **(B)** If an EV has that specific sRNA, it will release the quencher and fluoresce. Different color QDs (green, red) can be used to detect different sRNAs. **(C)** The QD-loaded EVs will be used to track EV uptake, subcellular localization, and movement in sorghum and its fungal pathogen, *Colletotrichum sublineolum*. Courtesy Jeffrey L. Caplan, Roger W. Innes, and B. C. Meyers.

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

Principal Investigators: Andreas E. Vasdekis[1] (PI), Armando G. McDonald,[1] Luke Sheneman,[1] and Scott E. Baker[2]
Participants: Nava R. Subedi[1] (postdoc), Gurkeerat Kukal[1] (student), and Erin Bredewig[2] (staff)
Organizations: [1]University of Idaho and [2]Pacific Northwest National Laboratory
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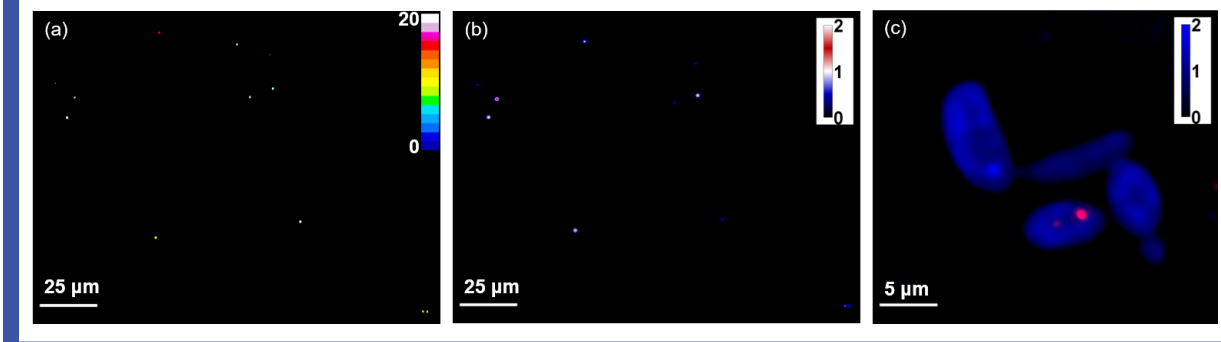
Research Plans: Strain optimization without disrupting growth represents a major challenge for biofuel production with high titers and yields. To address this challenge, the project is constructing and validating an optical imager that quantifies yields and titers with single-cell resolution, thus generating data regarding the role of metabolic pathway compartmentalization and systems-level cellular noise. The team's approach involves hardware, software, and biomarker development. In regard to hardware, the team is integrating quantitative-phase imaging (QPI) for precision metabolic profiling with light-sheet fluorescence and Raman imaging for quantifying enzyme, metabolite, and nutrient uptake dynamics. In parallel, this study is developing machine-learning approaches for label-free organelle identification by QPI, as well as Raman and gene-encoded fluorescent biomarkers for 4D quantification of enzymes, metabolites, and nutrient fate.

Current and Anticipated Accomplishments: The project has demonstrated QPI's enhanced precision in informing about growth and biodiesel precursor production in single cells. Since then, it has integrated QPI with light-sheet fluorescence microscopy, relying on an optical quasi-lattice for increased imaging throughput rates and resolution of single cells (see figure). Further, the team has developed machine-learning approaches for recognizing organelles that store biodiesel precursors directly in QPI images with high precision. In the context of biomarker development, the team has expanded its existing palette of green fluorescent protein (GFP)–tagged strains to include proteins that are active in central carbon metabolism, as well as "doubly-tagged" strains with two enzyme reporters in one cell. Similarly, the team has investigated deuterium-based Raman tags, finding similar consumption rates to natural glucose, as well as sufficient deuterium incorporation into key vibrational bands. The immediate next steps in the project are to (1) expand project hardware to include Raman spectroscopy and imaging and evaluate it for compatibility with microfluidics; (2) continue to augment the project's catalog of gene-encoded biomarkers to include sensors using riboswitch-controlled reporters of the concentration of specific metabolites; (3) investigate the role of deuterium in the fatty acid content of accumulated lipids by Raman spectroscopy and mass spectrometry; and (4) identify improved supervised learning methods for faster label-free organelle recognition of multiple QPI targets that are possible to execute on desktop computers.

Benefits and Applications: By minimizing photobleaching and phototoxicity, this project will acquire multivariate molecular information pertaining to the titers and yields of biodiesel precursor production at the single-cell level. As such, the project improves its fundamental understanding in optimizing biofuel production. The proposed imager development is executed on standard inverted microscopes, thereby making this technology accessible to the broader scientific community, including nonspecialists.



(a) Light-sheet imaging of 1-µm fluorescent beads; color coding denotes the z position of each particle (in µm). **(b)** Same target as in (a) but in quantitative-phase imaging (QPI). **(c)** *Yarrowia lipolytica* imaged by QPI (blue), with the localization of a fluorescent central carbon metabolism enzyme quantified by light-sheet imaging (red). Courtesy University of Idaho and Pacific Northwest National Laboratory.

