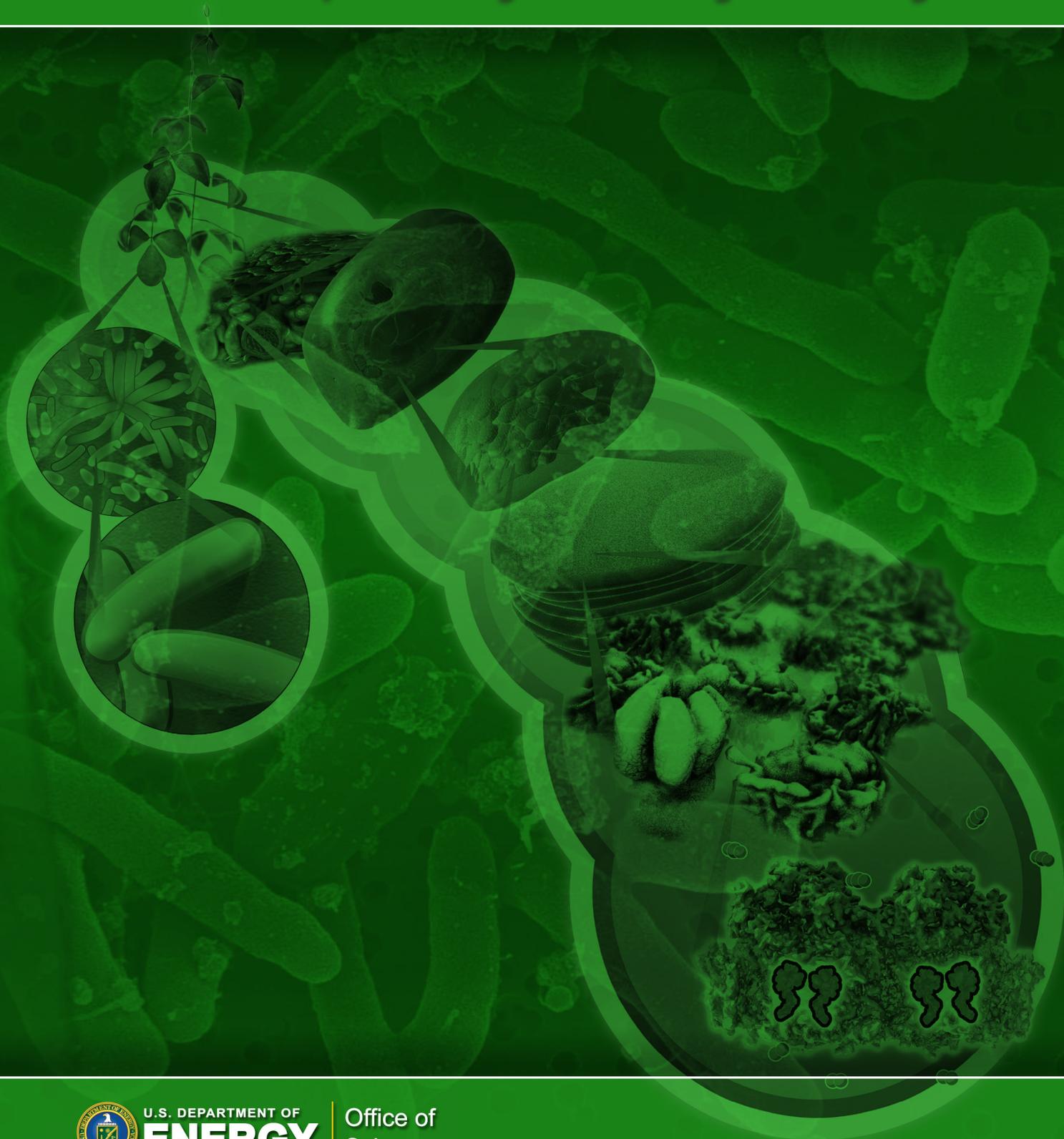


Mesoscale to Molecules: Bioimaging Science (Technology) Program 2016 Principal Investigator Meeting Proceedings



U.S. DEPARTMENT OF
ENERGY

Office of
Science

Office of Biological and Environmental Research

Mesoscale to Molecules: Bioimaging Science (Technology) Program

Principal Investigator (PI) Meeting

August 2–3, 2016
Gaithersburg, Maryland

Program Manager

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

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Cover illustration: Metabolic processes integrate genetically programmed molecules into structures that span different physical scales (**left:** microbe and microbial community, **right:** plant). Imaging and measurement technologies that can resolve multiple key metabolic processes over time within and among cells will enable the linking of molecular-scale information to whole-cell, systems-level understanding. [Plant component image adapted from Iowa State University figure, p. 2 of BER. 2015. *New Bioimaging Technologies for Plant and Microbial Systems*. Microbe and microbial community images adapted from Fig. 2.1 of BERAC. 2013. *BER Virtual Laboratory: Innovative Framework for Biological and Environmental Grand Challenges; A Report from the Biological and Environmental Research Advisory Committee*, DOE/SC-0156.]

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Bioimaging Science (Technology) Program
2016 Principal Investigator (PI) Meeting Proceedings**



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Preface

The first annual Mesoscale to Molecules: Bioimaging Science (Technology) Program Principal Investigator (PI) Meeting was held at the Gaithersburg Marriott Washingtonian Center, Gaithersburg, Maryland, on August 2–3, 2016. The program's mission is to understand translation of genomic information into the mechanisms that power living cells, communities of cells, and whole organisms. The program's goal is to develop new imaging and measurement technologies to visualize the spatial and temporal relationships of key metabolic processes governing phenotypic expression in plants and microbes. Annual program PI meetings are intended to bring together the investigators contributing to the program, review progress and the current state-of-the-art in bioimaging research, and provide a platform for stimulating discussion and exchange of ideas among program investigators. The meeting's proceedings provide an outline of the program's current state and potential future directions and opportunities.

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Table of Contents

List of Funded Projects	vi
Executive Summary	1
Program Update	1
Potential Future Directions and Opportunities.....	3
Abstracts	4

List of Funded Projects

Universities

**Multifunctional Plasmonics Nanoprobes
for Cellular Sensing and Imaging**

Duke University

**Integrated and Dynamic Multispectroscopic
In Situ Imaging of Plant Metabolism at the Level
of Subcellular Compartments**

Iowa State University

**Development of Biosensors to Measure the Spatial and
Temporal Concentration Profiles of Inorganic Phosphate
in Plants During Arbuscular Mycorrhizal Symbiosis**

*Texas A&M University and Boyce Thompson Institute
for Plant Research*

**Multiscale Dynamics of Water Regulation by
Bacteria in Synthetic Soil Microsystems**

University of Connecticut

**Development and Refinement of an *In Situ*
“Molecular Microscope” Utilizing
Ultrahigh-Resolution Mass Spectrometry**

University of Missouri, Columbia

**The Transparent Soil Microcosm: A Window into
the Spatial Distribution and Dynamics of Carbon
Utilization and Microbial Interspecies Interactions**

University of North Carolina at Chapel Hill

**Development of a Novel High-Precision,
High-Resolution SIMS Platform for Elemental
and Isotopic Characterization of Microbial Cells
at a Systems Level**

Washington University in St. Louis

National Laboratories

Small Worlds

Argonne National Laboratory

Adaptive Biosystems Imaging

Oak Ridge National Laboratory

**Systems Biology Based on an Integrated,
Mesoscale Imaging and Analysis Framework**

Pacific Northwest National Laboratory

**SLAC Mesoscale Integrated Biology Pilot Project:
MFX Station at LCLS**

SLAC National Accelerator Laboratory

Executive Summary

The Mesoscale to Molecules Bioimaging Technology Program, within the Department of Energy's (DOE) Office of Biological and Environmental Research (BER), sponsors research that addresses grand challenges for biology. These grand challenges are centered around developing a better understanding of the spatial and temporal distributions of key metabolites and biomarkers associated with microorganisms and plants related to bioenergy and environmental systems. The Bioimaging Technology Program is advancing new and improved instruments that are being designed and constructed from the ground up, adapted from other scientific disciplines, or upgraded with new capabilities to create a comprehensive and versatile toolbox for imaging biological dynamics and chemistry. These instrument development approaches involve (1) hypothesis-based technologies focused on solving particular challenges for plant biology, biofuels, and biogeochemistry and (2) broad-based systems and design-based technologies that can lead to new discoveries. Both approaches are being pursued with versatility, transferability, and portability in mind to foster widespread adoption by the larger science community for future field and laboratory studies.

