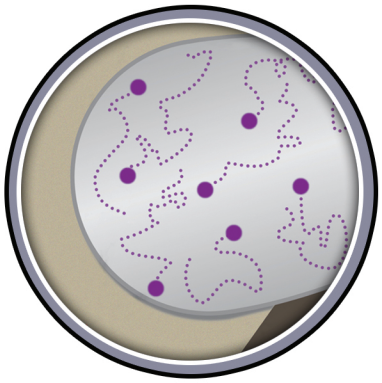

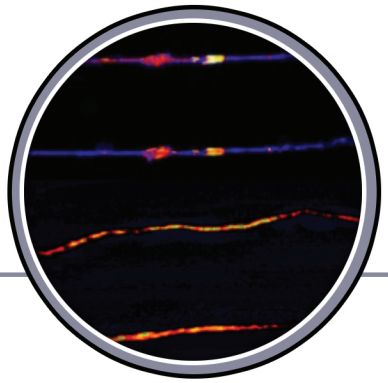

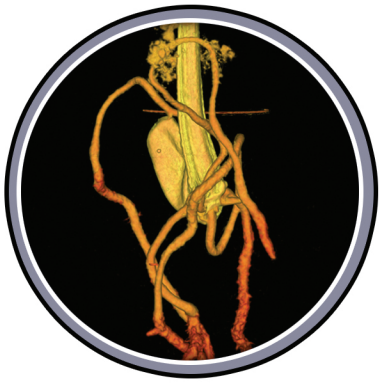

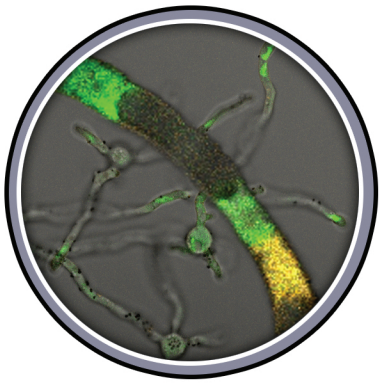

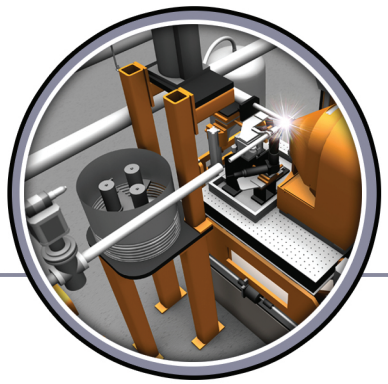

# New *In Situ* Imaging and Measurement Technologies for Biological Systems

U.S. DEPARTMENT OF ENERGY | Office of Science

Office of Biological and Environmental Research

August 2014

U.S. DEPARTMENT OF ENERGY | Office of Science

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# New *In Situ* Imaging and Measurement Technologies for Biological Systems


### Contact for Programmatic Information

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



This brochure is available at science.energy.gov/ber/news-and-resources/.


## About the Cover

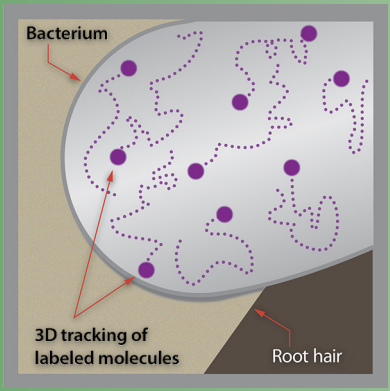
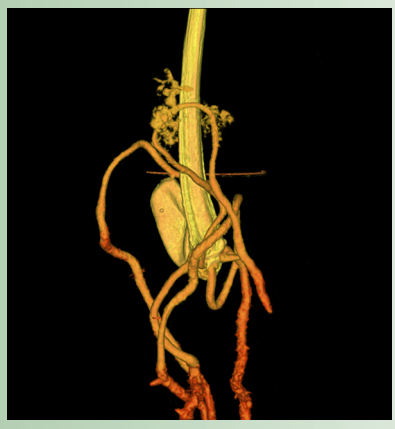
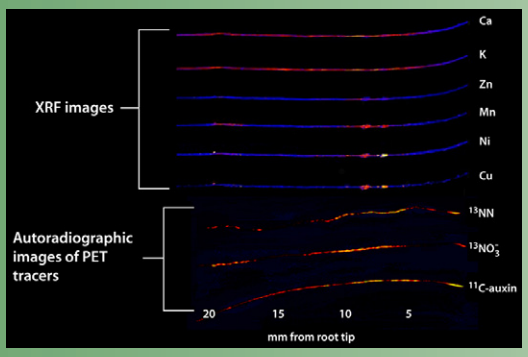