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

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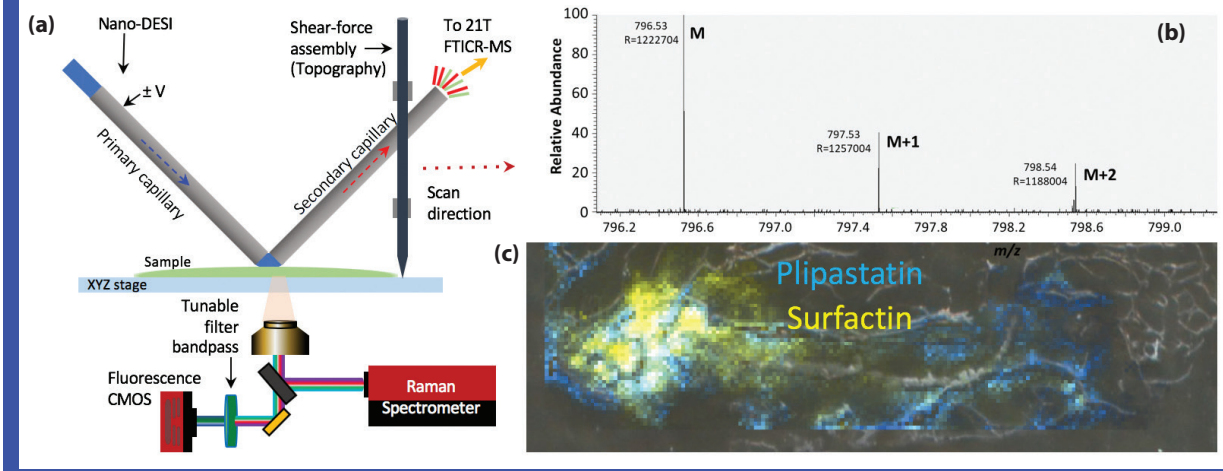
Research Plans and Progress: The planet's ecosystems rely on the activities of soil microbes. However, scientists' ability to directly observe the enzymatic and metabolic activities of microbes within soil is limited, both by the complexity of microbial communities as well as the lack of experimental tools to study them and their molecular interactions *in situ*. The goal of this project is to create an integrated platform (see figure panel a) that combines fluorescence microscopy, Raman spectroscopy, and nanoDESI-MSI (nanospray desorption electrospray ionization–mass spectrometry imaging). Using this instrument, the team will use fluorescent- and stable-isotope labels to investigate microbial activities and molecular transformations occurring in soil and soil-like model environments. There are three aims: (1) Develop a test system for instrument validation. (2) Construct a high-resolution isotopic microscope that integrates Raman, nanoDESI-MS, and fluorescence imaging for *in situ* measurements of carbon transformation. (3) Interrogate polysaccharide decomposition in model and native soils. For this project period, the team focused on aims 1 and 2.

Current and Anticipated Accomplishments: Construction of this high-resolution multimodal instrument is well underway. The team has created a customized mobile platform to integrate the optical microscopy components into the nanoDESI system, which is designed to work with a variety of MS systems. The project has incorporated a shear-force component to control the nanoDESI probe-to-surface distance, permitting accurate and high-resolution images of samples with complex topography. Several modifications from traditional nanoDESI platforms have been required. Specifically, it proved necessary to mitigate excess vibrations from the MS instrument with a decoupled vibrational isolation cart to keep the shear-force probe and nanoDESI capillaries stable. The team has obtained data from the 21 Tesla–Fourier transform ion cyclotron resonance–mass spectrometer (21T-FTICR-MS) exhibiting ultrahigh resolving power [R > 1.2 million at 700 mass to charge ratio (m/z)] and a mass accuracy of <1 mDa directly (see figure panel b) and ion mobility spectrometry (IMS) data at high spatial resolution from complex biological samples (see figure panel c). Work is ongoing to incorporate the fluorescence and Raman aspects of this novel platform. The project has concomitantly developed a simplified agar-based model to spatially interrogate the degradation of xylan and the metabolic interactions of *Bacillus subtilis* and *Bacillus cereus*, both of which will be used by the team to benchmark the data obtained with its instrument against that obtained from existing dual-modality microscopes.

Potential Benefits/Applications: The capacities provided by this potentially transformational technology will enhance understanding of the microbial and metabolic interactions occurring in soil communities that are relevant to carbon degradation. This instrument is being built at EMSL, where it will be available to the entire EMSL user base, enabling a range of DOE-relevant systems to be interrogated in the future.



(a) Design of high-resolution isotopic microscope integrating fluorescence, Raman, and nanoDESI modalities. **(b)** NanoDESI spectrum from a single position showing the ultrahigh mass resolution obtainable. **(c)** IMS image of cross-section of a bacterial colony showing the distributions of two known specialized metabolites. Schematic design by Venky Prabhakaran and Chris Anderton; Mass spectra collected by Jessica Lukowski; IMS data collected by Dusan Velickovic.