Modalities spanning electron, ion, optical, Raman, and X-ray microscopy and spectroscopy are being used with dynamic, *in situ*, cryogenic, or fixed samples and analyzed with both nondestructive and destructive approaches for holistic bioimaging. A major focus for the Bioimaging Technology Program are new optical developments advancing multimodal optical and fluorescence microscopy and Raman microspectroscopy. These activities are complemented by innovative applications of electron and ion microscopy as well as X-ray absorption spectroscopy and microscopy and femtosecond crystallography at DOE-sponsored user facilities. Imaging mass spectrometry capabilities also are being further enhanced to yield highly selective, sensitive, and quantitative chemical maps that identify intra- and extracellular gradients and the distribution, abundance, and fate of stable isotopes, natural elements, and metabolites. This work supports simultaneous observations using conventional microscopies for correlated structural and chemical imaging and the interpretation of biological function.

Complementary novel fluidic devices are creating controlled environments to visualize how biosystems sense and respond to environmental changes. New tracers, probes, and sensors are further expanding the impact of the new instrumentation and fluidic devices by enabling dynamic tracking of targeted cells, organelles, enzymes, biomarkers, and small molecules. Both label-based and label-free imaging approaches are being pursued to visualize complex pathway dynamics occurring in living biosystems while also detecting chemical signatures. These new technologies will help track cellular responses to environmental perturbation, spatially localize complex mechanistic pathways, and visualize structural dynamics of macromolecules.

In addition to pursuing advances within each of these respective techniques (e.g., optical, electron, X-ray, and ion-based

approaches), a major continuing focus of the Bioimaging Technology Program should center on the integration of these different approaches, linking the resulting complementary data to create a holistic picture of the biological systems being imaged. For such cross-platform imaging, the incorporation of methods that permit indexing and registration of images (such as multifunctional tracers, probes, and sensors to act as cross-platform fiducial markers) is required so that disparate datasets of different formats, magnifications, or resolutions for the same sample can be overlaid and co-referenced in a meaningful fashion. Realization of all these technologies will produce a diverse set of bioimaging data that requires integrated data processing algorithms, visualization, and modeling to properly interpret and connect the imaging data to omics-based organism models or pore-scale lattice Boltzmann models and root-scale water flux models.

Finally, biological imaging is inherently transdisciplinary, and successful teams must continue to reflect this approach by integrating researchers in the imaging, technology development, nanoscience, and computational communities with structural biologists, genomic scientists, ecologists, and biogeochemists as a critical step toward translating laboratory-developed technologies into the natural environment. This process will address the need to dynamically image complex (and often unknown) native microbial populations and to investigate community organization and how multiple metabolisms and processes co-occur in space and time.

Program Update

BER's Mesoscale to Molecules Bioimaging Technology Program incorporates sponsored research at four national laboratories and seven universities across the United States. All the projects are addressing a grand challenge for biology to enable *in situ* and dynamic bioimaging across a range of spatial and temporal scales. As no single instrument spans the complete spatiotemporal landscape for bioenergy and environmental bioimaging needs, multiple new instruments, methods, and techniques are being developed and employed to bridge current gaps.

At the heart of bioimaging technology platforms is core instrumentation. New instruments are being developed, and existing instruments are being applied (or adapted from other scientific disciplines) and upgraded to complement other modalities in an effort to create a versatile and powerful toolbox for imaging biological dynamics and chemistry occurring across scales. These scales range from small unicellular organisms to complex microbial and fungal community interactions with plants. Due to their noninvasiveness and ability to record dynamic events (in many cases nondestructively), optical methods have been the predominant *in situ* bioimaging methods. Consequently, a major focus for BER's Bioimaging Technology Program are new developments advancing confocal, super-resolution/subdiffraction, interferometric, multifocal and hyperspectral microscopy, and Raman microspectroscopy (spontaneous, surface-enhanced scattering, and stimulated and coherent

anti-Stokes scattering). Both label-based and label-free imaging approaches are being commissioned to visualize complex pathway dynamics occurring in living biosystems while also detecting target chemical signatures.

Innovative applications of X-ray absorption spectroscopy, nanotomography, and macromolecular femtosecond crystallography using DOE-sponsored synchrotron and X-ray free electron laser facilities, along with scanning and transmission electron microscopy, are being pursued to complement the optical approaches. These applications will provide ultrastructural, whole-cell context as well as detailed structures and mechanisms of macromolecular complexes. *In situ* molecular, elemental, and isotopic imaging (atmospheric pressure laser ablation electrospray ionization [LAESI]/matrix-assisted laser desorption ionization [MALDI], 21 Tesla Fourier transform ion cyclotron resonance, and secondary ion mass spectrometry) are being developed to provide important insight into metabolite identities, spatial distributions, and rates of metabolic activity with single-cell spatial resolution.

Along with new instrumentation that supports the framework for capturing dynamic movies or time-lapsed images, novel fluidic devices are creating controlled environments to visualize how biosystems sense and respond to induced 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 as impacted by controlled changes to their local chemical and physical environment. In many of the instruments described previously, these fluidic devices are empowering *in situ* or correlative observations simply by creating controllable chambers in which biosystems can thrive while protecting the sample from inherent instrument constraints. For example, the need in many electron, ion, and X-ray microscopes to maintain high-vacuum conditions in order to optimize imaging beam stability and resolution typically prevents *in situ* observations. However, incorporating fluidic devices into these imaging modalities is heralding new avenues of research. For example, simultaneous quantitative tracking and comparison of multiple strains in equivalent environments during controlled chemical exchanges or depletion is being performed, providing insight into specific protein expression levels as a function of nutrient availability.

Finally, new tracers, probes, and sensors are expanding the impact of the new instrumentation and fluidic devices by enabling dynamic tracking of targeted organelles, enzymes, and small molecules. Inverse molecular sentinels, caged Raman and fluorescent probes, activity-based probes, functionalized quantum dots, engineered fluorescent proteins, optogenetic regulators, and Förster resonance energy transfer (FRET) inorganic phosphate and root exudate biosensors all are being designed, engineered, and implemented. The ultimate goal is to sense and track lipids, metabolites, enzyme activity, mRNA, and microRNA in living biosystems, in correlation with controlled changes to the local

chemical or physical environments or using light-based control of expression. The small size and varied nature of metabolites are a common challenge in tracking the fate and distribution of metabolites in biological systems. In many cases, their small size prevents physical labeling with extrinsic tags, while their relatively rapid transit and broad concentration range complicates analytical measurements. Thus, considerable efforts are ongoing to minimize or avoid any alteration of normal cellular function or mechanisms of small-molecule uptake, ultracellular localization, metabolism, or fate when exposed to novel tracers, probes, and sensors.

