New Bioimaging Technologies for Plant and Microbial Systems

U.S. DEPARTMENT OF ENERGY | Office of Science

Office of Biological and Environmental Research

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This brochure is available at science.energy.gov/ber/community-resources/bioimaging_technologies.pdf.

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; 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.]

Preface

The U.S. Department of Energy's (DOE) Office of Biological and Environmental Research (BER) supports fundamental research to advance a predictive understanding of complex biological and environmental systems relevant to DOE's missions in energy and the environment. Starting with the information encoded in organisms' genomes, BER-funded scientists seek to define the principles guiding translation of the genetic code into functional proteins and the metabolic and regulatory networks underlying plant and microbial systems. Concurrent with this research is a need for enabling technologies to place understanding of gene expression, regulation, and function into the spatiotemporal context of whole-cell environments. Imaging and measurement technologies that can resolve multiple key metabolic processes over time within and among cells will act as a crucial bridge toward linking molecular-scale information to whole-cell, systems-level understanding.

BER's current focus on developing a scientific foundation for plant biomass–based biofuel production requires a detailed understanding of cellular metabolism to incorporate, modify, or design beneficial properties into bioenergy-relevant plants and microbes. Likewise, capabilities for tracking materials and chemical exchanges within and among cells and their environment are crucial to understanding the activities of microbial communities in environmental settings. New imaging and measurement technologies that can track multiple metabolic processes will provide the integrative, systems-level data needed to gain a more predictive understanding of complex biological processes relevant to BER.

After initiating five pilot projects in FY 2014, BER's new Bioimaging Technology effort is targeted at developing novel multifunctional technologies to image, measure, and model key metabolic processes within and among microbial cells and multicellular plant tissues. The new program comprises eleven projects, including four at DOE national laboratories and seven at universities. These projects aim to create new *in situ*, dynamic, and nondestructive approaches to multifunctional imaging, quantitative flux measurements, and multiscale integrative analyses of plant and microbial systems relevant to BER's bioenergy and environmental research.

This brochure describes the current portfolio of projects and serves as an informational resource for BER's new and evolving Bioimaging Technology program.

Signed,

R. Todd Anderson, Ph.D.
Director
Biological Systems Science Division
Office of Biological and Environmental Research
Office of Science
U.S. Department of Energy

Contents

Multifunctional Plasmonics Nanoprobes for Cellular Sensing and Imaging

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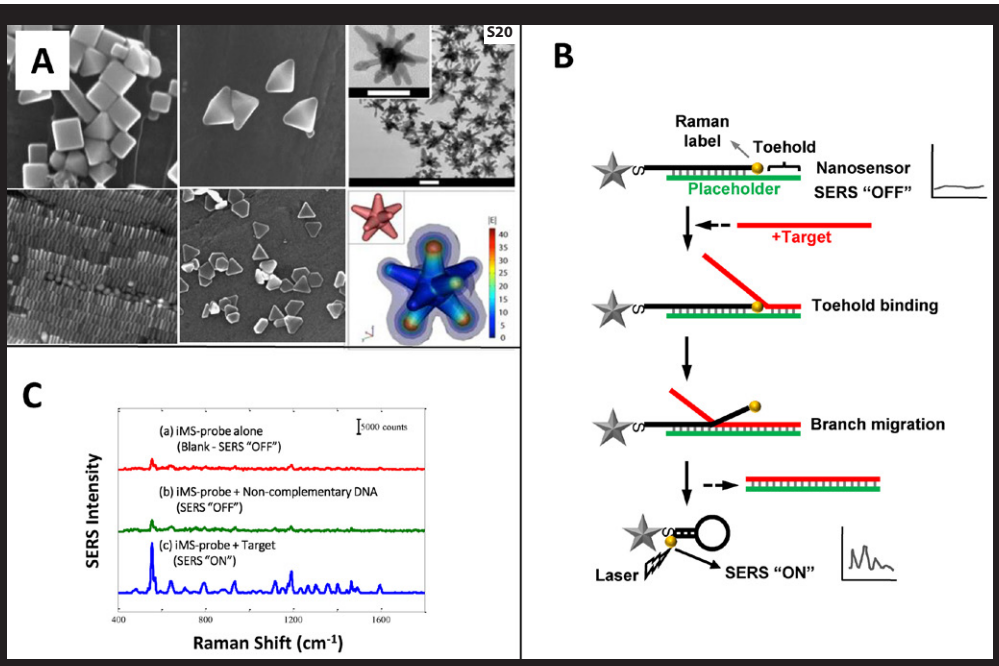
Collaborators: Zhen-Ming Pei and Tai-Ping Sun (Duke University); Kenneth Kemner (Argonne National Laboratory)

Project Summary: This project involves development of novel optical nanoprobes for functional sensing, tracking, and imaging of biotargets and molecular processes at the cellular and subcellular levels to analyze bioenergy-relevant plant systems. Under laser irradiation, labeled nanoprobes, which consist of metallic nanoparticles, exhibit strongly enhanced vibrational signals, often referred to as surface-enhanced Raman scattering (SERS). This nanoprobe technology will be developed and applied to monitor spatiotemporal expression of genes regulating plant flowering time and encoding master growth repressors, leading to a better understanding of plant systems for improved biomass production.

Objectives: (1) Develop a novel class of functional nanoprobes for cellular sensing and imaging using the SERS detection technique to enable selective and sensitive monitoring of target species within living plant cells. (2) Apply this sensing technique to probe the functional status of mRNA and microRNA expression to better understand cellular metabolic processes in plants.

