**Correlative High-Resolution Imaging and Spectroscopy to Characterize the Structure and Function of Microbial Biofilms** Research Team: Matthew Marshall, Alice Dohnalkova, Erin Miller, Zihua Zhu, James Evans Key Collaborators: James Carson, Mathew Thomas, Andrew Kuprat, Senthil Kabilan, Craig Szymanski, Xiao-Ying Yu (PNNL), David Kilcoyne (ALS), Xianghui Xiao, Barry Lai (APS)

# Purpose

- Illustrate how biofilms interact with liquids or substrates and reveal how microbe-scale interactions influence larger, pore-scale environmental processes
- Develop fundamental correlative capabilities for visualization, chemical analysis, and functional characterization of biofilms

# **Key Successes**

- Revealed architecture and chemistry of hydrated biofilms at the micro- and nano- scales to be a complex, biological system
- Multi-scale biofilm structural investigations provide understanding of bacterial environmental interactions with local fluid environments, solid surfaces, and as a dynamic microbial community
- Chemical imaging enabled interpretations of cell physiology relative to the biofilm's local environment

# **Research Accomplishments**

# Imaging



Cryogenic preparation of biofilm sections preserves excellent morphology and yields samples amiable for scanning transmission X-ray microscopy (STXM) analyses.

## Hydrated Biofilm Chemical Imaging



In situ microfluidic cell enables depth- and temporal-resolved, hydrated-state biofilm imaging using time-of-flight-secondary ion mass spectrometry (ToF-SIMS).

### **Biofilm Cryogenic Preparation and Chemical**

### X-ray Microtomography



conditions.





Developed high Z element staining to enhance the visualization of low Z biological materials in both dehydrated and hydrated

Constructed 3D renderings of biofilm morphology and imaged an intricate network of internal channels that facilitate fluid perfusion through the biomass.

Additional work will elicit biomass distribution as a function of distance from the nearest point of nutrient advection. This will demonstrate the relationship of nutrient advection and nutrient diffusion for sustenance across the biomass.

### **Gratings-Based Phase Contrast**





Used differential phase contrast tomography to image low Z hydrated biofilms without high Z contrasting agents.

- **Next Steps**
- \$2.5M)



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#### Project 1.1; October 2010 – September 2013

Phase contrast tomography setup at the APS.

Possible additional funding for multi-system analysis of subsurface biofilms from DOE-BER Early Career Research Award (5 years,

Apply approaches to pursue long-term support for spatial heterogeneity analysis in highcomplexity, microbial biofilm/mat systems Use methodologies to pursue extramural funding for host-pathogen biofilm research

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