Expanding the Utility and Range of Quantum and Polymer Dots for Multiplexed Super-Resolution Fluorescence Imaging in Plants

Principal Investigators: Gary Stacey[1] (PI), Zeev Rosenzweig,[2] Marcin Ptaszek,[2] Galya Orr,[3] Christopher Anderton,[3] Mowei Zhou,[3] and Dehong Hu[3]
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The emission spectra of plant pigments currently limit the number of fluorescent colors that can be imaged simultaneously and, therefore, the number of cellular components that can be imaged within a single experiment. Hence, developing fluorescent tags and a microscope system that can image beyond the visible range or near infrared (NIR) would greatly enhance plant imaging capabilities. Extending imaging into the NIR would support the interrogation of multiple proteins/molecules simultaneously, but it would inevitably decrease spatial resolution due to the increase of the diffraction limit of light. To address this need, the team is developing a super-resolution fluorescence imaging (SRFI) platform that would allow imaging of NIR-emitting molecules, as well as quantum dots (QDs) and polymer dots (Pdots).

The advantage of QDs over organic dyes is their broad absorption and narrow emission peaks, which supports multiplexed imaging with a single excitation wavelength. QDs also show higher photostability and photoblink—both required traits for single dot–based SRFI. The longest emission wavelength of commercially available semiconductor QDs is 800 nm. Advances in inorganic NIR-emitting QDs will be realized by forming stable shells around the QD cores and modifying their surface with passivating ligands to improve their oxidation stability in biological solutions. The QDs will be functionalized to support their use for antibody tagging, as well as for use in biological suspensions.

To further increase availability of NIR-emitting dots, this project is also developing luminescent Pdots, which are not available commercially. Pdots are carbon-based nanoparticles made of hydrophobic semiconducting polymers, with synthetically controlled diameters of 5 to 100 nm. Pdots have minimal cytotoxicity and are, thus, more suitable for biological applications than inorganic QDs. In order to advance biology and to fully demonstrate the utility of the resources developed from this work, this project is focusing on the challenging effort to identify and visualize plant receptors (proteins) within nanodomains on the plasma membrane (PM). These experiments seek to examine the dynamic nature of receptor movement and degradation at a single-molecule level as a result of environmental perturbations.

Practical use of bioenergy crops will require plants with high yield and tolerance to a variety of biotic and abiotic stresses. Plants are rooted in place and, hence, have evolved mechanisms to recognize environmental threats and respond. Environmental sensing is largely localized to the PM, where a plethora of receptors and associated proteins form complex and dynamic interactions in response to specific environmental stimuli. Paraphrasing the DOE Funding Opportunity Announcement, the innovative approaches the project is using include "QD-based imaging approaches and complementary optical imaging instrumentation for observation and characterization of multiple complex biological processes, including development of probes functionalized with specific, active molecules to bind with specific cellular targets that will enable dynamic localization and imaging to validate hypotheses related to cellular signaling while dramatically enhancing the ability to measure processes in and among living cells."

Hyperspectral Light-Sheet Raman Imaging of Leaf Metabolism

Principal Investigators: Keith Lidke (contact), David Hanson, Jerilyn Ann Timlin, and Jamey Young
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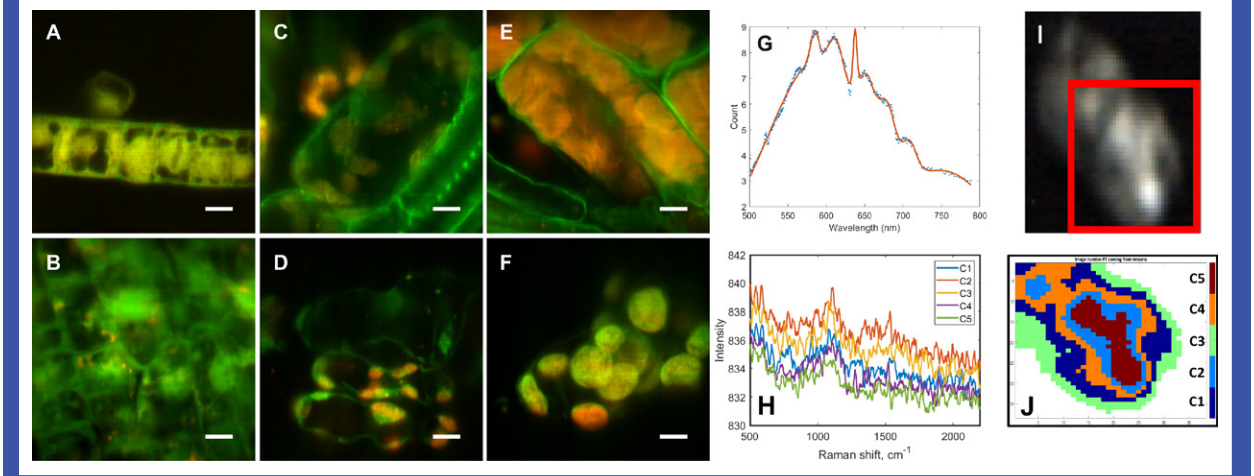
Research Plans and Progress: This project has finalized the custom design of the Raman light-sheet microscope and has procured the necessary components for the microscope. The framework of the microscope has been assembled, and the team is currently at the stage of testing and benchmarking microscope performance. Preliminary studies on plant cells using a hyperspectral fluorescence microscope are underway for noninvasive observations of plastids carrying photosynthetic pigment during the different phases of leaf cell development (see figure). These will aid understanding of the spatial distribution and biochemical and structural characteristics for Raman imaging. Data will be analyzed for their spectral components using software being developed at Sandia National Laboratories (SNL). Raman spectra of several key substrates and metabolites including $H^{13}CO_3^-$, glycine (1-^{13}C), L-serine (1-^{13}C), L-aspartic acid (3-^{13}C), and glycine (^{15}N) have been recoded with a Raman microscope. Raman spectral imaging has also been performed on a representative leaf cell sample (see figure). Analysis of the data to identify metabolite spectral signatures will soon be performed utilizing analysis software developed at SNL.