Approach: Develop a unique class of metallic nanoprobes including gold nanostars (see image A below), which exhibit a strongly enhanced electromagnetic field and thus produce intense SERS signals from molecules near the metal surface. As an optical spectroscopy technique, SERS uses laser light interacting with metallic nanostructures to excite molecules close to the nanoparticles and provide specific spectral fingerprints. These nanoprobes use a unique sensing technique, inverse Molecular Sentinels (iMS), which enables sensitive detection, tracking, and imaging of nucleic acid biomarkers associated with cellular regulation and function, including mRNA and microRNAs (see images B and C below). Thus, these nanoprobes can identify and tag nucleic acid targets and shine to enable monitoring and imaging of the expression of genes relevant to plant biomass production at high spatial and temporal resolution. X-ray microscopy imaging using the Advanced Photon Source at Argonne National Laboratory will be used to track and image the distribution of the nanoprobes in plant cellular systems.

Impact: A new type of multifunctional biosensing and imaging. The iMS molecular nanoprobe technology using SERS detection will enable better understanding and improved prediction of cellular function in plant growth. In addition to bioenergy applications, this technology could be used in other applications including medical diagnostics and environmental sensing.



Operating Principles of Inverse Molecular Sentinel (iMS) Nanoprobes. (A) Transmission electron microscopy images of various metallic nanoparticles including nanocubes (top left), nanorods (bottom left), nanopyramids (top middle), nanoprisms (bottom middle), and nanostars (top right); calculations indicate strongly enhanced electromagnetic fields at the branch tips of nanostars that produce intense SERS signals of molecules close to the nanoparticle (bottom right). **(B)** In the absence of a target, the iMS probe is open with a low SERS signal (top, "Off" state). Recognition and hybridization with the nucleic acid target sequence in the iMS probe's immediate vicinity result in displacement of the placeholder sequence, followed by a stem-loop closure, which brings the Raman dye close to the metal nanoparticle, thus producing a strong SERS signal (bottom, "On" state). **(C)** Demonstration of the iMS technique, illustrating the operating principle (shown in **B**): SERS spectra of the iMS nanoprobes **(a)** in the absence of an mRNA target sequence **(**Off state), **(b)** with a non-complementary sequence (Off state), and **(c)** in the presence of the target sequence **(**On state). [Image A courtesy Duke University. Images B and C adapted from Wang, Fales, and Vo-Dinh. 2015. *Nanomedicine: Nanotechnology, Biology, and Medicine*. DOI: 10.1016/j.nano.2014.12.012]

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

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

Project Summary: The integrated, multispectral molecular imaging technologies developed in this multidisciplinary project will enable dynamic imaging of metabolic processes that are asymmetrically distributed among different cellular and subcellular compartments of plant organs.

Objective: Integrate molecular imaging technologies spanning physical scales from 50 nm to 5 µm resolution to enable experimental observations of metabolic processes.

Approach: Integrate Raman and subdiffraction fluorescence imaging, dynamic fluorescent and Raman molecular probe imaging, and mass spectrometric imaging. This project will computationally unify these novel molecular imaging capabilities with traditional high-resolution visual imaging techniques through adaptation of the existing dynamic visualization Meta!Blast platform.

Impact: Imaging technologies that contribute to a more sophisticated understanding of how biomass-producing metabolic processes are interconnected and controlled. These technologies will be applied specifically to lipid metabolic processes and membrane trafficking that control spatially defined subcellular regions within plants and optimize plant biomass productivity.

Understanding Plant Metabolic Processes. Metabolic processes integrate genetically programmed molecules into structures that span different physical scales from nm to mm. How to read the genetic code that programs the individual components is well established, but understanding how these components integrate and provide chemical functionality still requires experimental observations. The technologies developed by this project will enable such experimental observations of metabolic processes. [Image courtesy Iowa State University]

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
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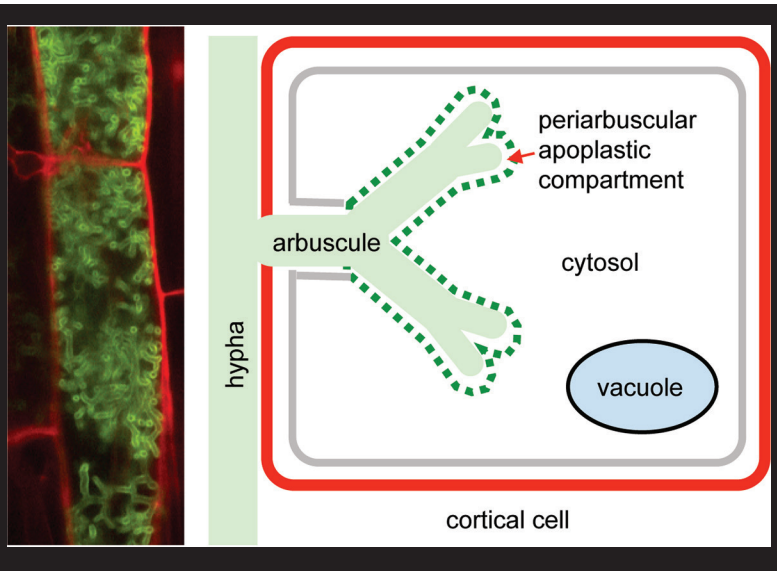
Project Summary: Arbuscular mycorrhizal (AM) fungi scavenge inorganic phosphate (Pi) from soil and then transfer this essential nutrient directly to plant roots through specialized cells that develop within the root cortical cells. Exploiting this symbiotic partnership to enhance Pi capture by plants could help make bioenergy crop production more environmentally sustainable. However, a comprehensive understanding of symbiotic Pi acquisition is limited by the current inability to monitor subcellular Pi concentration dynamics during AM symbiosis. This project is developing live Pi imaging tools and methods to address this critical limitation.