Any one of the platforms previously described can generate highly informative data on biosystem dynamics, but multimodal, correlative, and integrative approaches can be significantly more powerful. While new hybrid instruments are being developed to enable simultaneous acquisition of dynamics, chemistry, topology, and ultrastructure within the same instrument, most of BER's Bioimaging Technology Program projects involve multimodal or correlative imaging using a suite of separate instruments. Modalities spanning electron, ion, optical, Raman, and X-ray microscopy and spectroscopy are being used with dynamic, *in situ*, cryogenic, or fixed samples and analyzed with both nondestructive and destructive approaches for holistic bioimaging. For such cross-platform imaging, the incorporation of methods that permit indexing and registration of images is required so that disparate datasets of different formats, magnifications, or resolutions for the same sample can be superimposed and co-registered in a meaningful fashion. Multifunctional tracers, probes, and sensors are being exploited as cross-platform fiducial markers that provide both direct indexing and registration. For example, glycine-conjugated quantum dots are helping to identify rhizosphere microbial community structure and environmental ligands because they can be detected by fluorescent, electron, and X-ray imaging modalities.

Realization of all these technologies will produce a diverse set of bioimaging data that will require integrated visualization and modeling to properly interpret and connect the imaging data to omics-based organism models or pore-scale lattice Boltzmann models and root-scale water flux models. Such models are expected to improve mechanistic understanding of how biological systems are not only impacted by their environment, but also how they directly change and modify the larger ecosystem.

Combined in a comprehensive portfolio, the resulting capabilities will enable the imaging of key metabolites and molecular biomarkers across the hierarchies and dimensions of biological systems to provide understanding of a diverse array of biological and environmental processes. The primary focus of BER's Bioimaging Technology Program is to expand the frontier of new bioimaging, but validation of the new technologies also is planned using real-world biological applications of relevance to bioenergy and the environment (understanding and controlling quorum sensing, improving lipid feedstock yields, enhancing lignocellulosic deconstruction, or boosting feedstock sustainability and plant drought tolerance). Living plants (e.g., *Medicago truncatula* and *Brachypodium distachyon*), microbes (e.g., chemotrophs [*Bacillus subtilis* and *Yarrowia lipolytica*], phototrophs [*Cyanothece*, *Rhodospseudomonas*

palustris, *Ostreococcus tauri*, and *Chlamydomonas reinhardtii*] and mycorrhizal symbioses (*Methylobacterium*), and plant-microbe interactions (arbuscular mycorrhizal symbioses and *Glycine max* with *Bradyrhizobium japonicum*) are all being investigated to understand nutrient utilization and community and ecosystem interactions (e.g., soil water retention due to the presence or absence of particular organisms or biomass). These studies will lead to a better understanding of spatial and temporal metabolite distributions associated with growing microbial and plant systems.

Potential Future Directions and Opportunities

Biological imaging is inherently transdisciplinary, and the research and development process aimed at advancing DOE programmatic goals for the Mesoscale to Molecules Bioimaging Technology Program must continue to reflect this approach. Advances in biological imaging require integrating the expertise of the imaging, technology development, nanoscience, and computational communities. Also essential is reaching across biological disciplines to incorporate structural biologists and genomic scientists as well as ecologists and biogeochemists as imaging approaches are deployed in the natural environment.

BER's Bioimaging Technology Program spans a broad spectrum of technologies, including complementary nondestructive and destructive imaging modalities (e.g., optical, electron, X-ray, and ion-based approaches) that cover a range of spatial and temporal scales. In addition to pursuing advances within each of these respective techniques, a major focus of the program moving forward should center on the integration of these different approaches, linking the resulting complementary data to create a more holistic picture of the biological systems being imaged. Another critical need is to develop selective probes that allow identification, sensing, and functional imaging of key metabolites to molecular and genomic biomarkers in complex biological systems. Important advances include the simultaneous marking, tracking, and sensing of multiple players (elements, metabolites, and molecular biomarkers) in a given biological system. This capability also will provide essential flexibility to broaden the scope of investigations, opening new possibilities to discover key biomarkers or intermediates that are still unknown. Although probing a sample inherently perturbs it, methods based on selective, probe-induced perturbations of key biotargets or metabolic pathways of particular organisms could provide a unique means to investigate and understand complex native communities.

A major near-term challenge is translating laboratory-developed technologies into the natural environment. This process will involve incorporating the dynamics of microbially driven biogeochemistry (e.g., within the rhizosphere, biofilms, and other key biological interfaces), as well as imaging complex (and often unknown) native microbial populations to investigate community organization and how multiple metabolisms and processes co-occur in space and time.

The ultimate goal of such an approach is to generate spatially resolved snapshots of relevant cellular metabolomes, including both primary and secondary metabolites, as well as internal and secreted compounds. The real-time collection and interpretation of these integrated data will be a major advance in bioimaging technology—one that will provide new understanding for monitoring and understanding phenotyping in the laboratory and in complex natural environments.

To achieve these goals, advances in several key areas are needed, including (1) development of advanced probes that expand the monitoring capability for important biotargets ranging from key metabolites to molecular and genomic biomarkers; (2) development of associated delivery mechanisms (e.g., micro and nanofluidics) to more comprehensively sample key compounds and biomolecules; (3) development of new or improved imaging technologies capable of watching systems grow and evolve in their natural state, while acquiring real-time data across the full spectrum of relevant spatial scales; (4) development of cross-platform protocols for sample preparation, indexing and spatial registration, and data verification and correlation to increase the suite of complementary analyses that can be conducted on a given sample or suite of samples; and (5) improvements in data storage, processing, and visualization to enable effective extraction of critical biological and environmental information from the experimental data. Of special interest is a central clearinghouse for archiving experimental and simulation data and incorporating a standardized output and imaging framework for different analytical modalities that could be widely adopted. Such a data repository could be equipped to take advantage of advances in artificial intelligence designed to extract patterns from raw data for improved organization, interpretation, and representation.

The annual BER Bioimaging Technology Program's Principal Investigator (PI) Meeting provides a useful platform to increase horizontal (cross-platform) and vertical (cross-scale) synergies needed to achieve the aforementioned goals. Additional cross-team interactions (e.g., through teleconferencing or web conferencing) will help maintain this interactive momentum and catalyze new directions of investigation. Moreover, the creation of a Bioimaging Technology portal, detailing the diverse technological approaches and highlighting the applications for which they are best suited, would enable the program to impact a wider community of scientists who could use the new bioimaging approaches in their research. In particular, the creation of a "boot camp" (e.g., appended to relevant annual conferences) targeting young PIs, postdoctoral scholars, and graduate students from outside the current pool of DOE-affiliated researchers could dramatically expand the Bioimaging Technology Program's reach and have a transformational impact on the training of the next generation of scientists.

Abstracts

Multifunctional Plasmonics Nanoprobes for Cellular Sensing and Imaging

Principal Investigator: Tuan Vo-Dinh

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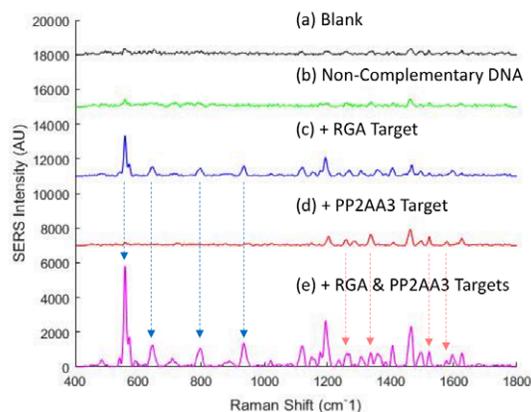
Collaborators: Zhen-Ming Pei and Tai-Ping Sun (Duke University); Kenneth Kemner (Argonne National Laboratory)

Project Summary: This goal of project is aimed at addressing the DOE FOA need to “develop and apply selective, nonperturbative probes to measure the spatial and temporal concentration profiles of nutrients, metabolites, signaling molecules, extracellular matrices and other biomolecules,” particularly as these are related to studies of plant and microbial biosystems relevant to DOE bioenergy programs.

