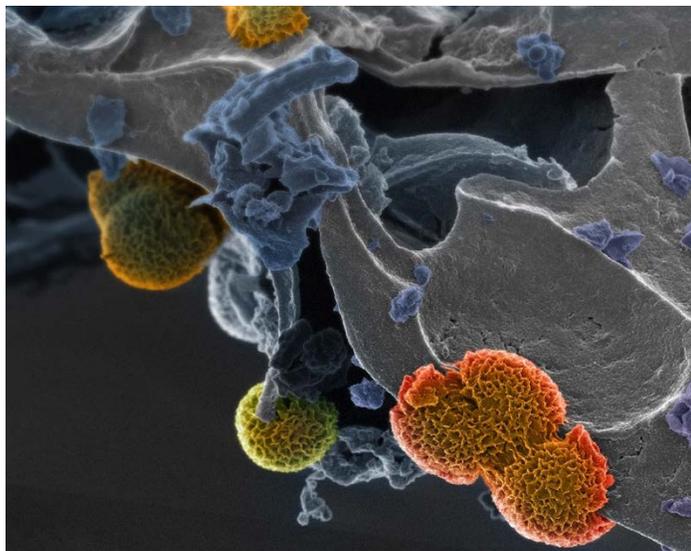


RHIZOSPHERE RESEARCH OPPORTUNITIES

EMSL scientists develop unique capabilities to provide the user community with a holistic view into the rhizosphere. Using multimodal approaches across length scales users can probe biogeochemical and hydrologic processes and dynamics from the molecular to pore scale. EMSL's unmatched multimodal analytical capabilities can be readily coupled with reactive transport models to provide multiscale simulations of rhizosphere processes and dynamics.



Plant Growth Facilities

For small stature plants EMSL's Plant Ecosystem Lab offers a variety of plant growth facilities including Conviron® walk-in rooms and Percival chambers. This allows growth and investigation of plants under environmentally controlled conditions with defined temperature, humidity, light intensity and CO₂ levels. The lab is currently under development and will be furnished with plant growth chambers equipped with automated imaging systems including visual red-green-blue and fluorescence cameras capable of revealing new transient or environment-specific phenotypes through replicating natural, dynamic or fluctuating environmental conditions. Sampling plant metabolites in root exudates is possible using hydroponic growth systems.

X-ray Computed Tomography

<https://www.emsl.pnl.gov/emslweb/instruments/x-ray-computed-tomography>

EMSL's X-ray computed tomography platform can characterize the pore structure of soils and sediments in rhizosphere cores as well as root architecture at spatial resolutions between a few hundred to tens of microns. A suite of data processing software reconstructs the set of 2-D projections into a 3-D image that can be segmented to quantify pore network tortuosity and connectivity or the surface area and volume of specified root orders. Reconstructed images can also be used as basis for reactive transport models.

Dynamic Multimodal Imaging

Fluorescence-based imaging:

<https://www.emsl.pnl.gov/emslweb/capabilities/cisa>

Mass spectrometry-based imaging:

<https://www.emsl.pnl.gov/emslweb/capabilities/mass-spectrometry>

<https://www.emsl.pnl.gov/emslweb/capabilities/microscopy>

EMSL offers unique capabilities for dynamic multimodal imaging of metabolites, lipids and proteins in microbial communities with (sub)cellular resolution, including fluorescence- and mass spectrometry-based imaging. Ambient ionization mass spectrometry imaging approaches facilitate *in-situ* molecular imaging of microbes (e.g. the rhizosphere) to directly map chemical distributions of intact molecular species (metabolites, proteins) employing liquid extraction surface analysis; laser ablation electrospray ionization; and nano-desorption electrospray ionization with 1-200 micron spatial resolution. Quantitative, laser scanning confocal, single molecule and super resolution fluorescence imaging, including structured illumination microscopy and Airyscan, can provide 3-D insights into dynamic processes in live cells, microbial communities and organisms with 300 nm – 120 nm spatial resolution. Insights with higher resolution (20 -30 nm) can be achieved by super resolution fluorescence stochastic optical reconstruction microscope (STORM) or photo-activated localization microscopy with intact hydrated cells. This approach supports quantitative gene expression analysis for multiple genes in intact cells using fluorescence *in-situ* hybridization (FISH). Combining nanoscale secondary ion mass spectrometry (nanoSIMS) with FISH can be used to quantify the expression of multiple genes of interest in individual cells to reveal mechanistic details about elemental flux within the microbiome with nanometer resolution. Combined MS, fluorescence and nanoSIMS experiments will enable linking biota with processes at the submicron scale, down to < 50 nm spatial resolution when combining STORM and nanoSIMS.

Extending to the nanoscale for both labeled and unlabeled components, helium ion microscopy provides nanoscale topographical and surface imaging ideal for studying plant/microbe interactions of intact specimens. Helios nanoLab scanning electron microscope coupled with energy dispersive spectroscopy (EDS) provides high-resolution imaging and chemical mapping and analyses. Cryo transmission electron microscopy (TEM) provides correlative high resolution imaging. It operates either at room temperature, or in cryo mode (-180C) for image acquisition of the rhizosphere components -- the plant root, the associated microbes and minerals, as well as the isolated macromolecules and other soil organic matter components. Coupled to the TEM, the samples can be further analyzed by EDS for elemental quantification, or by selected area electron diffraction mode for soil mineral characterization. Depending on the desired field of view and resolution, a sample preparation method is selected for the cross-sectional or whole mount imaging scheme. 3-D reconstruction of tilt series acquired by electron tomography facilitates virtual volume reconstruction, features segmentation and visualization.