Current and/or Anticipated Accomplishments/Deliverables for the Project Period: By the end of this project period, the team anticipates the hyperspectral light-sheet Raman microscope to be fully functional, the imaging performance characterized, and the system ready to proceed with plant leaf imaging. The design and production of a sample chamber for the leaf samples will be completed and fully integrated to the Raman hyperspectral microscope. Imaging will proceed on the selected plants available with a clear expectation of ^{13}C Raman spectral shifts of the key metabolites and substrates.

Potential Benefits/Applications: Energy and food crop productivity can be improved through engineering photosynthesis such as replicating C4 and related pathways in crops and other ways to reduce photorespiration. The project's imaging system and the group of species to be examined will demonstrate the power of this technology for assessing and understanding the effectiveness of these attempts to re-engineer photosynthesis.



Hyperspectral fluorescence images of bundle sheet and mesophyll cells from immature **(A, B)**, intermediate **(C, D)**, and mature **(E, F)** sorghum leaf sections. Scale bar = 5 µm. **(G)** Wavelength spectrum from immature bundle sheet cells showing signature peaks for cell components. Panel **(I)** shows Raman spectral shift image for young and dividing meristem cells of *Sorghum* apical meristem. Five Raman spectral shift components (C1 to C5) resolved for the area marked by red inset in panel **(I)** are shown in **(H)** along with their corresponding areas of occurrence in the image **(J)**. Courtesy Sandeep Pallikkuth and Roxana Khoshravesh Astaneh, University of New Mexico.

Metaoptics-Enabled Multifunctional Imaging

Principal Investigators: Paul Bohn, Anthony Hoffman, and Joshua Shrout
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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.

Accomplishments/Deliverables for the Project Period: (1) *Metaoptics*. Silver (Ag) ridge arrays (see Fig. 1a) have been fabricated by (*a*) direct electron beam lithography patterning and (*b*) deep reactive ion etching through an alumina grating hard mask. Reflectance (see Fig. 1b) and spectroscopic ellipsometry exhibit permittivity values (see Fig. 1c) with low-loss epsilon-near-zero (ENZ) and plasmonic modes. (2) *Behavior of* Myxococcus xanthus *bacteria*. Because little is known about *M. xanthus* biochemical profiles during surface colonization, the team used confocal Raman microscopy to profile growing cultures of *M. xanthus* as well as in dormant fruiting body spores. Dramatic changes in the Raman spectra (disappearance of bands at 1550 to 1560 per cm and appearance of a new feature at 1510 per cm) accompanied the transition from robust growth to nutrient-limited conditions and the formation of fruiting body aggregates. (3) *Electrochemically Modulated Bacterial Luminescence*. Luminescence images of individual *M. xanthus* drop-cast on a transparent indiumtin-oxide (ITO) electrode (see Fig. 2a) exhibit spatially heterogeneous emission along the cell body, suggesting that the origin of fluorescence is flavoproteins present within the cell. Interestingly, the team also observed dynamic physical reconstruction of individual bacteria (i.e., expanding versus shrinking of the individual cell), likely due to interfacial stress effects (see Fig. 2b, c).

Potential Benefits/Applications of DOE Funded Research: Taken together, the results shown here represent the fundamental building blocks needed to achieve *super-resolution imaging of electrochemically controlled bacterial luminescence using metaoptical structures*.