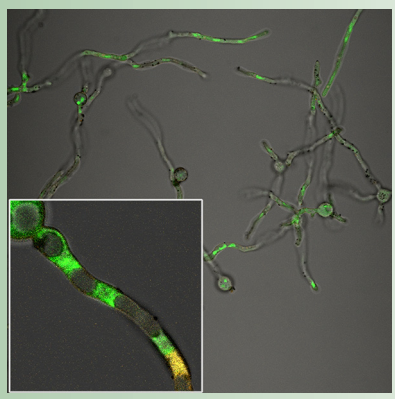

Illustration showing the tracking of labeled molecules within a rhizospheric bacterium. This example represents one step in a dynamic three-dimensional snapshot microscopy approach that can be applied to targeted organisms to reveal the process mechanics of complex molecular interactions. [Image courtesy Argonne National Laboratory]



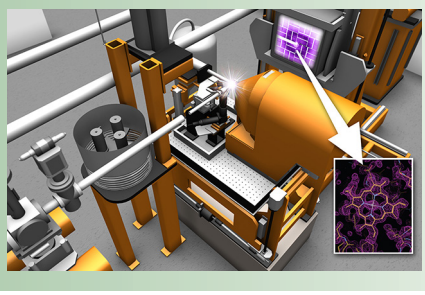
Three-dimensional neutron image of a wheat seedling with roots colonized by gadolinium-labeled *Pantoea* sp. YR343 approximately 5 days after inoculation. Root colonization is observed as a rough textural pattern (orange) along the root surface. Researchers can rotate the image on screen for more in-depth analysis. [Image courtesy Oak Ridge National Laboratory]




(Top) X-ray fluorescence (XRF) images showing concentrations of different metals along the length of a *Setaria* grass root. (Bottom) Autoradiographic images of three positron emission tomography (PET) tracers in three different *Setaria* grass roots. In both image sets, a color scale shows lower concentrations as blue and higher concentrations as red. All roots were exposed to *Azospirillum* (Hm053 hyper nitrogen fixing) bacteria. These results show how the metal concentrations, using XRF imaging, can be correlated with the uptake of PET radiotracers in plant root systems, illuminating the mechanism by which the association between these bacteria and the plant root system improves the plant's nitrogen uptake. [Image courtesy Brookhaven National Laboratory]



Overview and threefold-higher magnification (inset) confocal microscopy of filamentous fungi highlighting differential localization of green fluorescent protein (GFP) and Cy3 fluorophores during preliminary glucose uptake studies. [Image courtesy Pacific Northwest National Laboratory]



End-station concept of the macromolecular femtosecond crystallography instrument at the Linac Coherent Light Source. [Image courtesy SLAC National Accelerator Laboratory]

# 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 genetic potential encoded by organisms' genomes, BER 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 or among cells will act as a crucial bridge toward linking molecular-scale information to whole-cell, systems-level understanding.

