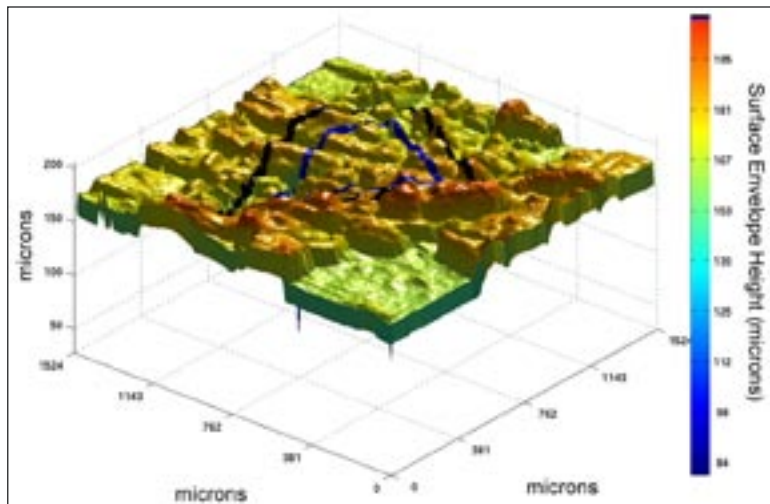


Advanced Cell and Molecular Imaging at Pacific Northwest National Laboratory

Most analytical techniques in biology provide information on populations of cells. However, to fully understand and model the behavior of biological systems, processes must be understood at the level of individual cells. Imaging technologies offer a powerful approach for observing and quantifying biochemical processes in living cells and bridge the gap between system and molecular-level studies.



3D topographical display of biofilm surface (data acquired by a scanning acoustic microscope).

To fully understand the behavior of a biological system, Pacific Northwest National Laboratory (PNNL) has developed a wide range of imaging technologies to probe biochemical processes using both living and fixed cells. Traditional microscopes analyze samples using a single imaging modality, usually with a single wavelength of light. We are building advanced instruments that combine the capabilities of multiple instruments, allowing different dimensions of information to be gathered simultaneously. We are also developing advanced algorithms to extract quantitative data from multispectral images.

Advances in microscopy require not only the development of more sensitive and specific instruments, but also the creation of software to operate the instruments and manage the large amounts of data they can generate. Scientists at PNNL are currently building the data infrastructure for storage, access, processing, and analysis of imaging data with the goal of turning advanced cell imaging into routine laboratory techniques.

A wide range of biological molecules can be detected at many different spatial scales using the imaging capabilities at PNNL. This provides researchers with the flexibility to apply imaging to a wide variety of different problems. Molecular detection capabilities include:

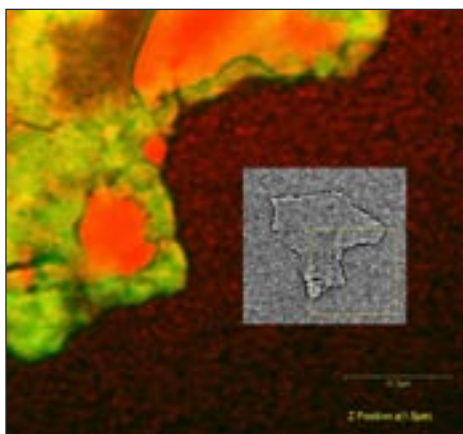
- Protein localization using fluorescent reporters and a variety of different epitope tags
- Metabolite distribution in biofilms and cell layers by NMR imaging
- Ion distribution using environmental scanning electron microscopy (ESEM)
- Protein dynamics in living cells using fluorescence resonance energy transfer (FRET)
- Single-molecule dynamics and spectroscopy.

Advanced biological imaging capabilities at PNNL enable the researcher to visualize both structures and protein activities within a cell, leading to insight about basic cellular mechanisms.

Imaging Technology and Instrumentation

A variety of imaging technologies allow researchers at PNNL to visualize biological processes down to individual molecules. Observing a sample with multiple technologies at one time allows the researcher to spatially correlate many different molecular activities. Capabilities include

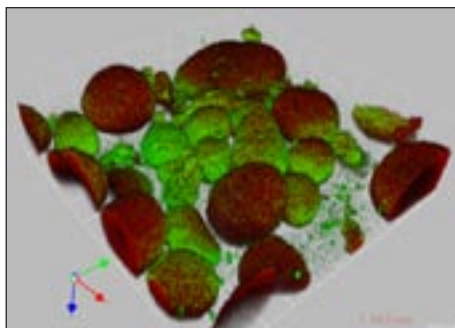
Combined CARS/two-photon confocal microscopy – This powerful instrument visualizes molecules based on their vibrational properties and by their fluorescent properties. It can visualize molecular species such as lipids and deuterated compounds.