Altogether, the array of imaging instrumentation at EMSL provides unique opportunities for correlative, multimodal and integrative bioimaging across scales perfectly suited for rhizosphere research.

Omics – Molecular Scale Characterization

<https://www.emsl.pnl.gov/emslweb/capabilities/mass-spectrometry>

https://www.emsl.pnl.gov/emslweb/capabilities/nmr_epr

<https://www.emsl.pnl.gov/emslweb/capabilities/cisa>

EMSL scientists and users are advancing the identification and interpretation of molecular signaling between microbes, fungi and plants as well as environmental interactions of microbes and microbial communities with soil and the atmosphere. EMSL houses a suite of measurement capabilities for metatranscriptomics, metaproteomics and metatranscriptomics in support of systems biology and multi-omics integration efforts. These include high-end MS instrumentation (i.e. Orbitraps) for quantitative metaproteomics, and next-generation sequencing systems such as the ion proton system for massively parallel unbiased sequencing and metatranscriptomics of complex microbial communities. The sequencing systems are also used for transcriptome analysis of small, spatially-defined bacterial clusters, isolated by laser capture microdissection, or defined subpopulations of cells, isolated by fluorescence activated cell sorter. EMSL also offers an ultra-high performance 21 Tesla Fourier transform ion cyclotron resonance mass spectrometer -- one of only two available world-wide. It has unmatched ability to identify and quantify diverse molecular species in complex systems, including intact proteins in fungal secretomes for top-down proteomics, allowing access to the functional capacity of the system, and analytes in complex environmental mixtures (i.e. dissolved or soil organic matter).

Top-down Proteomics

Top-down proteomics is aimed at functional characterization of intact proteins from natural or engineered plant or microbial systems to understand the effects of post-translational modifications and proteolytic processing events on their activity and stability. In contrast, the traditional bottom-up strategy, which involves the analysis of protein fragments, often destroys critical information about chemical modifications that alter protein structure and function. Similarly, activity-based proteomics enables highly specific tagging of microbial populations that participate in different metabolic/functional guilds of interest (e.g., lignocellulose degradation) detected by activity-based probes. Coupling activity-based probes with fluorescent tags allows imaging of these distinct populations with high spatial and chemical resolution, or sorting them using e.g. fluorescence activated cells sorting for further interrogation using multi-omics. This approach can determine identities and functional potential of distinct subpopulations and their specific roles within the ecosystem. Advanced microfluidic capabilities offer users the unique ability to perform omics measurements with limited amounts of material (down to very small

populations such as hundreds of microbial cells or a single eukaryotic cell).

EMSL has been at the forefront of proteomics and transcriptomics measurements at this level of sensitivity, opening up new possibilities such as proteomics of a single soil aggregate coupled with transcriptome analysis using approaches developed specifically for small amounts of starting RNA. Together, this suite of capabilities enables EMSL scientists and users to test hypotheses about the functional response of microorganisms, microbial communities and microbe-by-environment interactions.

Integrated Metabolomics

Integrated, state-of-the-art nuclear magnetic resonance spectroscopy and mass spectrometry of various types - gas chromatography, ion mobility spectrometry, and Fourier transform ion cyclotron resonance enable EMSL to provide increased identification coverage of the metabolomes of microbial communities, their hosts and their environments, and to identify the mechanisms by which metabolites involved in communication and interaction are generated, transported and sequestered within microbial communities.

Data Analytics, Modeling and Simulation

<https://www.emsl.pnl.gov/emslweb/capabilities/computing>

EMSL is expanding capabilities to couple computational resources with data generation. We are coupling metabolomics measurements with molecular dynamics simulations (NWChem code) to achieve accurate identification of metabolites without the use of chemical standards, thereby expanding the number and diversity of metabolites identified by mass spectrometry. We are performing genomic sequence analysis and data mining to improve the depth of coverage from proteomics studies. We are reconstructing images to provide input to reactive transport models. The extensive expertise at EMSL in multi-scale reactive transport modeling spans the pore-to-basin scale; in particular, our modeling expertise encompasses experience with a diverse suite of software systems, including smoothed particle hydrodynamics and transient energy transport hydrodynamic simulator for pore-scale simulation and PFLOTRAN, Amanzi and eSTOMP for continuum-scale simulation. EMSL is making codes for data analysis, modeling and simulation available on the Cascade high-performance computer, and we are applying them to rhizosphere samples and processes to track the transport of carbon and critical micronutrients in pore fluids within the rhizosphere and the chemical interactions with extracellular enzymes and mineral surfaces.

For more information about EMSL: www.emsl.pnnl.gov/

March 17, 2017

EMSL, the Environmental Molecular Sciences Laboratory, is a national scientific User Facility sponsored by the Department of Energy's Office of Biological and Environmental Research in the Office of Science. Located at Pacific Northwest National Laboratory in Richland, Wash., EMSL offers an open, collaborative environment for scientific discovery to researchers around the world.