This new technology development effort in BER is targeted at creating novel multifunctional technologies to image, measure, and model key metabolic processes within and among microbial cells and multicellular plant tissues. BER's current focus on developing a scientific basis for plant biomass-based biofuel production requires detailed understanding of cellular metabolism to incorporate, modify, or design beneficial properties into bioenergy-relevant plants and microbes. Likewise, the ability to track materials and chemical exchanges within and among cells and their environment is crucial to understanding the activity of microbial communities in environmental settings. New imaging and measurement technologies that can characterize multiple metabolic transformations will provide the integrative systems-level data needed to gain a more predictive understanding of complex biological processes relevant to BER.

In FY 2014, five pilot projects were initiated at separate DOE national laboratories to develop *in situ*, dynamic, and nondestructive approaches to multifunctional imaging, quantitative flux measurements, and multiscale integrative analysis of biological systems. These projects will serve as a prelude to initiating a longer-term technology development program. BER intends to follow up this initial effort at the national laboratories with a similar funding opportunity announcement (FOA) to the academic community, pending availability of funds, in FY 2015.

This brochure outlines the pilot projects at the DOE national laboratories and the investigators leading these efforts. This initial description of BER's new effort serves as an informational resource for the evolving imaging and measurement technology program.

Signed,

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

# Small Worlds

**Principal Investigators:** Kenneth M. Kemner and Mark Hereld
**Organization:** Argonne National Laboratory (ANL)
**Email:** kemner@anl.gov; hereld@anl.gov

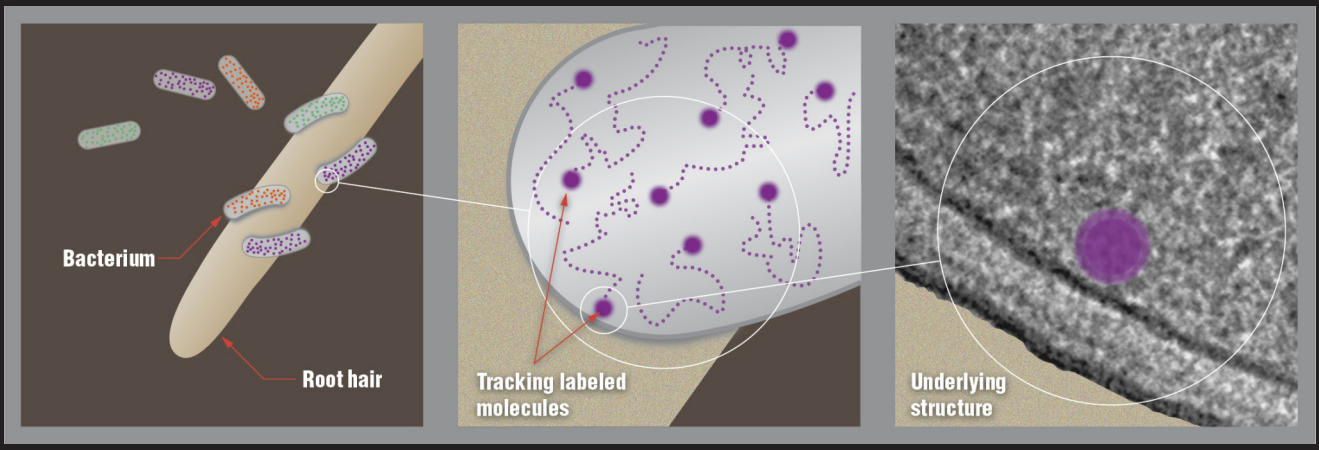
**Collaborators:** Frank Collart, Nicola Ferrier, Robin Graham, Sarah O'Brien, Elena Rozhkova, and Rosemarie Wilton (ANL); Oliver Cossairt (Northwestern University); Benjamin S. Glick and Norbert F. Scherer (University of Chicago)

**Project Summary:** The contingent of capabilities developed in this project will enable construction of dynamic experiments that can track and correlate interrelated molecular actors in complex processes, while providing detailed corroboration and supplementary data across physical 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: *three-dimensional* (3D), *multimodal imaging* and *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. This new microscope will be able to obtain the information required for reconstruction of 3D multiscale volumetric data of complex systems in a single "snapshot" measurement. This dynamic 3D snapshot microscopy will be complemented by new correlative tomographic methods in two separate imaging modalities: (1) electron tomography will provide high-resolution matching of dynamic processes to detailed cellular structure, and (2) X-ray tomography will enable development of larger-scale intercellular correlations for studying cross-organismal processes. 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 markers that function across modalities.