1) Research Plans and Progress, Including Objectives and Goals for the Project Period. We have developed multifunctional plasmonics-active nanoprobes, called inverse Molecular Sentinels (iMS), which can provide tracking and sensing capabilities for use in the analysis of bioenergy-relevant plant systems. The multiplex capability of SERS is an important feature due to the narrow Raman bandwidths, which provides significant advantages over other methods. We have demonstrated the multiplexing capability of the iMS technique, two iMS nanoprobes were designed to target RGA and PP2AA3 genes and labeled with two different Raman dyes, Cy5 and Cy5.5, respectively (see figure at right). RGA gene belongs to a 5-gene DELLA family in Arabidopsis, which plays a critical role in controlling plant biomass. We have also used synchrotron-based X-ray absorption spectroscopy (XAS), which is a nondestructive, noninvasive method that can probe the speciation of elements in any environment of interest (e.g., in hydrated geo- and biomaterials, in solution, or in dried solids), to analyze gold nanospheres and nanostars used in the iMS probes.

2) Current and/or Anticipated Accomplishments/Deliverables for the Project Period. (These should be in terms of expected products probes, instrument, tools and technology development and not in descriptions of experimental design, procedure or presentation of manuscripts). We have demonstrated the feasibility of the inverse Molecular Sentinels (iMS) n technology, which can provide tracking and sensing capabilities for use in the analysis of bioenergy-relevant plant systems.

Multiplex Detection using a Mixture of RGA & PP2AA3 iMS



SERS spectrum of a mixture of two iMS nanoprobes targeting to RGA and PP2AA3 (PP2A). Spectrum (a): blank (in the absence of any targets). Spectrum (b): in the presence of non-complementary DNA. Spectrum (c): in the presence of RGA target. Spectrum (d): in the presence of PP2AA3 target. Spectrum (e): in the presence of both RGA and PP2AA3 targets. The arrows illustrate the increased intensity of the major SERS peaks in the presence of corresponding targets.

3) 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 study will contribute significantly to effective strategies to better control flowering time and increase biomass accumulation for biofuels and crops. In this study, the development of DELLA-specific nanoprobes will provide powerful tools to monitor spatial and temporal regulation of DELLA expression during plant growth and will contribute significantly to effective strategies to manipulate plant development to increase biomass for biofuels and for agricultural improvements.

Integrated and Dynamic Multispectroscopic *In Situ* Imaging of Plant Metabolism at the Level of Subcellular Compartments

Principal Investigator: Basil J. Nikolau
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Email: dimmas@iastate.edu

Collaborators: Diane Bassham, Young-Jin Lee, R. S. Houk, Arthur Winter, and Eve S. Wurtele (ISU); Jacob W. Petrich and Emily Smith (Ames Laboratory)

This project is developing and applying integrated molecular imaging technologies that can be used to monitor membrane lipid remodeling. Understanding the remodeling of membrane lipid topology in plant cells has major consequence in optimizing plant biomass productivity. The integrated molecular imaging technologies will be developed in the biological context of autophagy that remodels membrane lipid topologies that control spatially defined subcellular regions within plant cells and optimize plant biomass productivity during environmental stresses, which limit biomass productivity. Genetic stocks that will enable the dissection of membrane lipid dynamics have been identified and analyzed to identify specific target lipid molecules for molecular imaging. Analytic technologies for imaging these specific target lipid molecules via fluorescence, Raman and mass spectroscopy have been established. These imaging technologies are being developed in the context of computational capabilities that will integrate multi-spectral imaging with genome scale models. We have established an infrastructure that ensures transparent collaboration among the students of different collaborating groups. An integrated strategy is presented to address the goals of the project. These goals are being addressed by fulfilling the following tasks, and progress to date is indicated.

Task 1: Genetic and biochemical analysis of defined autophagy and lipid metabolism genes. The initial analyses focused on identifying specific lipid molecules that are affected by the autophagy-induced dynamics of cellular membranes. The rationale being that these specific lipid molecules will be targeted for imaging via the technologies that will be developed in Tasks 2-4.

Task 2: Develop and apply *in situ* optical imaging platforms. The team has demonstrated the first-of-its-kind, red-releasing photocage. Photocages are compounds that release a cargo or generate a change in a signal when exposed to light. For use in plant systems, it is desirable

to design photocages that release their cargo when exposed to a range of visible light wavelengths. This may enable multiple cargos to be released independently with different wavelengths of light or to increase the penetration depth of the light that generates the signal. Longer wavelengths of light are associated with deeper penetration depths in tissues, so a red-release photocage has this benefit. The team synthesized and demonstrated the use of the red photocage in a biological system.

Task 3: Chemical synthesis and tuning of self-destructing fluorophores for the *in situ* visualization of dynamic events. A new class of fluorescent chemical imaging probes capable of *in situ* imaging have been synthesized. In particular, we have designed and synthesized a new class of photocages derived from BODIPY dyes capable of dynamic fluorescence imaging using visible light. These probes release compounds with visible light irradiation with wavelengths spanning the visible and entering the near-IR.

Task 4: Spatial mapping of metabolites via mass-spectrometry. We have focused on optimizing the performance of atmospheric pressure mass spectrometry imaging (MSI) and apply the imaging technology to spatially map metabolites. These optimizations have reduced the laser spot size to approximately 50 μm , as compared to the 125 μm spot size that was available at the start of the project. In addition, we have focused on finding a technique that can be used to integrate information from different imaging platforms, i.e. mass spectrometry, Raman and fluorescence microscopy, and optical microscopy, which is essential for multimodal image comparison. This is especially important because MSI has far lower image resolution than optical imaging techniques.

Task 5: Develop computational imaging visualization platform. We are establishing a database with a visualization platform that the broader research community in different fields of chemistry, chemical engineering, biochemistry, and biology can readily access, comprehend and explore the data obtained from the combined and diverse analytical chemistries in the context of the biological materials under study. The major initial focus was the implementation of imaging visualization software.

Development of Biosensors to Measure the Spatial and Temporal Concentration Profiles of Inorganic Phosphate in Plants During Arbuscular Mycorrhizal Symbiosis

Principal Investigators: Wayne K. Versaw and Maria J. Harrison
Organizations: Texas A&M University and Boyce Thompson Institute for Plant Research
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Research Progress: Activities in year 1 of this project have focused on three areas. First, we have been engineering inorganic phosphate (Pi) biosensor constructs for expression in our target mycorrhizal host species, *Medicago truncatula* and *Brachypodium distachyon*. Different promoters in these constructs and their related controls will enable either constitutive expression in plants or specific expression in mycorrhizal roots. Transformation of *M. truncatula* with several of these constructs was initiated, and we expect the first batch of stable lines to be available by fall 2016. Second, we used in vitro assays to identify mutant Pi biosensors that can function in acidic conditions. We are currently using these “acid insensitive” biosensors to test strategies to target the proteins to acidic cell compartments, i.e., apoplast and vacuole. Third, we conducted an extensive study of imaging and image analysis methodology using available *Arabidopsis thaliana* lines that express related Pi biosensors.

Accomplishments/Deliverables: We developed methods to quantify variation in Pi-dependent FRET signals within populations of transgenic plants, to quantitatively account for nonspecific changes in FRET, and finally, to assess and correct for fluorescence quench that can occur in pigmented cells/cell compartments. A description of these methods was recently published: *Microscopy & Microanalysis* (2016) 22: 300-310.

Potential Benefits/Applications: The Pi biosensors and associated methodology we are developing are providing the first insights into the distribution and concentrations of Pi in plant cells. This information is of immediate importance to the plant/biofuel research community since Pi is frequently the limiting factor for plant productivity. A presentation of our work at the International Workshop on Plant Membrane Biology held in Annapolis, MD in June 2016 also highlighted the broad applicability of our imaging methods to other fluorescence-based biosensors, particularly our approach to *in vivo* biosensor calibration using microinjection.