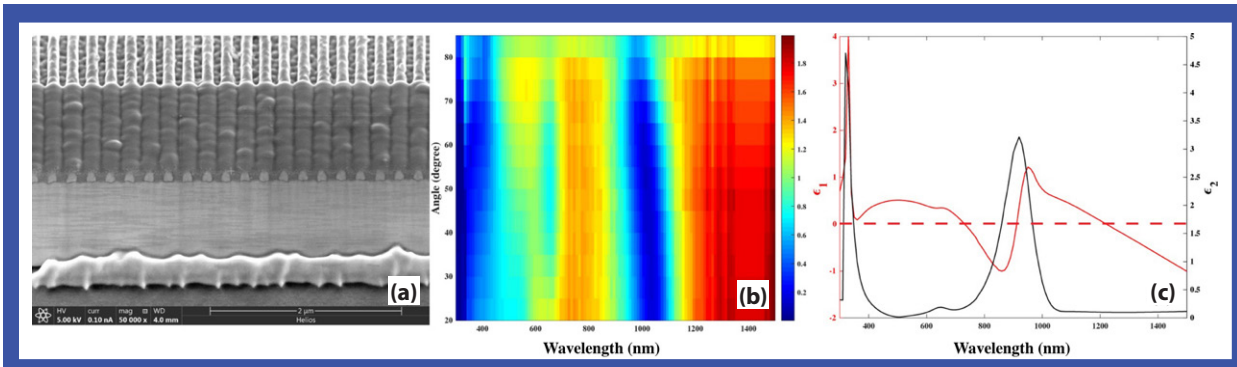

Fig. 1: (a) Scanning electron microscope (SEM) image of metaoptic silver (Ag) ridge structure; **(b)** reflectance, $R(k,\omega)$; and **(c)** dielectric response spectrum, $\varepsilon(\omega)$. Courtesy Milan Palei and Anthony Hoffman.

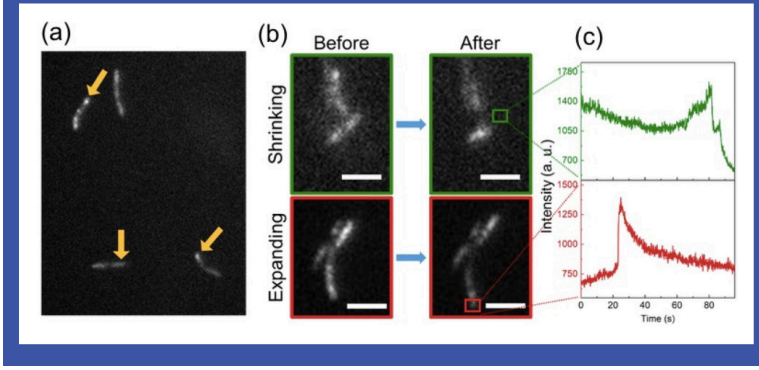

Fig. 2: Dynamic interfacial reconstruction of *Myxococcus xanthus*. **(a)** Spatially anisotropic luminescence along individual cell bodies. **(b)** Images and **(c)** intensity-time plots showing dynamic reconstruction under potential control. Courtesy Vignesh Sundaresan and Paul Bohn.

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

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Research Plans and Progress: Plant tissues are often 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 project 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. The team 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. The project will use it to address biological problems connected to cell wall assembly in grasses. Most specifically, the team 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 (SRS) signals; (2) to determine a unique combination of fingerprints for various cell wall components and selected metabolites; and (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/Deliverables:
Progress to date includes:

1. Development of a fully functional open-source multiphoton scanning system
   a. Added open-source fluorescence-lifetime imaging microscopy (FLIM) imaging acquisition in the project's OpenScan system (manuscript in preparation)
   b. Added open-source FLIM Analysis package – FLIMJ (manuscript in preparation)
2. Implementation of a fast two-channel time domain–based FLIM system with polarization control
   a. Currently supports four detection channels at <1-MHz scanning frequency
   b. Published Fast FLIM optimization based on nonparametric Bayesian model (Wang et al. 2019)
   c. Polarization-based imaging of autofluorescence (manuscript in preparation)
3. Development of a novel fiber-based spectral detector
   a. Published spectral detection proof of principle (Sagar et al. 2019)
   b. Ongoing work on autofluorescence- and scattering-based contrast
   c. Ongoing work to use GPU-based real-time spectral contrast
4. Development of a novel scheme to detect forward and backward second harmonic generation
   a. Implemented (manuscript in preparation)
   b. Testing with plant samples ongoing
5. Development of hyperdimensional imaging (HDIM) microscope for multiparametric signature registration to record emission spectrum, fluorescence lifetime, and rotational anisotropy.

Potential Benefits/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, the project 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.

References of Published Papers in Last Funding Year

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2. Sagar, M. A. K., et al. 2019. "Optical Fiber-Based Dispersion for Spectral Discrimination in Fluorescence Lifetime Imaging Systems," *Journal of Biomedical Optics* **25**(1), 1–17. DOI:10.1117/1.JBO.25.1.014506.
3. Otegui, M. S. "Imaging Polyphenolic Compounds in Plant Tissues," *Recent Advances in Polyphenol Research* **7**, Chapter 11. In press.