**Small Worlds Workflow Applied to a Transport Problem in the Rhizosphere.** (Left) X-ray tomography will aid identification of target organisms in a genetically marked sample. (Center) Dynamic three-dimensional imaging will then study molecular interactions to uncover process mechanics. (Right) Electron tomography will correlate detailed dynamics with high-resolution cellular structure. [Image courtesy Argonne National Laboratory]

# New Mesoscale Multimodal Imaging of Cellular Communication Between Microbe and Plant in the Rhizosphere

**Principal Investigator:** Sean McSweeney
**Organization:** Brookhaven National Laboratory (BNL)
**Email:** smcsweeney@bnl.gov

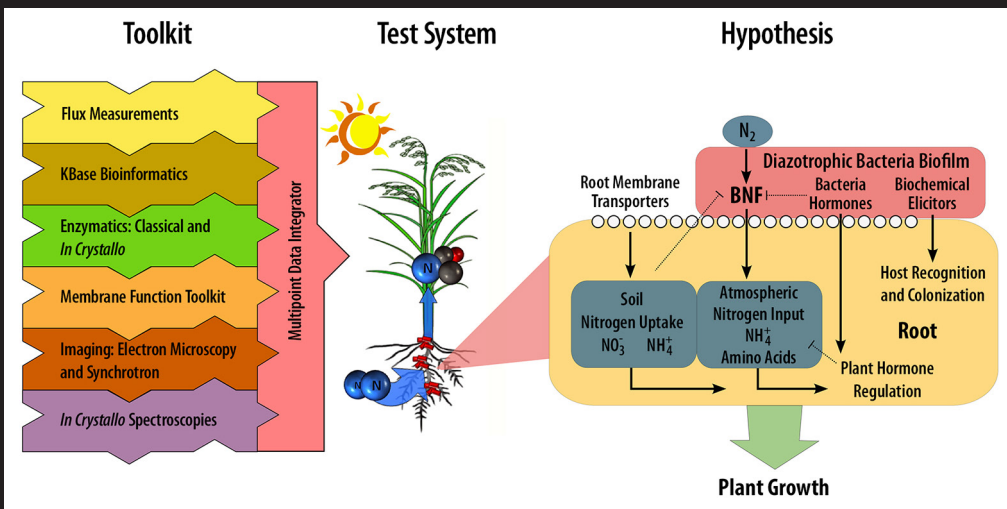
**Collaborators:** Richard Ferrieri, Huilin Li, Sergei Maslov, Lisa Miller, Allen Orville, Eric Stach, Ryan Tappero, and Lin Yang (BNL); Gary Stacey (University of Missouri, Columbia); Wayne Hendrickson and Qun Liu (Columbia University); Steve Almo (Albert Einstein College of Medicine); and Chris Anderton and Ljiljana Paša-Tolić (Pacific Northwest National Laboratory)

**Project Summary:** The interaction between plants and their environment is of key importance in promoting sustainable, healthy, and robust plant growth. Underlying the ability of plants to cope with a variety of stresses and often suboptimal conditions are sophisticated mechanisms to establish and maintain cellular homeostasis, as well as nurture beneficial symbiotic partnerships while defending against pathogenic exploitation. However, the interface between plants and their environment remains a poorly understood, complex microenvironment. Gaining insights into these interactions thus requires a combination of intercalated scientific techniques.