Multiscale Dynamics of Water Regulation by Bacteria in Synthetic Soil Microsystems

Principal Investigator: Leslie M. Shor

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Collaborators: Daniel J. Gage and Yongku Cho (University of Connecticut); Jessica F. Chau (Benedict College)

The objective of this project is to develop a multifunctional *in situ* platform to link gene expression, regulation, and function with the spatiotemporal context of the rhizosphere. Using this platform, we aim to assess and model the impact of microbial exopolysaccharide (EPS) production on soil water retention and ecosystem resiliency.

Research Plans and Progress, Including Objectives and Goals for the Project Period:

During year 1, we have developed a synthetic rhizosphere microhabitat to measure pore-scale microbially mediated moisture retention. Devices feature physical microstructures that replicate aggregated and nonaggregated sandy loam soil. We are nearing completion of baseline microhabitat experiments performed without optogenetic control. We have also validated passive particle tracking to measure EPS characteristics *in situ*. We have also developed optogenetic tools for control of EPS production *in situ*. We have prepared host strain deletion of *exoY* and *expA*, and have established a control interface for optogenetic control in an open, non-structured setting. Finally, we have developed and validated a Lattice Boltzmann model of pore scale water evaporation in both

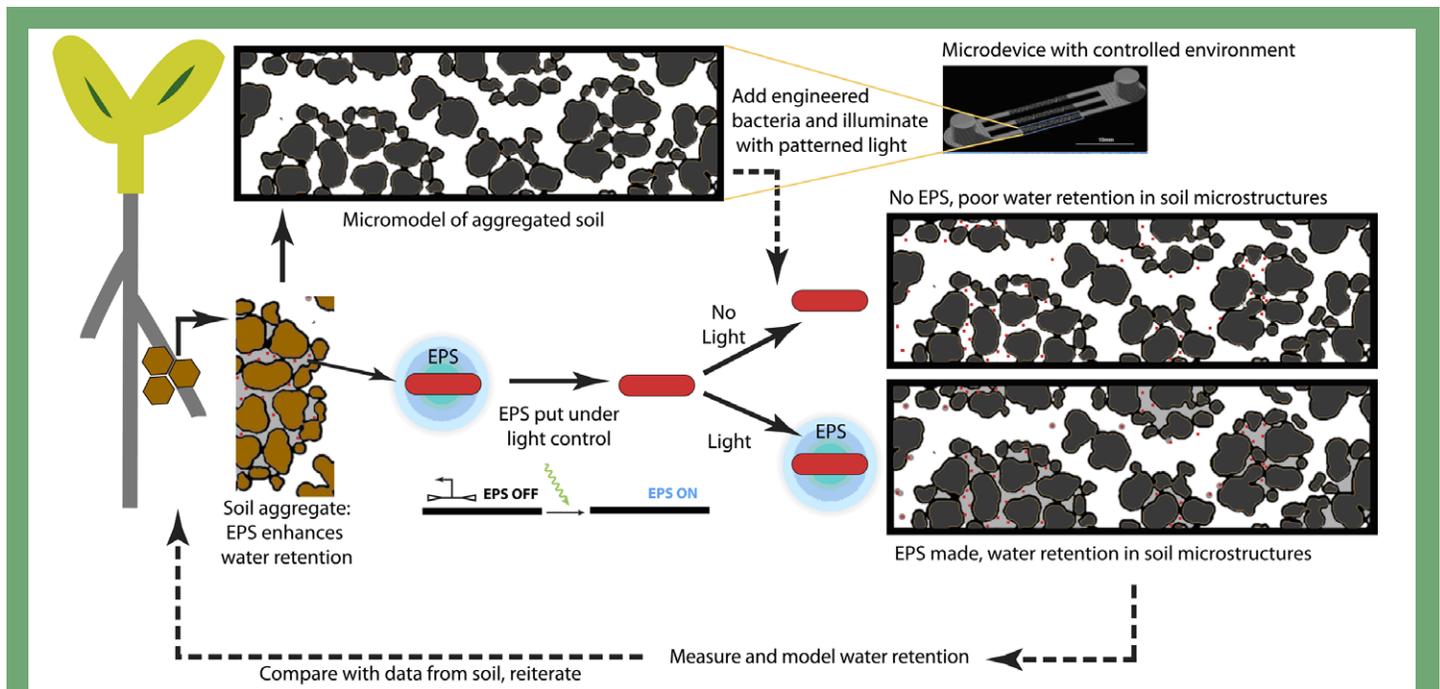
aggregated and non-aggregated sandy loam soil with hydrophilic and hydrophobic surface characteristics.

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

- A synthetic rhizosphere microhabitat—soil microdevices enabling optical imaging of pore-scale water retention and gene expression control, coupled with a humidity control system.
- Optogenetic tools for controlling gene expression in soil bacteria, with spatial and temporal resolution that enables the control of pore-scale EPS synthesis.
- A model of soil water transport that incorporates the effect of pore-scale geometry and surface hydrophobicity.

Potential Benefits/Applications and Deployment of Bioimaging Technology:

This project will develop a generalizable experimental platform to link gene function and micro-scale extracellular habitat conditions with the overall function of terrestrial microbial communities. Here, light addressable control of gene expression in soil bacteria within a defined microscale habitat will be integrated with a multi-scale model of water dynamics in soil. Specific intersections with ongoing ORNL and PNNL research projects have been identified. Results will continue to be disseminated to the broader community through conference papers, peer-reviewed journal publications, and private sector collaborations.



Multifunctional *in situ* platform to link gene expression, regulation, and function with the spatiotemporal context of the whole cell environment. Our approach includes fabrication of synthetic micro-habitats and development of novel optogenetic control capabilities. This projects offers an generalizable experimental platform and contributes to predictive understanding of modulate moisture retention and promote plant resiliency to drought conditions.

Development and Refinement of an *In Situ* “Molecular Microscope” Utilizing Ultrahigh-Resolution Mass Spectrometry

Principal Investigator: Gary Stacey

Organization: University of Missouri, Columbia

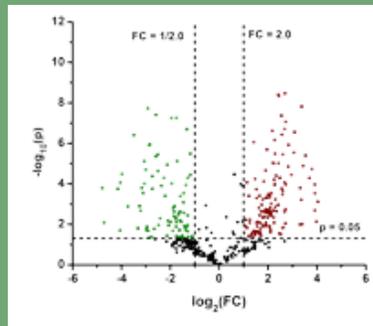
Email: staceyg@missouri.edu

Collaborators: Akos Vertes (George Washington University); Ljiljana Paša-Tolić, Christopher Anderton, and David W. Koppenaal [Environmental Molecular Sciences Laboratory (EMSL) and Pacific Northwest National Laboratory]

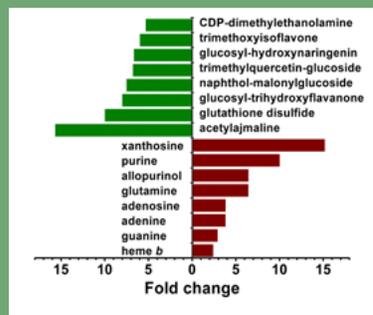
Project Summary: The ability to measure diverse biomolecules (e.g., proteins, metabolites, and lipids) in a single cell or several cells, their variation over time, and their response to environmental perturbations remains an exciting scientific challenge. This project will develop a new approach to image and observe these biomolecules simultaneously in their native cellular compartments, and to monitor their movement and fluxes, enabling a significantly enhanced understanding of how biological systems function, respond, and adapt.

Objectives: (1) Develop an advanced laser ablation electrospray ionization (LAESI) source, which is capable of unlocking the entire range of biomolecules within a single live cell. (2) Combine this source with ultrahigh-resolution mass spectrometry to (a) achieve unprecedented levels of molecular information with exceptional spatial detail in complex biological systems and (b) further demonstrate and validate *in situ* analysis using well-characterized plant-microbe interaction systems as models.

