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Integrated Dynamic 3D Imaging of Microbial Processes and Communities in Rhizosphere Environments: The Argonne Small Worlds Project

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Microscopic imaging is an important and powerful method for learning about the world around us and gaining mechanistic understanding of how complex biological and biogeochemical systems function. A main goal of a project based at Argonne National Laboratory entitled Small Worlds is the development of a new multi-modal imaging capability for studying complex multi-agent processes in cells and systems of cells across spatial and temporal scales. There are three general scientific questions that drive the development of technologies within the Small Worlds project. These include 1) What is the spatial arrangement and dynamics of metabolic function within bacteria and bacterial communities relative to a plant root?, 2) What is the spatial arrangement and biochemical dynamics of bacterial and community response to root exudates or other environmental stimuli such as light or nutrient stress?, and 3) What is the spatial distribution of bacteria and bacterial metabolism within pore structure of an opaque soil aggregate?

Within the Small Worlds project we are developing and integrating three imaging methods that, when used together, we expect will lead to new understanding of the organization and dynamic function of a wide range of complex biological systems. The imaging methods make use of three different types of probes for querying biological systems. The first approach, scanning x-ray fluorescence (XRF) imaging, allows sub-micron scale measurements of intact complex systems in their native environment, even if that environment is opaque to visible light. The second method, correlative electron-optical imaging, allows ultra-resolution imaging of whole organisms by transmission electron microscopy (TEM) and 3D spatial correlation with optical imaging of the identical (fixed) samples. The third development, multifocal microscopy (MFM), enables optical 3D microscopy of dynamic living systems with nanoscale resolution by interferometry, plus multi-scale volumetric imaging in a "snapshot" mode for quantitative determination of transport on nanometer to 100 μm scales. The three approaches are synergistic when used in tandem with reporters that function across one or more of the imaging methods.

System-specific molecular-scale reporters and methods for using them in multi-modal experimental protocols are being developed to enable introduction of a wide range of tracking, indicating, affecting, and registration functions into biological systems under study. Quantum dots (QDs) enable correlation across all three microscopies; solute binding proteins (SBPs) with FRET (Förster resonance energy transfer) capability provide the foundation for observing interactions with small molecules of interest (e.g., nutrients) and are of biological interest in their own right; self-labeling proteins enable

unprecedented resolution in correlative optical-electron microscopy; robust fluorescent proteins (FPs) enable multi-color tagging of molecules for dynamical study while surviving the rigors of sample preparation required by scanning transmission electron microscopy (STEM) tomography.

To dynamically image biological systems such as the root-microbe interface as the plant responds to stimuli, it is critical to position the plant within the viewing area of the microscope while also enabling real time stimulation and maintaining the viability of the plant. Therefore, we are also developing a new microfluidic root-microbe interaction sampler chip to enable performance of state-of-the-art confocal and MFM imaging involving timed interaction between organismal communities and nutrients, other organisms, and light in this new setting.

We have developed a green fluorescence arabinose sensor within the periplasmic space of a rhizobacteria (*P. fluorescens* SBW25) to signal the presence of arabinose near root cells. We also have developed a MFM capable of simultaneously imaging 25 focal planes separated by as little as 50 nm. These 25 planes can be imaged at a rate of 25 frames per second. Also, as a proof of principle, we have high-pressure frozen a sample of pure bacterial culture expressing a green fluorescence protein reporter that was embedded in plastic resin, sectioned, and examined by confocal microscopy. The section was subjected to electron tomography and the 3D fluorescence reconstruction was overlaid on the tomogram. Finally, we have used quantum dots consisting of a CdSe core that are conjugated to glycine to label bacteria. Next, using XRF microtomography, we have imaged the spatial distribution of QD-containing bacteria within a ~600-micron-sized soil aggregate that the bacteria were allowed to colonize.

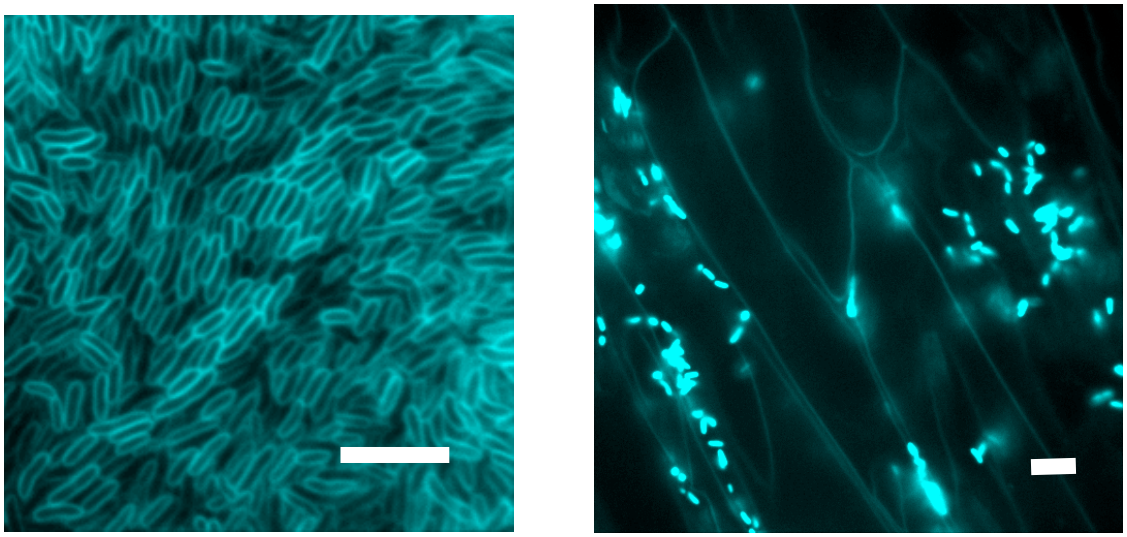

Figure 1. Confocal microscopy image of rhizobacteria (P. fluorescens SBW25) expressing fluorescence solute sensor in periplasmic space (left). Confocal microscopy image of cytoplasmic fluorescence-labeled P. fluorescens SB@25 colonizing root within microfluidic root-microbe interaction chamber (right). (scale bar = 10 microns).

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