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

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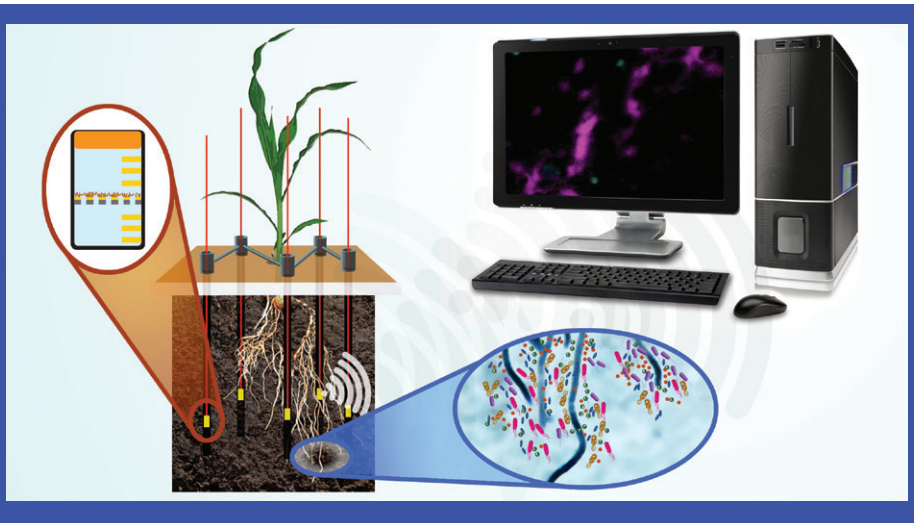
Overview: Interactions between plants and microbes define the growth and health of individual plants and plant communities. Most of these interactions involve communicating chemicals that travel through the rhizosphere. Communications within single species are also picked up by other species in the community. Despite the importance of these communications to agriculture, the environment, and the development of clean energy sources, little is known about these activities in the rhizosphere.

Research Plans: The 4DMAPS project will provide the means to probe the distributions and time-dependent changes of these chemical communicators and their changes in response to perturbations of the system, such as occurs with variations in nutrients, water, temperature, and pathogen presence. Molecular sensors located in and close to the rhizosphere will detect specific communicating molecules and relay that information to the 4DMAPS instrument, which will integrate input from a large number of electrochemical sensors that are distributed throughout the soil and vertically mobile to produce real-time images of the locations and concentrations of the signaling molecules. The molecular specificities 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.

Progress: Over this past year, the overall objective has been to develop and test the component parts of the 4DMAPS system. To that end, this project has achieved the following: (1) sensors have been created and demonstrated to function in the salt concentrations and pH found in soil to measure a protein and two small molecules, (2) two molecular targets for aptamers have been synthesized, (3) aptamers are in the process of selection against one signaling molecule, (4) sensors have undergone the first steps of miniaturization, and (5) a microbiome has been identified that can be used in a synthetic soil in a mixed community containing plants.

Potential Benefits/Applications: The goal of this project is to develop an instrument that can image in real time the chemicals in the rhizosphere that govern a 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 agricultural applications to monitor nutrient transformations and rhizosphere microbial metabolic activities that contribute to the growth of healthy, productive plants.



4DMAPS aptasensors are attached to moveable rods in shafts around a plant root. The sensors report wirelessly 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 it changes with time. Courtesy Ames Laboratory.

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

Principal Investigators: Ryan Tappero[1] (PI), David (Peter) Siddons,[1] Mourad Idir,[1] Sunny Liao,[2] Jenny Bhatnagar,[3] and Rytas Vilgalys[4]
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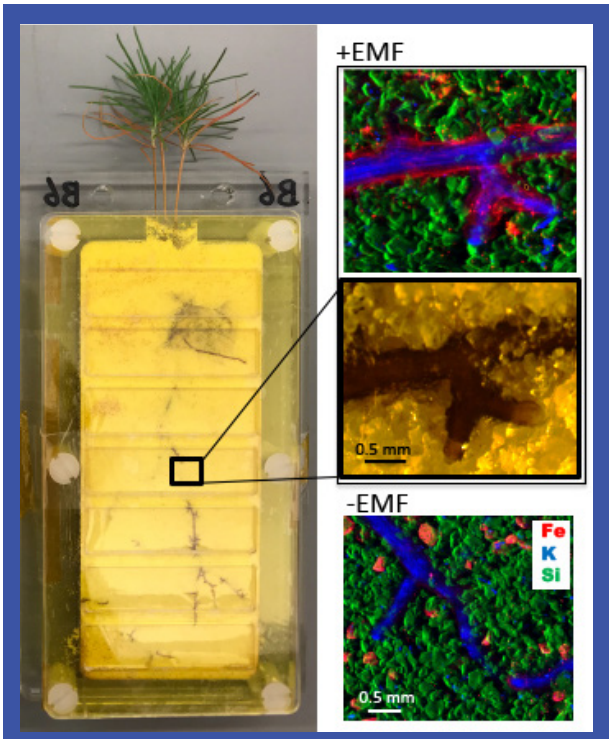
Research Plans and Progress: This project's goal is to develop a full-field X-ray fluorescence imaging system (FFFI) for studying spatial and temporal dynamics of trace elements in complex biological systems. Conventional X-ray fluorescence (XRF) imaging is performed in a scanning-probe modality. A limitation of scanning XRF is the time required to record images one pixel at a time. The team is developing 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/Deliverables: Progress to date includes (1) development of model soil and rhizosphere microcosms, (2) benchmark and time-course measurements of *Pinus-Sullius* model system (compatible/incompatible pairs), (3) design simulations/evaluation of different detector magnification system solutions (Wolter, coded aperture), (4) detector readout system design, (5) detector ASIC design [130-nm process] and redesign (65-nm process), and (6) pink beam imaging tests with polycapillaries and MURA (coded aperture) using commercial TimePix3 sensor.