Approach and Progress: *In situ* mass spectrometry imaging investigations within the natural environment of biological specimens require new atmospheric pressure ion sources. LAESI is a new technology for direct microsampling and molecular imaging of biological tissues and cells. Two versions of this source are being developed and tested. First, the conventional LAESI source is being equipped by multimodal imaging capabilities through a long distance microscope. Simultaneously observing fluorescence and bright field images help us selectively target cells of particular phenotypes. The second LAESI source version will rely on laser pulse delivery through a sharpened optical fiber. This will facilitate the selective analysis of targeted cells in plant tissues. We began the development of the LAESI source by studying a well-characterized model plant-rhizobium system, specifically soybean (*Glycine max*) with a common mutualistic soil bacterium (*Bradyrhizobium japonicum*). This system was chosen due to its convenience and, more importantly, a wealth of baseline information that can be used to validate the measurements made using the LAESI system. Figure 1 shows the results of our initial studies that confirm distinct metabolic



Volcano plot comparing the metabolites identified significantly regulated in soybean nodules infected with a *NifH* mutant (unable to fix nitrogen) vs. those infected with the wild type.



Some examples of metabolites differentially regulated in the WT vs. *nifH* mutant comparison.

profiles between soybean root tissue infected with the bacterial wild-type, relative to similar tissue infected with a *nifH* mutant unable to fix nitrogen. Well over 200 different metabolites were identified from data derived from the LAESI-MS measurements. An example is heme, derived from leghemoglobin, a well-known, symbiotic-specific protein in rhizobial infected tissues.

Currently, we are in the process of putting together the dual modality microscope, for simultaneous bright field and fluorescence microscopy, which will be used for targeted LAESI-MS imaging via fiber optic ablation or transmission mode ablation. Project staff are now on-site at EMSL, beginning the process of combining the LAESI technology with high magnetic field Fourier transform ion cyclotron mass spectrometry (FTICR MS), which is uniquely available at EMSL. By interfacing a LAESI source to the high field FTICR MS at EMSL, *in situ* imaging of metabolites, lipids, peptides, and proteins will be feasible on a variety of Department of Energy (DOE)-relevant systems.

The Transparent Soil Microcosm: A Window into the Spatial Distribution and Dynamics of Carbon Utilization and Microbial Interspecies Interactions

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Research Progress and Plans

Overall Objectives: Establish a multi-modal imaging platform to enable the visualization of the spatiotemporal dynamics of microbial community interactions and carbon flow in soil-like environments.

Project Period Objectives: Build imaging chamber prototypes and optimize components for fluorescence and Raman microscopy; image microbes in transparent soil chambers. Progress to date: We have successfully met the initial objectives established in Aim 1 (“Establish a multi-modal microfluidic imaging platform to visualize activity in soil-like environments”) by using fused filament fabrication to generate chamber prototypes from ABS plastic. Troubleshooting these prototypes allowed us to optimize the size of the wells and ports, hydration requirements, imaging specifications, and O₂ limitation. Chambers to image microbe-microbe interactions (MMIs) are now being generated using laser-cut acrylic adhered with VaLaB to glass-bottom tissue culture dishes and sealed with an air-permeable polycarbonate membrane; fiducial markers are embedded on the glass and microcosm hydration is maintained via a potassium sulfate “moat.” Chambers to image microbe-root-carbon interactions (MRCs) have been designed to contain a top-loading plant port for seedling growth; roots descend into the chamber below formed by coverslips sealed with VaLaB and containing fluid flow ports to allow chamber hydration and the addition of nutrients, dyes, or bacteria (Fig. A). We are optimizing plant growth conditions using this device but anticipate fabricating future chambers using an alternative Form 2 SLA 3D printer, which is capable of creating high-resolution objects in intricate detail using diverse resins. Method development has also been successfully completed for optimizing the media and fluorescent dyes for analyzing bacteria and transparent soil using confocal fluorescence and Raman microspectroscopy. The growth medium MSg has been shown to be compatible with both fluorescence microscopy and Raman-based metabolic activity measurements, including analyses performed on liquid samples in the presence of transparent soil (Fig. B). In addition, we have begun successfully imaging fluorescently-labeled bacteria grown in transparent soil using these devices, including on natural sand particles spiked into the transparent soil (Fig. C).

Current and Anticipated Accomplishments/Deliverables: The current results are being directly translated into technology development (testing and refining the bioimaging chambers) for broader

A

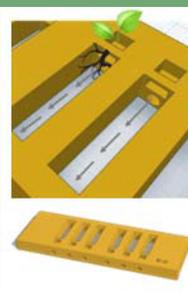
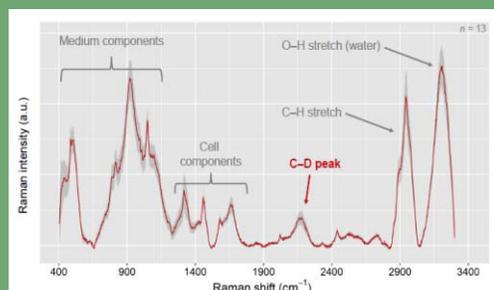


Fig. A. Depiction of MRC flow chamber.

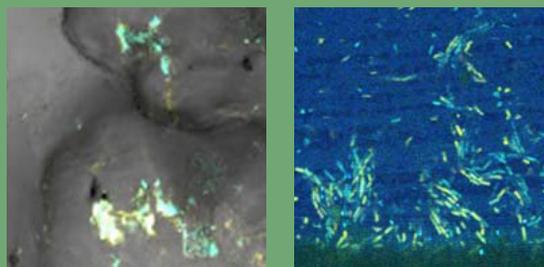
Fig. B. Raman spectrum of *B. subtilis* in MSg, transparent soil, and deuterated water; the C-D peak is indicative of metabolic activity.

Fig. C. *B. subtilis* biofilms on microcosm periphery and on sand spiked into the chamber.

B



C



dissemination. With *in situ* measurements of metabolic activity shown to be feasible via Raman microspectroscopy, we anticipate the MMI and MRC chambers to provide a means to accomplish Aim 2 (“Visualize the assembly of interspecies and interkingdom soil communities”) and Aim 3 (“Explore the movement of carbon in soil communities”).

Potential Benefits/Applications: Our data demonstrate that the imaging chambers (and associated novel analytical methods) are likely to find widespread use among groups involved in plant and microbial research, advancing our understanding of microbial community assembly and ecosystem processes within soil habitats. Due to its low manufacturing cost and versatility with a variety of imaging systems, these platforms are also expected to benefit other research areas, where they could be adapted to investigate questions in nutritional science or medical microbiology.

Development of a Novel High-Precision, High-Resolution SIMS Platform for Elemental and Isotopic Characterization of Microbial Cells at a Systems Level

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This project will develop a new analytical platform for rapid, high-precision determination of the elemental and stable isotopic composition of microbial cells at sufficiently high spatial resolution to localize and quantitatively map bio-essential elements within individual cells.

Research Plans and Progress: The research plans focus on characterizing three model microbial systems (cyanobacteria, purple photosynthetic bacteria, and *Methylobacterium*) that are promising for sustainable bioenergy production to validate this platform. We have spent year 1 characterizing these systems on a current state-of-the-art platform while the instrument upgrades at the heart of our proposal (new ion source and detector system) are being developed. We have optimized protocols for preparing microbial cells for analysis by SIMS (e.g., comparing fixed vs. non-fixed cells; liquid deposition vs. embedded and microtomed). During analysis of cyanobacteria we are able to resolve not only individual cells, but conduct subcellular elemental localization (such as determining the abundance, size, and distribution of internal polyphosphate bodies). For smaller cells (e.g., *R. palustris*) we are not currently able to adequately resolve sub-cellular distributions with the existing instrumentation. We are however able to obtain precise whole-cell measurements of the incorporation of stable isotope labels (C and N) to quantify rates of microbial C and N fixation—and the variance in these values within microbial populations growing under ostensibly identical conditions (both for *R. palustris* and *Methylobacterium*).