**Objective:** Achieve a more comprehensive understanding of how plant adaptation is influenced by the genome(s) and local environment. Such an understanding would potentially enable the engineering of more tolerant plants, optimization of cultivation practices to improve yield and productivity, and strategies allowing important plant species to flourish within marginal or otherwise challenging environments.

**Approach:** Understanding complex biological systems over many temporal and spatial scales presents a significant scientific challenge. This project will develop, extend, and integrate technologies to generate a "toolkit" capable of investigating length scales—from atoms to organisms—and dynamics—from microseconds to growth seasons. In providing these tools, the project will exploit (1) new capacities afforded by the National Synchrotron Light Source II (NSLS-II) at BNL for structural biology, imaging, and spectroscopy; (2) capabilities for creating and measuring short-lived radioisotopes including $^{11}C$, $^{13}N$, $^{15}O$, $^{18}F$, and several transition metals; (3) multilength-scale synchrotron imaging correlated with electron microscopy; and (4) partnerships with academic researchers to extend the toolkit's application.

**Impact:** Ultimately, a toolkit of general applicability in plant science to enable overlapping, quantitative investigations across length scales spanning seven orders of magnitude (millimeter to angstrom resolution). Details of these molecular processes and their dynamics also will be used to develop predictive models of metabolism and transport processes within and between symbiotic partnerships.




**Modular Plant Toolkit for Studying Complex Biological Processes.** Using state-of-the-art facilities available through the National Synchrotron Light Source II and other resources at Brookhaven National Laboratory (BNL), a set of interacting tools will be created to test hypotheses in complex biological systems. The driving force for the toolkit's initial development will be an investigation of plant sustainability through symbiosis with $N_2$-fixing plant growth promoting rhizobacteria (PGPR). The toolkit is needed to gain knowledge about these unique plant-microbe interactions from a mechanistic perspective. *Setaria viridis* (A10.1) is a PGPR growth-responsive C4 grass whose genome has been sequenced by the Department of Energy's Joint Genome Institute, making this genetically tractable model organism a good test system. [Image courtesy BNL]

# Adaptive Biosystems Imaging

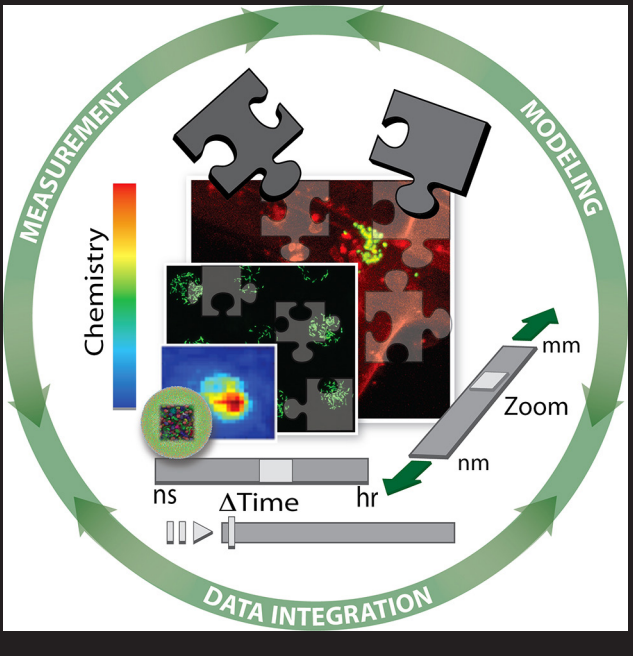
**Principal Investigator:** Mitchel J. Doktycz
**Organization:** Oak Ridge National Laboratory (ORNL)
**Email:** doktyczmj@ornl.gov
**Collaborators:** Jeremy Smith and Paul Langan (ORNL); Jonathan V. Sweedler and Zaida (Zan) Luthey-Schulten (University of Illinois, Urbana-Champaign); Xiaoliang (Sunney) Xie (Harvard University); Paul W. Bohn (University of Notre Dame); and Tessa R. Calhoun (University of Tennessee, Knoxville)