Objectives: (1) Develop a suite of genetically encoded Förster resonance energy transfer (FRET)–based Pi biosensors that can be targeted separately to key subcellular compartments in plants. (2) Establish methods to conduct quantitative live imaging of these biosensors in host transgenic plants to measure spatial and temporal Pi concentration profiles during AM symbiosis.

Approach: (1) Using *in vitro* assays, identify Pi biosensors from screens of a mutant biosensor library. These biosensors will have affinities and pH response ranges optimal for the subcellular compartments directly involved in symbiotic Pi transport (periarbuscular apoplastic compartment) and subsequent Pi storage and metabolism (vacuole and cytosol). (2) Modify biosensor genes for subcellular targeting in plants and verify Pi-dependent FRET responses using transient assays. Validated biosensors and location-matched controls will be expressed from AM-specific promoters in two plant species: *Medicago truncatula* and *Brachypodium distachyon*. (3) Inoculate transgenic plants with AM fungi and then use confocal microscopy to monitor subcellular Pi profiles during AM symbiosis.

Impact: Pi biosensors with a range of characteristics suitable for measuring Pi in distinct subcellular compartments of plant cells, along with essential controls. These biosensors will provide the first insights into Pi concentrations in root cells during AM symbiosis, contributing to a holistic view of Pi transport and functioning of AM symbiosis. Standardized imaging and analysis methods will ensure that this Pi biosensor technology is widely accessible.



Live Pi Imaging During AM Symbiosis. FRET-based Pi biosensors are being developed for quantitative imaging in the periarbuscular apoplastic compartment (shown in green in the micrograph at left). These biosensors will monitor Pi transfer from fungus to plant cell, as well as the dynamics of Pi metabolism and storage in the cytosol and vacuole of the colonized plant cell, during AM symbiosis. [Images courtesy Texas A&M University/Boyce Thompson Institute for Plant Research]

Multiscale Dynamics of Water Regulation by Bacteria in Synthetic Soil Microsystems

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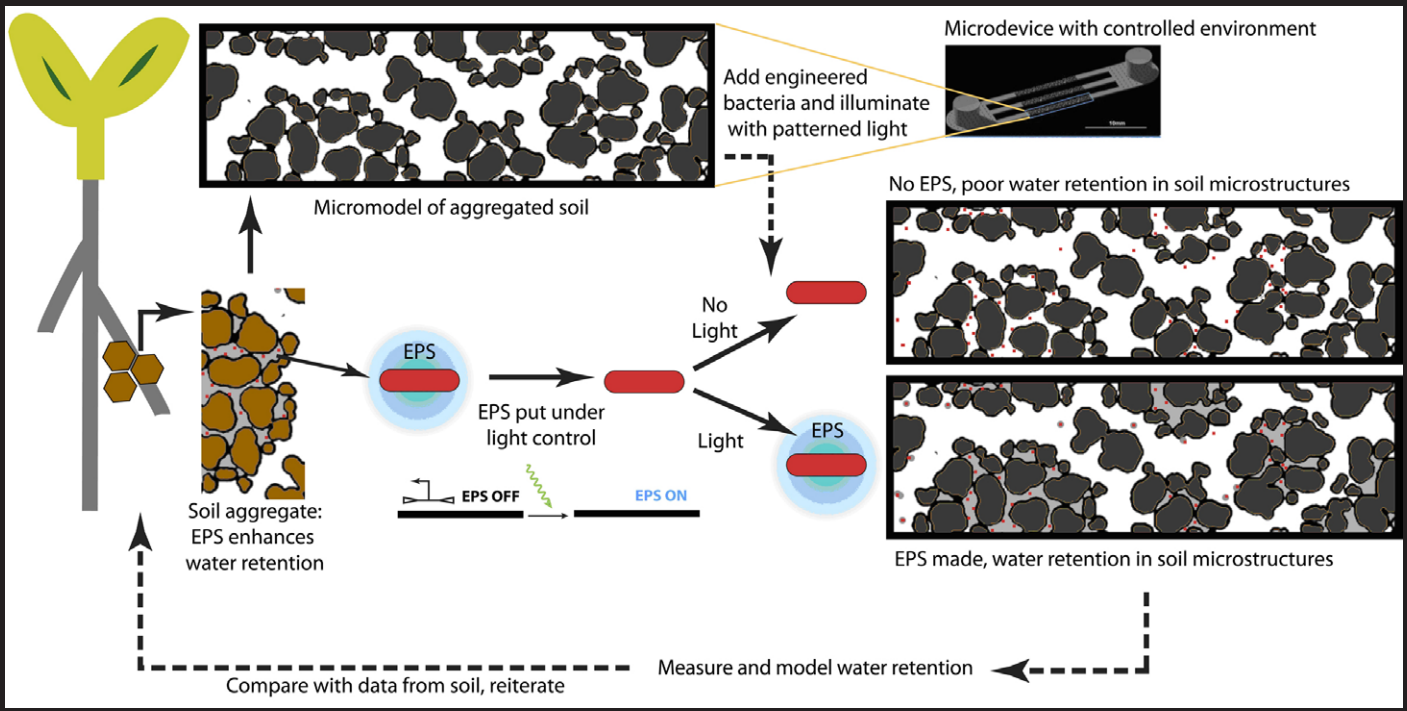
Collaborators: Daniel J. Gage and Yongku Cho (University of Connecticut); Jessica F. Chau (Benedict College)

Project Summary: Microbial processes are essential to the productivity of terrestrial ecosystems and to the growth, health, and resilience of bioenergy-relevant plants. Of particular concern are anticipated decreases in soil moisture associated with global climate change. Natural microbial processes may modulate water loss. To harness the potential of microbes for promoting terrestrial ecosystem resiliency, this project will improve understanding of microbial moisture regulation *in situ*.