One slice of 3D multiphoton image set, showing CARS image (red) and two-photon excited fluorescence (green) of *Shewanella oneidensis* MR-1. The bacteria are unstained and are visible by their hydrocarbon content and appear densely both within and outside the fluorescent matrix. The inset is a larger-area bright field image of the matrix, which is attached to the cover slip of the flow cell. A wide, shallow biofilm surrounds the dense matrix region.

Combined confocal and magnetic resonance microscope – Winner of a 2001 *Discover Magazine* Technology Award, this powerful microscope can simultaneously visualize gene expression patterns and metabolism and is currently being used to study medically relevant biofilms.

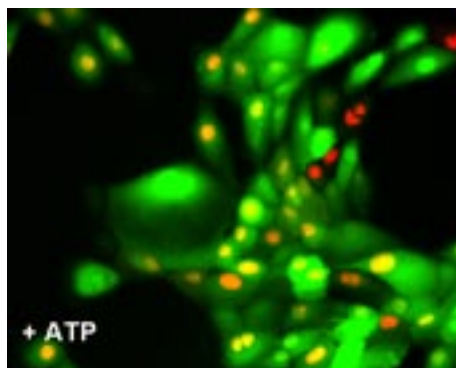
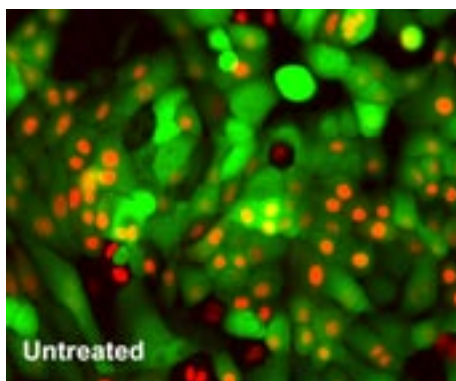
High-speed multispectral confocal microscope for high-throughput cell screening – Using customized software, this microscope allows real-time compartmental analysis of dozens of samples simultaneously.



Live imaging by confocal microscopy reveals 3D structure of biofilms in this developing biofilm of *Shewanella*. Red color indicates DNA in cells, and green color indicates extracellular polysaccharides that play a structural role in the biofilm and may actively and passively sequester metals and radionuclides from the environment. Each unit represents 40 microns.

Multispectral confocal microscopy for 3D imaging of living cells and tissues – Standard and multiphoton capabilities are available.

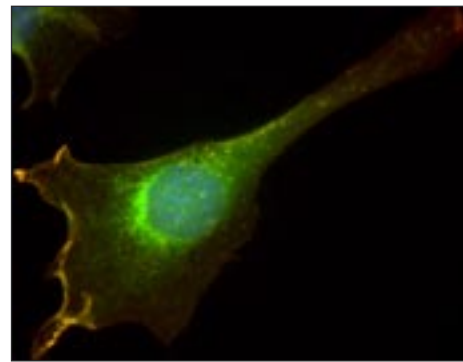
Single-molecule imaging and spectroscopy – The dynamics of individual molecules can be followed at the cell surface using photobleaching and total internal reflection fluorescence (TIRF).



Nuclear translocation of mitogen-activated protein kinase (MAPK) protein following stimulation with adenosine triphosphate (ATP) for 10 minutes. Nuclei are labeled with mRFP.

Atomic force microscopy-enhanced fluorescence imaging microscopy – This combined microscope uses the optical/AFM approach and a high-sensitivity far-field microscope to provide atomic-level reaction rate measurements.

Scanning acoustic microscope – Provides non-invasive, rapid measurements and is very sensitive to localized changes of density, stiffness, and viscosity, even in optically opaque samples such as biofilms.



Pulse chase experiments using multi-use affinity tags track changes in localization patterns and protein stability. Cells were labeled with a pulse of a red tag to label existing protein and relabeled 5 hours later with a green tag to label newly synthesized protein.

Electron microscope suite –

- LEO 982 ultrahigh-performance field-emission scanning electron microscope
- ElectroScan Environmental Scanning Electron Microscope (ESEM)
- Environmental Field Emission (FEI) Scanning Electron Microscope
- EOL 2010 high-resolution transmission electron microscope.

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