Current and/or Anticipated Accomplishments/Deliverables for the Project Period: We have developed the protocols and proof-of-concept demonstration to probe the uptake, assimilation, and subcellular

localization of key elements, isotopes, and biomolecules associated with important metabolic processes (respiration, nitrogen fixation, light harvesting, and photosynthesis). This also includes development of custom vacuum-compatible, transparent, conductive sample-mounting discs, suitable for both microbial analysis via SIMS, as well as optical microscopic inspection; these have now been manufactured, tested and incorporated into routine microbial imaging work. We are now poised to evaluate the improvements associated with the installation of the new detector system and ion source. The new detector (RAE imaging system) and associated pumping system have been delivered to Washington University and are awaiting installation (anticipated July 2016). The plasma micro-oxygen ion source has now been delivered to the vendor; engineering is currently underway to adapt this source to the 7f instrument platform and we expect installation in late 2016.

Potential Benefits/Applications of DOE-Funded Research: This approach will enable the integration of molecular-scale chemical and isotopic information with biological function to generate a new whole-cell, systems-level understanding of these model systems. Specifically, the following advances are anticipated to have wide bioimaging applications: (1) imaging over a relatively large field-of-view (100umx100um), which could include >1000 microbes of about 1 um, with rapid analysis for isotopic labeling with a relative precision of <1%; (2) relatively rapid acquisition of trace element ion images with micron spatial resolution; and (3) spatially co-registered images of major (e.g., C, N, P) constituent elements paired with corresponding trace element (e.g., Fe, Mo) distribution. This will improve our ability to determine rates of uptake for key metabolic processes (e.g., C/N fixation) as well as to understand how these processes may depend on the abundance of essential trace metals and ambient environmental conditions (e.g., pH, light exposure). The results of our work will hopefully lead to improved systems-level understanding of a variety of microbial systems that are promising for sustainable bioenergy development. The ability to leverage these results to aid in bioenergy development will have broad benefits to society and the environment by helping to provide safe, renewable, carbon-neutral energy.

Small Worlds

Principal Investigators: Kenneth Kemner and Mark Hereld

Organization: Argonne National Laboratory

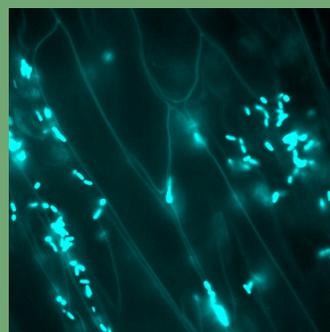
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Collaborators: Frank Collart, Nicola Ferrier, Robin Graham, Philippe Noirot, Sarah O'Brien, and Rosemarie Wilton (ANL); Oliver Cossairt (Northwestern University); Benjamin S. Glick and Norbert F. Scherer (University of Chicago)

Research Plans and Progress: To develop a new multimodal imaging capability for studying complex multi-agent processes in cells and systems of cells across physical and temporal scales. A scientific driver of understanding detailed interactions among synergistically functioning organisms, particularly bacteria and roots, provides a focus for imaging technology development that will enable the development of models that make it possible to enhance the growth and health of a wide range of plants. To create this new experimental capability, the project will develop two major technological axes: (1) *three-dimensional (3D), multimodal imaging* and (2) *multi-agent molecular sensor systems capable of targeting several elements of a process at once*. The combination of these two technologies—with supporting software for image reconstruction, volumetric data fusion, and quantitative analysis—will enable scientists to target complex processes in a wide range of biological systems.

Current and/or Anticipated Accomplishments/Deliverables:

Progress to date includes: 1) introduction of self-labeling fluorescent proteins within rhizobacteria and cloning of metabolite sensors for root exudates, 2) development of 2D X-ray fluorescence microscopy approach to image bacteria labeled with CdSe quantum dots (QDs) and initiation of the development of 3D imaging of QD-labeled bacteria within opaque soil aggregates, 3) development of root-microbe microfluidic interaction chamber integrated into optical microscopes, 4) development of microscope for video rate capture of 3D imagery from snapshot frames, 5) development of computational algorithms for reconstructing 3D volumes from snapshots, 6) method for preparation and processing of robustly labeled root-microbe samples for correlative fluorescence and tomographic electron microscopy.



Confocal microscopy image of rhizobacteria (*P. fluorescens* SBW25) expressing fluorescence solute sensor in periplasmic space.



Confocal microscopy image of cytoplasmic fluorescence-labeled *P. fluorescens* SBW25 colonizing root within microfluidic root-microbe interaction chamber.

Potential Benefits/Applications: A platform for studying a range of complex dynamic processes in cellular and intercellular systems in 3D. This platform will systematize creation of sensor systems capable of simultaneously tracking, sensing, and controlling several aspects of a complex process in a single experiment. It also will enable correlation of image volumes by providing nanoscale markers (quantum dots) that function across modalities.

Adaptive Biosystems Imaging

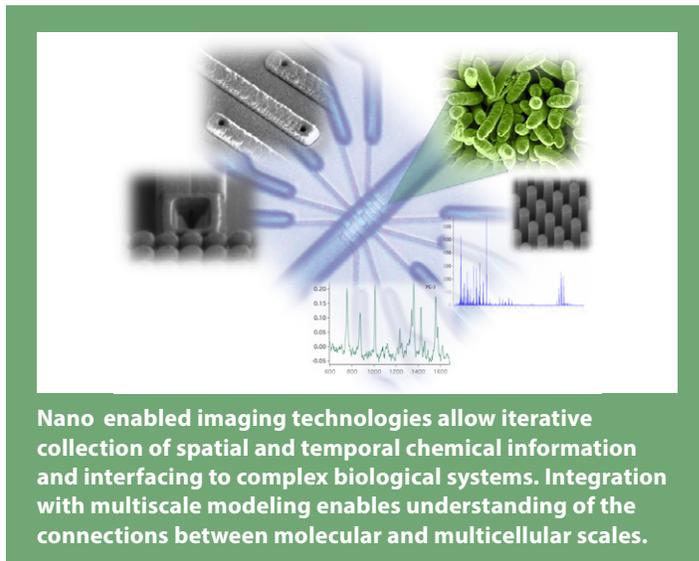
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Tracking the fate and distribution of metabolites is recognized as the key link between genomic information and functional processes occurring at different biological system hierarchies. However, the small size and varied nature of metabolites prevent labeling with extrinsic tags, while their relatively rapid transit and broad concentration range challenge analytical measurements. Our strategy is to develop and implement an Adaptive Biosystems Imaging capability that advances a new, nano-enabled technology for imaging metabolites. An arrayed nanofluidic sampling assembly is being created to spatially and temporally capture metabolite information for sensitive detection and analysis by analytical measurement tools. The easily accessible technology permits simultaneous observation by conventional microscopies for correlated imaging and interpretation of biological function. To realize this new technology, we are concurrently optimizing operational aspects of the imaging system while applying the system to various biological measurement challenges. We are systematically examining the design elements and implementation approaches that influence spatial resolution, sensitivity and dynamic range. Throughout these efforts, computational fluid dynamics, coupled with Brownian models, are being used to guide our imaging system designs. These efforts are being combined with novel approaches to enhancing the selectivity and sensitivity of metabolite detection by mass spectrometry and Raman spectroscopies. We are



Nano enabled imaging technologies allow iterative collection of spatial and temporal chemical information and interfacing to complex biological systems. Integration with multiscale modeling enables understanding of the connections between molecular and multicellular scales.

benchmarking the performance of metabolite imaging measurements against a well characterized, quorum sensing based, fluorescence reporter system and extend these studies for understanding spatial and temporal metabolite distributions associated with growing microbial and plant systems. Multiscale computation is being used to interpret the observed metabolite distributions and connect the imaging data to genome-based models of the organisms, thereby improving mechanistic understandings of both the biological systems examined and the imaging system. The resulting capability will enable the imaging of metabolites across the hierarchies and dimensions of biological systems to provide understanding to a diverse array of biological and environmental processes.