Objectives: Develop a multifunctional *in situ* platform linking gene expression to overall system function within the extracellular context of a realistic soil microenvironment. This project focuses on spatial regulation of genes encoding exopolysaccharide (EPS) production to understand the moisture-regulating function of microbes in soils and its potential to increase terrestrial ecosystem resiliency.

Approach: (1) Employ advanced manufacturing techniques to create and control a synthetic rhizosphere microhabitat physically similar to real soil. (2) Develop novel optogenetic tools to place the genes of soil microbes under the control of light and enable microscale spatiotemporal control of microbial genetic capabilities *in situ*. (3) Build systems-level understanding of the microbial water-regulating function through iterative experiments and modeling, employing both a pore-scale lattice Boltzmann model and a root-scale water flux model.

Impact: An experimental platform linking overall ecosystem function with *in situ* microscale gene control. This platform will enable light addressable control of gene expression in various cellular systems important to biological and environmental research.



Multifunctional *In Situ* Platform Linking Gene Expression to Overall Ecosystem Function. Fabrication of synthetic microhabitats and development of novel optogenetic control capabilities will place microbial genes under the control of light within a synthetic soil microenvironment. Iterative experiments and modeling will build systems-level understanding of the water-regulating function of soil microbes in real soil systems. This platform and its enabling tools can be applied to a host of different cellular systems important to biological and environmental research. [Image courtesy University of Connecticut]

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

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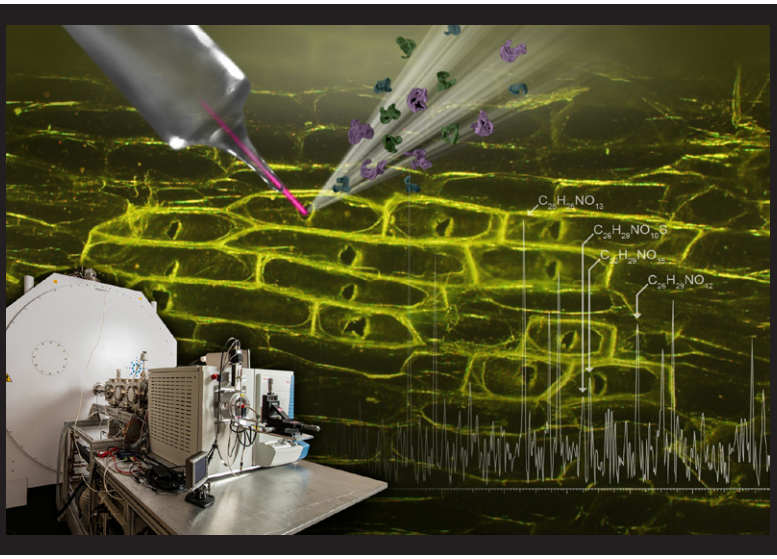
Collaborators: Akos Vertes (George Washington University); Ljiljana Paša-Tolić, Christopher Anderton, and David W. Koppenaal [Environmental Molecular Sciences Laboratory (EMSL) and Pacific Northwest National Laboratory]

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: *In situ* mass spectrometry imaging investigations within the natural environment of biological specimens require new atmospheric pressure ion sources. One such source is the recently developed LAESI, which currently can provide cell-by-cell imaging for large cells [i.e., sizes of 50 to 200 micron (1 to 3 nanoliter volume)]. Handling more typical plant cell volumes in the tens of picoliter volume range requires improvements to this ion source, as well as enhanced sensitivity, dynamic range, and interference reduction in mass spectrometry detection. High magnetic field Fourier transform ion cyclotron resonance mass spectrometry (FTICR MS) now delivers low parts-per-billion mass accuracy and a resolving power of >3 million R [mass (m)/Δm], a milestone achievement for biomolecular analysis applications. This performance level facilitates confident identification of cellular and extracellular components and even specification of elemental molecular composition in biological samples. A high magnetic field (21 Tesla) FTICR MS recently installed at EMSL enables truly unique, spatially resolved, ultrahigh-resolution molecular measurements and observations. By interfacing a LAESI source to this FTICR MS, *in situ* imaging of metabolites, lipids, peptides, and proteins will be feasible on a variety of Department of Energy (DOE)–relevant systems. With the increased sensitivity and advanced tandem MS methods that EMSL's high magnetic field FTICR MS offers, the goal of single-cell imaging of "omics" in ambient conditions becomes feasible. As this new imaging approach is developed, this project will explore plant-microbe rhizosphere interactions, focusing on well-characterized root systems (e.g., *Arabidopsis*, sorghum, and switchgrass) and increasingly complex synthetic and natural root biofilm associations, to demonstrate the power of the new tools.

Impact: Novel techniques that can visualize and map a wide range of molecules involved in plant-microbe interactions fundamental to plant growth and sustainability. These new capabilities will be available to the broader scientific community via DOE EMSL, a national user facility.