Potential Benefit/Applications: Project seeks to deliver a first-generation, working prototype of the 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., iron, copper, and zinc). Dynamics and transport processes on this time scale can be studied. Another expected outcome is a design blueprint for future development of a second-generation, sub-10 micron resolution model of the FFFI detector system that could be deployed on benchtop X-ray sources.



(Left) Rhizosphere microcosm developed for live-plant XRF imaging of trace elements at the plant-soil interface. **(Right)** Scanning-probe XRF images of iron (Fe, red), silicon (Si, green) and potassium (K, blue) in pine rhizosphere with (+EMF) and without (–EMF) its host-specific ectomycorrhizal fungi. Microcosms *without* EMF contain predominantly unaltered grains of iron-coated sand following two months of growth, while those *with* EMF contain severely altered grains that have largely disintegrated, and roots show fungal sheath enriched with iron. Spatially resolved X-ray absorption spectroscopy has identified an Fe(III) organometallic complex within the fungal sheath. A ligand-promoted dissolution mechanism is used for Fe acquisition in the *Pinus-Sullius* system. Courtesy of FFFI Science Team.

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

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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. The project is using 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. Project objectives are to (1) develop a label-free method of visualizing microbial cells and organic matter in mineral and soil matrices; (2) develop AO 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 the project's AO-multiphoton microscope to rhizosphere and soil pilot studies. The goals of this performance period were to build a multimodal multiphoton microscope, model optical aberrations caused by soil minerals, and design an adaptive optics system for correcting these aberrations.

Accomplishments: In this first year, the project hired two excellent postdoctoral researchers, who are working with the microscopy and AO teams. The microscopy team built the project's multimodal microscope, including the coherent anti-Stokes Raman scattering, stimulated Raman scattering, and two-photon excitation systems, and three of the five detection systems. The team achieved first light and detection with this system. The team also parameterized the optical scattering characteristics of well-constrained mineral samples. The AO team used these characteristics to model the aberration properties. In turn, the AO team used the model to evaluate AO correction algorithms and design an AO system optimized for soil minerals. The team then built an AO testbed based on this design, which researchers will use to experimentally validate their model, test AO configuration and algorithms, and finalize the AO system design.

Potential Benefits Beyond DOE: AO, 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. Project research will focus on extending these approaches to highly scattering materials. Therefore, this research has the potential to improve methods for imaging in and through skin, bone, and other highly scattering biological materials. The team anticipates 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 AO with multiphoton microscopy.

In addition, agricultural, microbial, and environmental researchers are interested in the specific application that this project is targeting—rhizosphere and soil processes. A major part of this research will be determining the optimal modes of imaging in these systems. Therefore, the project's 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, the LLNL team is 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

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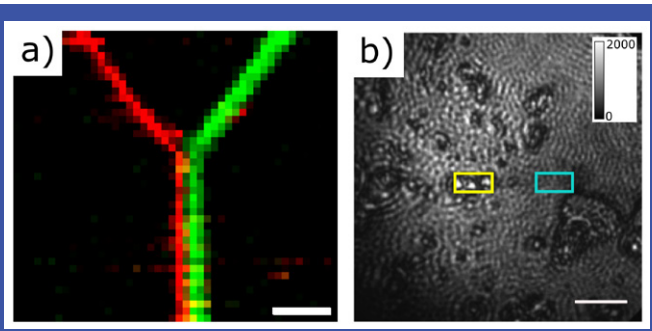
Research Plans: This project aims to develop a multimodal imaging platform integrating novel and existing analytical capabilities whose synergy yields 3D spatiotemporal chemical information in the bulk and at the interface in biological systems *in situ*. The objectives are to (1) develop new bioimaging modalities, vibrational sum-frequency generation (vSFG) microscopy, and *in situ*–liquid extraction–mass spectrometry (*in situ* LE-MS); (2) couple vSFG, coherent anti-Stokes Raman spectroscopy (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/Deliverables: The project has successfully developed the first-of-its-kind *in situ* LE-MS imaging platform that utilizes a porous membrane microfluidic surface in combination with a continuous LE sampling probe. Several microfluidic devices having known channel geometry were fabricated for *in situ* LE-MS imaging and supplied with a constant flow of model aqueous compounds (e.g., indole-3-acetic acid, caffeine, and propranolol) for validation of the technique. The *in situ* LE-MS system was shown to continuously extract nL/min flow rates for mass spectrometric analysis at any point along the porous membrane surface. This technology was validated by chemically imaging the solvent composition inside a two-channel microfluidic device via microsampling of the sample solvent flow. Research into improving the spatial resolution of the technique has led to the development of a moveable sampling probe having a long (several-meter) line without compromising the sensitivity and throughput of the technique.