**Project Summary:** Understanding how observable biological processes, carried out over wide-ranging temporal and spatial scales, arise from molecular-scale events represents a grand challenge facing biological and environmental research. To address this challenge, ORNL is leading a pilot project 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 will 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 neutron sciences, nanofabrication, and high-speed computation to create new instrumentation, 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 through a robust data architecture system 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 can link 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.** Iterative collection of measurement data and integration with modeling information will lead to understanding the connections spanning diverse spatial, temporal, and chemical dimensions. [Image courtesy Oak Ridge National Laboratory]

# Systems Biology Through an Integrated Multimodal Imaging and Analysis Framework

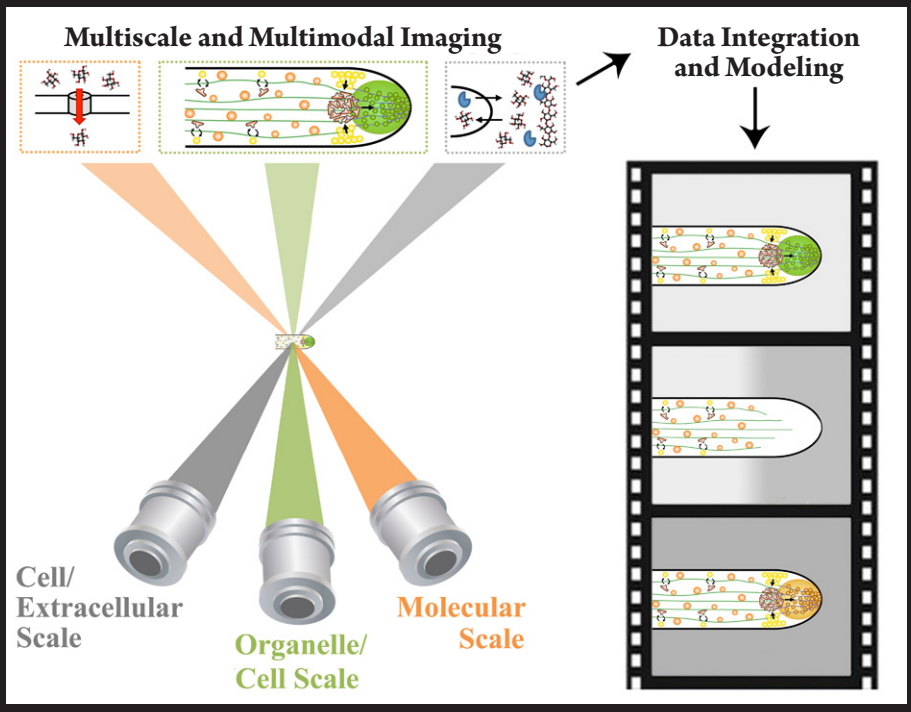
**Principal Investigator:** James E. Evans
**Organization:** Pacific Northwest National Laboratory (PNNL)
**Email:** james.evans@pnnl.gov
**Collaborators:** Scott Baker, William Cannon, Ziyu Dai, Ryan Kelly, Matthew Marshall, Galya Orr, Ljiljana Paša-Tolić, H. Steven Wiley, Aaron Wright, and Zihua Zhu (PNNL); Diane Lidke and Keith Lidke (University of New Mexico); and Mark Bathe (Massachusetts Institute of Technology)

**Project Summary:** Organisms have evolved a number of spatially localized processes for intra-, extra-, and intercellular communication to detect and respond to their environment. Unfortunately, no single technology exists that spans all the relevant spatial and temporal scales to observe the many mechanisms employed by cells that control tiered communication and cellular regulation. This project seeks to meet this challenge by establishing a multiscale, multimodal imaging and analysis platform.