***In Situ* Molecular Microscope.** Ultrahigh-resolution molecular measurements of ablated cellular material will be possible using a newly developed microscopy system. Biological materials can be sampled, as depicted, at single-cell and several-cell levels using a LAESI probe. Biomolecular components then are analyzed with exquisite mass resolution using a high magnetic field (21Tesla) FTICR MS system developed at EMSL (lower left), enabling new insights into biochemical and bioenergy transformations. [Image courtesy Environmental Molecular Sciences Laboratory / George Washington University]

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

Principal Investigator: Elizabeth Anne Shank
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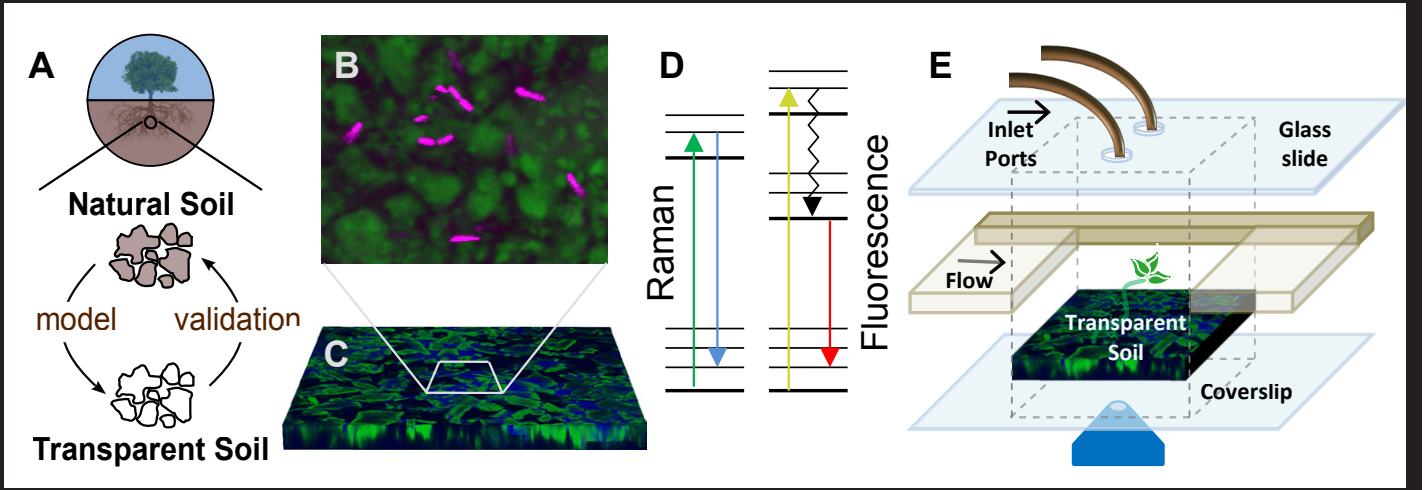
Collaborators: Carol Arnosti and Jeff Dangl (University of North Carolina at Chapel Hill); David Berry (University of Vienna); Jennifer Pett-Ridge (Lawrence Livermore National Laboratory)

Project Summary: Soil supports a rich ecosystem of interacting microbes and plants. The metabolic activities of these soil inhabitants can profoundly affect processes such as global carbon and nutrient cycling. However, understanding of how microbes mechanistically exert these critical ecosystem-level processes is lacking, in part due to the inability to visualize their spatiotemporal and chemical interactions at the single-cell level within soil environments. This project aims to overcome this challenge by developing an innovative imaging platform to enable real-time, nondestructive visualization of microbial activities in a native-like model soil system.

Objective: Establish a novel multimodal visualization system for nondestructive and dynamic observations of how microbes interact and influence one another and plants via chemical signaling and nutrient exchange in native-like microenvironments.

Approach: (1) Create microcosms composed of optically transparent, soil-like particles, plant roots, and microbial communities that can be perturbed using microfluidics and monitored using confocal fluorescence microscopy, Raman microspectroscopy, and isotopic carbon tracing. Incorporating fluorescently or isotopically labeled microbes and plants into these microcosms will yield a critical capability to directly monitor and perturb the spatiotemporal dynamics of single cells and individual metabolites within native-like microenvironments. (2) Demonstrate the application of this new system by exploring how microbes physically and chemically interact with one another in heterogeneous environments, how microbes interface with the surfaces of artificial and real plant roots, and how carbon is used and degraded in the context of synthetic microbe-plant communities.

Impact: A unique multimodal imaging system that bridges the gap between traditional laboratory and field experiments. This robust, practical, and adaptable method for exploring microbial interactions and activities *in situ* will provide a deeper understanding of the microscale processes occurring within soil that relate to global carbon and nutrient cycling.



Transparent Soil Microcosm. (A) A transparent soil substrate captures the physical and chemical heterogeneity of natural soil, yet remains optically transparent when hydrated. **(B, C)** These transparent soil micro-environments will enable visualization of individual cells, metabolites, and nutrients in three dimensions using various forms of microcrosopy **(D)**. Robust imaging chambers **(E)** will be created to facilitate use of this approach by the broader scientific community. [Images courtesy 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

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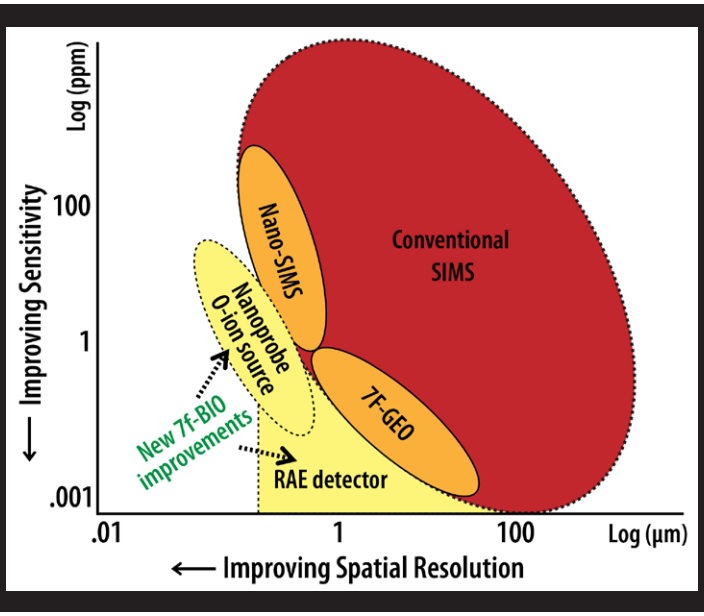
Collaborators: Arpita Bose, Alexander Bradley, and Himadri Pakrasi (WUSTL)

Project Summary: 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 bioessential elements within individual cells.