Systems Biology Based on an Integrated, Mesoscale Imaging and Analysis Framework

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Collaborators: Scott Baker and Christer Jansson (PNNL); Michael Knoblauch (Washington State University, Pullman)

Research Plans: Our project aims to develop innovative instrumentation, novel chemical probes and new correlative imaging and modeling technologies to yield an unrivaled view of biosystem dynamics when combined with 'omics analyses. Specifically, we plan to: 1) construct a hybrid Stimulated Raman/Coherent Anti-Stokes Raman Scattering and Helium Ion Microscope (SRS/CARS/HeIM) for simultaneous observation of cell structure and quantitative label-free chemical imaging; 2) design and install a ponderomotive phase plate on a unique Dynamic Transmission Electron Microscope (DTEM) to visualize macromolecular dynamics with atomic to near-atomic spatial resolution and 10 nanosecond to millisecond temporal resolution; 3) manufacture new nanofluidic devices to enable higher-throughput and reproducible *in situ* bioimaging studies; 4) fabricate advanced microfluidic devices for nondestructive *in situ* and quantitative bioimaging in precise chemical environments to tease apart the effects of cell-cell variability versus phenotypic response; 5) design novel activity-based probes for highly selective *in situ* chemical imaging using label-based and label-free approaches; 6) synthesize multifunctional probes for multimodal microscopy and 'omics applications; 7) perform correlated *in situ* dynamic and cryogenic bioimaging and systems biology analysis to gain an all-inclusive view of cellular response to changing environments; 8) implement *in situ* hyperspectral fluorescence and Raman imaging to track lipid biogenesis in real-time nondestructively; and 9) build an iterative experimentation approach of physics-based simulations and multimodal imaging/systems biology to inform and validate biodesign principles.

Accomplishments/Deliverables for Project Period: While the large instrumentation under development builds upon equipment specifically localized to PNNL (hybrid SRS/CARS/HeIM & DTEM), all of the

remaining deliverables are directly portable and will be easily transferred to other institutions. This includes: 1) nanofluidic devices with 25x larger field-of-view and patterned features for improved window stability and low-dose focusing aides (immediately adoptable by owners/users of commercial *in situ* electron microscopy holders); 2) directed flow 3- and 5-port nanofluidic platforms empowering controlled mixing for dynamic *in situ* electron or ion microscopy (immediately compatible with FEI, JEOL and Zeiss electron, optical and ion instruments); 3) microfluidic devices for (a) filamentous organisms, (b) unicellular organisms and (c) plant/plant microbe interactions (all compatible with commercial optical and Raman microscopy systems); 4) glucose, arginine (and other small molecule) activity-based probes (being validated using multiple organisms to enhance portability); 5) multimodal/multifunctional reporter probes for cross-platform bioimaging and 'omics analysis (compatible with standard electron, ion, optical, Raman, and X-ray microscopy workflows as well as Mass Spectrometry and Nuclear Magnetic Resonance); and 6) protocols for expanded *in situ* multimodal/correlative imaging, systems biology and iterative modeling.

Potential Benefits/Applications: Once realized, the new technologies will help track cellular responses to environmental perturbation, spatially localize mechanistic pathways and visualize structural dynamics of macromolecules. While the three main focus areas of this project were designed for advancing bioenergy relevant applications (such as improving lipid feedstock yields, enhancing lignocellulosic deconstruction or boosting feedstock sustainability and plant drought tolerance) the overall portfolio will be extensible to broad biological applications spanning the interrogation of complex microbiomes, interactions within the rhizosphere, synthetic biology and even biomedical applications. In addition to disseminating results through publications, we have also initiated an outreach program to transfer microfluidic devices to external collaborators for rapid adoption of these new and maturing technologies. Similar outreach will be pursued for other portable probes and devices. Ultimately, most of the capabilities will fold into the EMSL user program with the stand-alone CARS/SRS microscope and microfluidics being made available as early as mid 2017.

SLAC Mesoscale Integrated Biology Pilot Project: MFX station at LCLS

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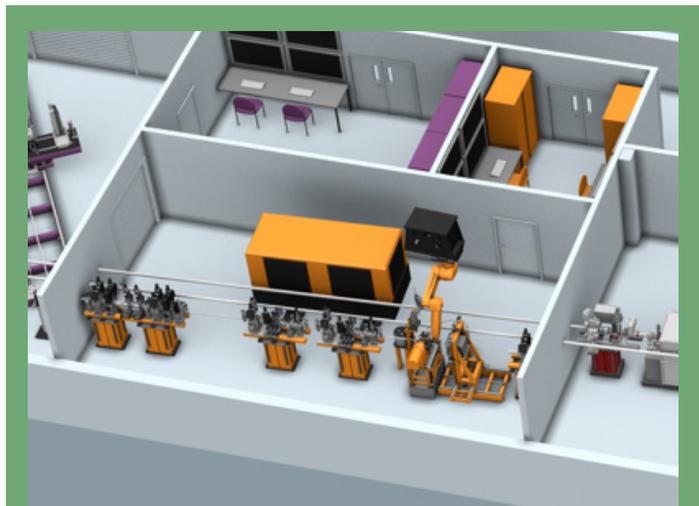
Collaborators: Sébastien Boutet, Axel T. Brunger, Aina E. Cohen, David M. Fritz, Britt Hedman, Keith O. Hodgson, S. Michael Soltis, and William I. Weis (SLAC/Stanford University)

Research Plan: Synchrotron radiation has transformed biology over the past few decades by providing brilliant beams of X-ray light for probing the structures of molecules. Now X-ray free electron lasers (XFELs) promise to usher in another new era, allowing scientists to tackle important questions previously out of reach. With beams 10 billion times brighter and pulses 1,000 times shorter than those available at synchrotron light sources, XFELs can provide structural information from crystallized samples by enabling diffraction data collection before samples are damaged or destroyed by XFEL pulses. The project aims (1) to develop a new instrument for diffraction, scattering, and imaging at the world's first operational XFEL, SLAC's Linac Coherent Light Source (LCLS) with a 2-year time frame, and (2) to optimize the macromolecular femtosecond crystallography (MFX) instrument for biological research. As part of an integrated biology platform being developed at SLAC, MFX will open new frontiers in biology, medicine, bioenergy, and environmental science, enabling researchers from the United States and around the world to investigate complex biological phenomena.

Progress: The MFX station was initiated as a multipartner project in spring 2014 and the beamline and experimental hutch have been commissioned early 2016. On January 12, 2016, the first X-ray beam was delivered to the beamline and first diffraction images were recorded on March 12.

Current and Anticipated Deliverables: MFX began real experiments with the start of the user program on July 1, 2016. Various sample delivery and data acquisition systems are currently being implemented, which will enable femtosecond serial crystallography, scattering and spectroscopy and promise to reveal the structures and dynamics of complex biomolecules or assemblies. MFX also can take X-ray snapshots of organelles and cells at medium resolution. The availability of a dedicated station for these types of experiments provides an optimized infrastructure for the most effective and efficient use of LCLS beam time.

Impact: Substantial expansion of the overall capacity and efficiency of LCLS that, when integrated with other imaging approaches, will help fill the gap between the vastly expanding wealth of genomic data and the limited structural knowledge available on the control of cellular and subcellular processes. MFX also will provide new opportunities for



Concept of the MFX instrument at LCLS.



Recent photograph of the MFX instrument.

collaborations with other DOE user facilities, relevant programs within the DOE's Office of Biological and Environmental Research (BER), BER Virtual Laboratory and KBase. These collaborations will enable high-impact research investigating, for example, how photosynthesis works; how bacteria fix carbon and nitrogen, break down cellulose, and transform toxic metals such as mercury into less toxic forms; how enzymes work together to catalyze metabolic processes; and how the structures and shapes of black carbon particles and other aerosols affect air quality and human health.