Simultaneously, a wide-field CARS microscopy was developed for rapid and simultaneous acquisition of CARS images across an entire field of view. Using a total internal reflection (TIR) excitation scheme incorporating a femtosecond titanium:sapphire (Ti:sapphire) oscillator to generate both spectrally asymmetric pump and temporally chirped broadband near-infrared Stokes pulses, sufficiently strong excitation fields necessary to drive the third-order optical responses over the large focal region were achieved. The wide-field CARS microscopy allows access to a wide range of Raman modes spanning ~1,000 to ~3,500 per cm and a straightforward means to spectrally select specific modes with vibrational frequencies within this range. The unique capabilities of this novel wide-field CARS microscope were validated through acquiring high-quality CARS images from model and complex biological samples, including *Pantoea* sp. YR343 cells. Lastly, the *in situ* LE-MS and CARS imaging modalities are in the process of being combined.

Potential Benefits/Applications: The *in situ* LE-MS imaging modality allows broad chemical analysis of the liquid flow inside a microfluidic device without affecting the operation of it. The team believes that this capability will enable a number of biological systems to be chemically monitored *in situ* in an untargeted manner. Further, this technology can be readily adapted by many laboratories. The wide-field CARS technology enables greater temporal imaging than is capable with current systems. Both *in situ* LE-MS and CARS developments have resulted in patent applications and could become commercial products in the future. Together, these imaging technologies would enable currently unobtainable insights into the chemical dynamics and localization in living systems such as plant-microbe dynamics occurring in the rhizosphere.



(a) *In situ*–liquid extraction–mass spectrometry image of propranolol (red) and caffeine (green) in a continuously flowing microfluidic device. **(b)** Chirped pulse–CARS microscopy of fixed *Pantoea* sp. YR343 cells at 2,880 per cm without the use of molecular labels. Courtesy John F. Cahill and Yingzhong Ma. Reprinted with permission from Cahill, J. F., et al. 2020. "*In Situ* Chemical Monitoring and Imaging of Contents Within Microfluidic Devices Having a Porous Membrane Wall Using Liquid Microjunction Surface Sampling Probe Mass Spectrometry," *Journal of the American Society of Mass Spectroscopy*. DOI: 10.1021/jasms.9b00093. Copyright 2020 American Chemical Society.

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

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Research Plans and Progress: This project is developing 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 completed, this technology will be initially tested using model systems of relevance to BER's bioenergy and environmental microbiology research thrusts.

Current and Anticipated Accomplishments: The project's novel spectral imaging platform provides significant advantages over the currently employed approaches to metabolic mapping, simultaneously achieving (1) tunable spectral resolution (0.1 to 1 nm) for narrow-band/high-resolution measurements; (2) fast acquisition speeds to track, for example, microbial motion and nutrient uptake in real time; (3) high detection sensitivity to facilitate both high-speed acquisition and to empower low–light level registration; and (4) ease of switching between nanoscopic and macroscopic probing volumes.

These desirable properties are realized through the development of novel spectroscopic-grade hyperspectral/spectral imaging detectors coupled to a flexible custom-built optical platform that affords multimodal spectral imaging in a single setup across nano-micro-meso-macroscopic spatial domains.

To date, the project has constructed a multimodal spectral imaging platform equipped with three optical axes that support optical-absorption/dark-field, fluorescence, and Raman imaging using various light sources. With this setup, the team has been able to detect absorbance signals less than 10 mOD in magnitude at low incident powers and using integration times on the order of 5 milliseconds/pixel in a point-scanning hyperspectral imaging scheme. The team is currently in the process of upgrading its software and detection system to facilitate full spatiospectral mapping at significantly improved acquisition rates of ~5 milliseconds per line. In parallel, the team is implementing a line-scanning hyperspectral imaging modality that will achieve similar acquisition rates at much higher pixel densities.

The dark-field scattering imaging modality boasts a signal-to-noise ratio of ~1,000:1 and full incident light rejection by virtue of the project's excitation/collection geometry without the use of filters in either the excitation or detection paths. The project's newly discovered technique—dark-field fluorescence-based hyperspectral imaging—enables collection of high signal-to-noise images and will potentially allow collection of multiplexed, multifluorphore images without the need for filters in the excitation and collection arms. The team is currently benchmarking its system on single-cell systems and will commence analysis of plant cell wall composition over the next few months.

Potential Benefits: The team anticipates its platform will result in an unprecedented level of molecular-level insight into metabolic pathways in microbial communities and plants. Achieving this goal will provide technology critical to understanding 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, this project would be able to build a knowledge base that would put it 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.

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