Objectives: (1) Develop an analytical technique 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). (2) Employ a suite of model microbial systems (e.g., cyanobacteria, purple photosynthetic bacteria, and *Methylobacterium*) that are promising for sustainable bioenergy production to validate this platform. Taken together, the resulting data 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.

Approach: This analytical approach utilizes secondary ion mass spectrometry (SIMS), a technique in which the sample is bombarded with a focused ion beam, generating secondary ions from the sample. The ions then are electromagnetically focused onto a series of collectors. New capabilities are being developed for subcellular localization of bioessential elements to specifically probe key metabolic processes by upgrading an existing state-of-the-art SIMS platform: a Cameca 7f-GEO instrument. The resulting instrument, a "7f-BIO," will contain (1) a novel ion source for increased spatial resolution and (2) improved detector capabilities for coregistration of diverse elemental and isotopic data obtained using different ion sources and analytical conditions. Both of these improvements will make the 7f-BIO uniquely suited for rapid analyses of biological specimens in a wide field of view.

Impact: An instrument platform that enables the linkage of molecular-scale chemical and isotopic information with biological function to generate whole-cell, systems-level understanding that currently is not achievable using standard SIMS instruments. This and successor instruments will be used for addressing key questions about the uptake, assimilation, and subcellular localization of elements, isotopes, and biomolecules associated with important metabolic processes in a range of microbial systems of interest for sustainable bioenergy production.



Comparison of Analytical Resolution and Detection Limit of Widely Used Imaging and Related Techniques. Shown is the range of conventional SIMS (red oval), current SIMS 7f-GEO and Nano-SIMS instruments (orange ovals), and the dual new capabilities being developed for the 7f-BIO instrument: (1) novel radio frequency nanoprobe oxygen (O^-) ion source (yellow, upper left) and (2) new resistive anode encoder (RAE) detector for stigmatic imaging (yellow, bottom center). This new analytical platform will enable rapid, high-precision determination of the elemental and stable isotopic composition of microbial cells at sufficiently high spatial resolution to localize and quantitatively map bioessential elements within individual cells. [Illustration courtesy Washington University in St. Louis]

Small Worlds

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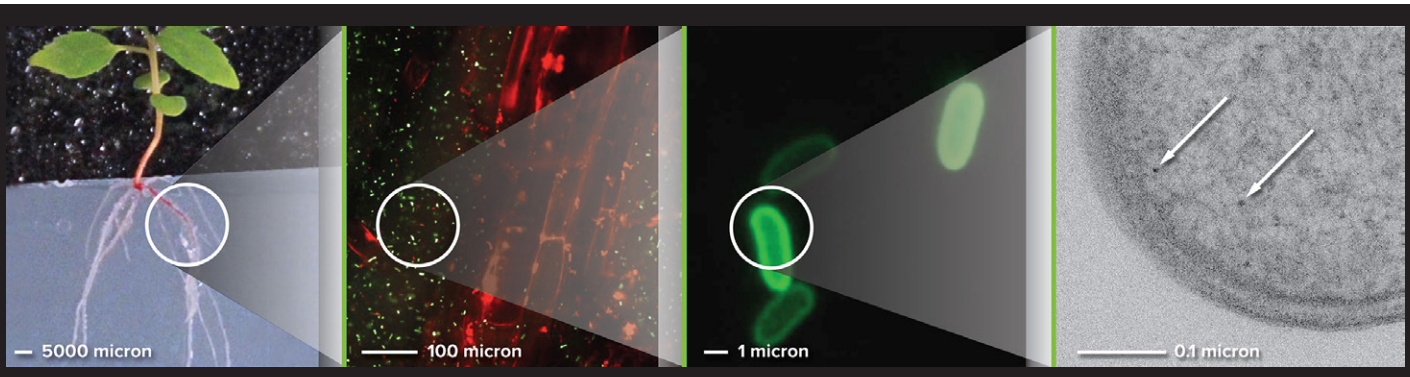
Collaborators: Frank Collart, Nicola Ferrier, Robin Graham, Philippe Noirot, Sarah O'Brien, and Rosemarie Wilton (ANL); Oliver Cossairt (Northwestern University); Benjamin S. Glick and Norbert F. Scherer (University of Chicago)

Project Summary: The contingent of capabilities developed in this project will enable construction of dynamic systems biology experiments that can track and correlate interrelated molecular actors in complex processes, while providing detailed corroboration and supplementary data across physical and temporal scales with qualitatively different imaging modalities.

Objective: Develop a new multimodal imaging capability for studying complex multi-agent processes in cells and systems of cells across physical and temporal scales. For example, understanding detailed interactions among synergistically functioning organisms, particularly bacteria and roots, 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.