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

**Approach:** Develop a multiscale, multimodal platform for structural, functional, chemical, and dynamic imaging of biological systems. This platform will allow transfer of samples among a suite of ion, electron, and optical technologies while maintaining indexing and registration for correlative analysis. Custom-designed probes and novel nano- and microfluidic chambers will enable dynamic and live-cell tracking of biological systems in response to chemical gradients. The initial focus will be on single-celled yeast and cyanobacteria and multicellular filamentous fungi to examine how chemical communication pathways, internal cellular organization, and protein localization in these organisms change in response to dynamic environments. Along with innovations in data integration and modeling, long-term goals are to expand the imaging and analytics to more complex biological systems, demonstrate broad applicability for linking the atomic to the mesoscale, and refine computational predictions of how cells interact with the environment.

**Impact:** Novel multifunctional probes and labeling chemistries, unique technologies, and integrative analytics for visualizing spatially regulated processes with relevant spatial and temporal scales and high chemical sensitivity. Ultimately, this platform will be made available to investigators within the Department of Energy's (DOE) Office of Biological and Environmental Research, and advancements will be published to benefit the larger scientific community. The expected scientific leadership and technology platform will help define the frontier for understanding the spatiotemporal control exhibited by cells in response to changing environments and guide the future design of biological systems to meet DOE mission needs.




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

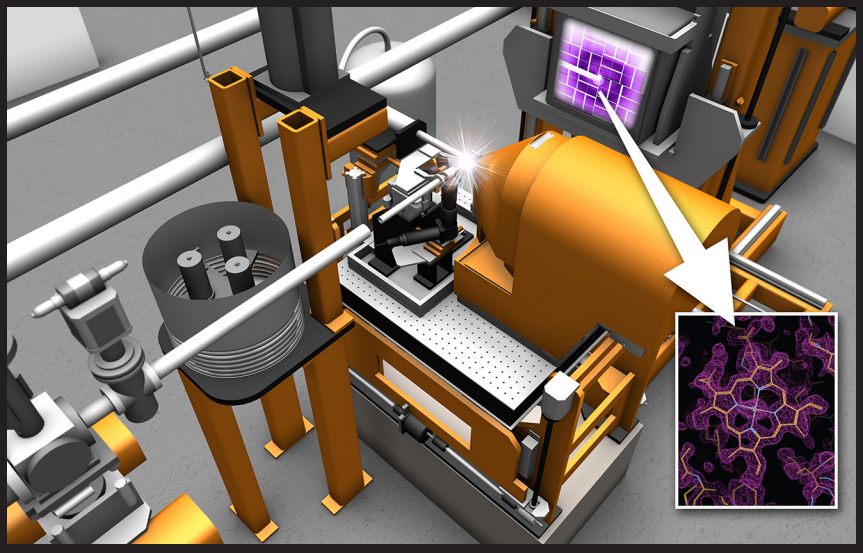
# SLAC Mesoscale Integrated Biology Pilot Project: MFX Station at LCLS

**Principal Investigator:** Soichi Wakatsuki
**Organization:** SLAC National Accelerator Laboratory (SLAC)
**Email:** soichi.wakatsuki@stanford.edu
**Collaborators:** Sébastien Boutet, Axel T. Brunger, Aina E. Cohen, David Fritz, Britt Hedman, Keith O. Hodgson, S. Michael Soltis, William I. Weis, and Garth Williams (SLAC/Stanford University)

**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 researchers from the United States and around the world to investigate 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. These 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. The availability of 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.



**End-station** concept of the macromolecular femtosecond crystallography instrument at the Linac Coherent Light Source. [Image courtesy SLAC National Accelerator Laboratory]

**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 federally funded user facilities, relevant programs within the Department of Energy's (DOE) Office of Biological and Environmental Research (BER), BER Virtual Laboratory, and DOE Systems Biology Knowledgebase (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.