Approach: Develop a 3D snapshot interferometric holographic microscope (3D-SIHM) capable of imaging whole live cells in fluorescence and brightfield modes simultaneously in 3D. Use of the new 3D-SIHM microscope will enable the capture of information required for reconstruction of 3D multiscale volumetric data of complex systems in a single "snapshot" measurement. This dynamic microscopy will be complemented by new correlative tomographic methods in two separate imaging modalities: (1) electron tomography to provide high-resolution matching of dynamic processes to detailed cellular structure and (2) X-ray tomography to enable development of larger-scale intercellular correlations for studying cross-organismal processes within opaque media such as soil. Supplementing these advances in 3D multimodal imaging will be the development of new multi-agent molecular sensor technology.

Impact: A platform for studying a range of complex processes in cellular and intercellular systems. 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.



Early Results of Multiscale Imaging of Root-Bacteria Symbionts. Left to right: Plant with roots; root stained red with bacteria containing green fluorescent protein (GFP); GFP within periplasmic space of bacteria for future use as a sensor for root exudates; transmission electron micrograph of a thin section of bacterial cell (arrows indicate position of quantum dots inside cell). [Images courtesy Argonne National Laboratory]

Adaptive Biosystems Imaging

Principal Investigator: Mitchel J. Doktycz
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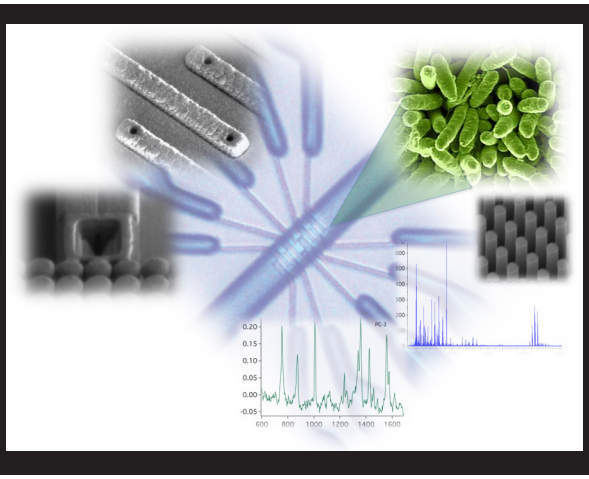
Project Summary: Understanding how observable biological processes, carried out over wide-ranging spatial and temporal scales, arise from molecular-scale events represents a grand challenge facing biological and environmental research. To address this challenge, ORNL is leading an effort to develop and apply an Adaptive Biosystems Imaging (ABI) capability that will address how to collect and interpret molecular imaging data over diverse spatial and temporal scales for understanding how processes detected on smaller scales lead to larger-scale phenomena.

Objective: Develop and apply an adaptive approach to imaging, wherein computational modeling and simulation guide interactive, molecular imaging measurements that enable coupling of systems models to observable phenomena. Iterative collection of measurement data and integration with modeling information will lead to understanding the connections spanning diverse spatial, temporal, and chemical dimensions. The resulting capability will serve as a framework for designing and implementing bioimaging experiments that reach across the hierarchies and dimensions of biological systems to provide understanding of a diverse array of biological and environmental processes.

Approach: Integrate unique resources in nanofabrication, neutron sciences, and high-speed computation with new measurement approaches, stable-isotope probes, and supercomputer-driven simulation tools capable of tracing the production, transport, and fate of selected metabolites in biological systems. The project's first main thrust is to enable interactive imaging by specifically advancing three analytical technologies: nano-enabled imaging, multimodal spectroscopic imaging, and neutron imaging. These tools share capabilities for performing direct measurements, without the need for extrinsic chemical probes, and can capitalize on spectral shifts induced by stable isotopes. The second thrust is to construct and evaluate dynamic, multiscale systems models that incorporate genomics-based information with imaging and analytical measurements. Specifically, the project will link and apply simulation techniques that address different spatial and temporal scales in which larger-scale, coarse-grained methods are informed by results from finer-detail computations.

Impact: A new means of acquiring chemical image data that enables linking of molecular events to biological outcomes and behavior. This adaptive imaging capability will enable assembly of intracellular, extracellular, and phenotypic data and information, collected across multiple platforms and across multiple decades of length and time, into a coherent understanding of biological systems. The resulting framework will be applicable to virtually any cell- or community-level system and can be extended for whole-plant, multi-organism assemblages and ecosystem-scale questions.



Adaptive Biosystems Imaging. 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. [Image courtesy Oak Ridge National Laboratory]

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

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PNNL Co-PIs: William Cannon, Ryan Kelly, Matthew Marshall, Hongfei Wang, and Aaron Wright

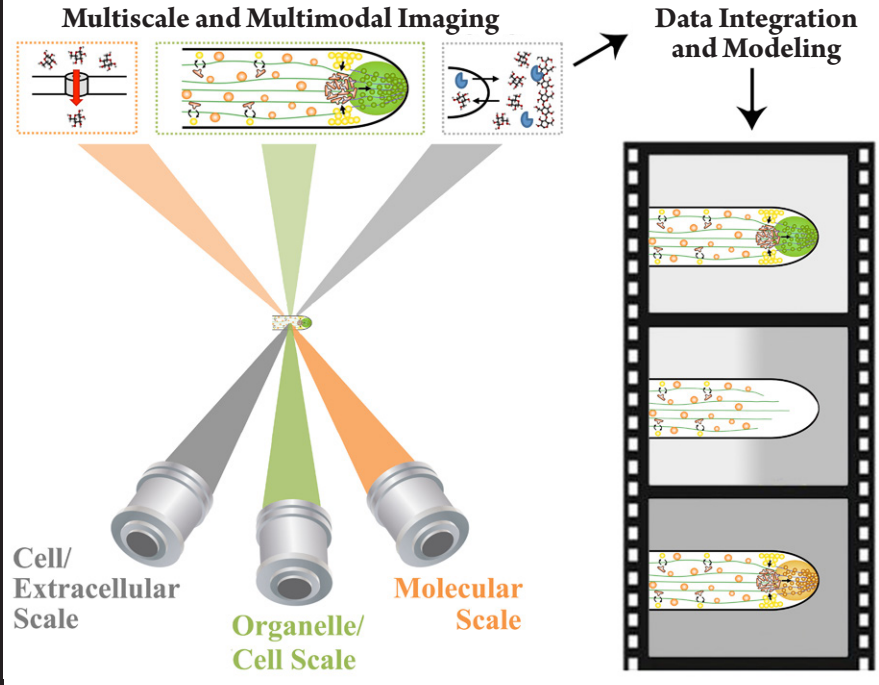
Collaborators: Scott Baker and Christer Jansson (PNNL); Michael Knoblauch (Washington State University, Pullman)

Project Summary: Organisms rely on coordinated metabolic and physiological processes that span atomic, molecular, cellular, extracellular, and multicellular scales to detect and respond to their environment. Unfortunately, no single technology exists that spans all relevant spatiotemporal scales needed to observe critical mechanisms employed by cells for governing tiered communication and regulation. This project seeks to establish a unified systems biology and chemical imaging approach by placing chemical data within a spatial and temporal whole-biosystem context to enhance bioenergy applications.

Objective: Develop an integrative imaging platform that combines unique chemical probes, *in situ* environmental chambers, and advanced imaging instrumentation to monitor and quantify the dynamics of cellular processes with unprecedented clarity.

Approach: Correlate multiscale structural, functional, chemical, and dynamic imaging of biological systems by transferring samples among a suite of electron, ion, optical, and X-ray technologies, while maintaining sample registration under controlled environments. Custom-designed probes and novel nano- and microfluidic chambers will enable dynamic and live-cell tracking of biological response to chemical gradients for organisms ranging from unicellular algae to plant roots. The multimodal imaging data then will be computationally joined with proteomic and metabolomic data to iteratively refine modeling predictions of how cells interact with their environment.

Impact: Novel multifunctional probes, unique nondestructive and dynamic imaging technologies, and integrative analytics for visualizing spatially regulated processes with relevant spatial and temporal scales and high chemical sensitivity. This expected scientific leadership and technology platform with its other advancements will benefit the larger scientific community, helping to define the frontier for understanding the spatiotemporal control exhibited by cells in response to changing environments and guide the future design of biological systems.



Integrated Multimodal Imaging and Analysis Framework. This *in situ* imaging project will generate multidimensional imaging data to inform predictive biology through a tight coupling between experiments, observations, simulations, models, and theory. [Image courtesy Pacific Northwest National Laboratory]

SLAC Mesoscale Integrated Biology Pilot Project: MFX Station at LCLS

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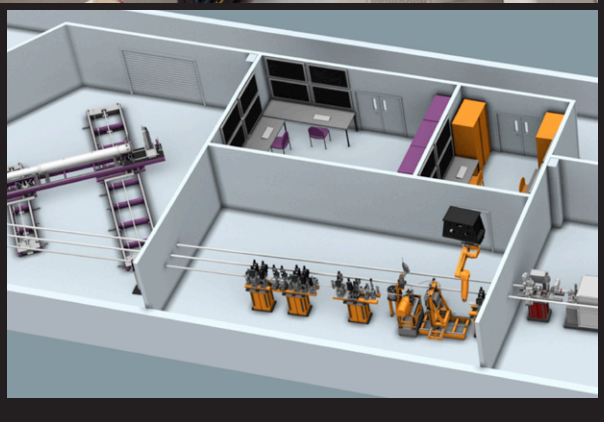
Project Summary: Synchrotron radiation has transformed biology over the past few decades by providing brilliant beams of X-ray light for probing the structures of molecules. Now X-ray free electron lasers (XFELs) promise to usher in another new era, allowing scientists to tackle important questions previously 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.

Objectives: (1) Develop a new instrument for diffraction, scattering, and imaging at the world's first operational XFEL, SLAC's Linac Coherent Light Source (LCLS). (2) 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 investigations of complex biological phenomena at many levels and scales.

Approach: Build the MFX station as a multipartner project within a 2-year time frame. The station will record diffraction patterns from micro- and nanosized crystals. The crystals are delivered into the XFEL beam by various means, from fixed targets (at both cryogenic and ambient temperatures) to liquid jets (at atmospheric pressure and ambient temperature), and include media mimicking the lipidic environment of cell membranes. These delivery techniques enable femtosecond serial crystallography and promise to reveal the structures of complex biomolecules or assemblies that do not form crystals of sufficient size or quality for study at synchrotrons. In other modes, MFX can be used to study how biological molecules change shape in response to external stimuli and how the active metal centers in proteins transfer electrons to carry out photosynthesis and other biological processes. MFX also can take X-ray snapshots of organelles and cells at medium resolution. Having a dedicated station for these types of experiments provides an optimized infrastructure (i.e., enhanced optics, state-of-the-art detectors, automated alignment, and real-time data monitoring and analysis) 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 collaborations with other relevant federally funded activities. 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.



Photograph (top) and End-Station Concept (bottom) of the Macromolecular Femtosecond Crystallography Instrument at the Linac Coherent Light Source. [Image courtesy SLAC National Accelerator Laboratory]

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