Correlative High-Resolution Imaging and Spectroscopy to Characterize the Structure and Function of Microbial Biofilms

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**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**

**Biofilm Cryogenic Preparation and Chemical 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).

**X-ray Microtomography**

- Developed high Z element staining to enhance the visualization of low Z biological materials in both dehydrated and hydrated conditions.
- Constructed 3D renderings of biofilm morphology and imaged an intricate network of internal channels that facilitate fluid perfusion through the biomass.

**Gratings-Based Phase Contrast**

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

**Next Steps**

- Possible additional funding for multi-system analysis of subsurface biofilms from DOE-BER Early Career Research Award (5 years, $2.5M)
- Apply approaches to pursue long-term support for spatial heterogeneity analysis in high-complexity, microbial biofilm/mat systems
- Use methodologies to pursue extramural funding for host-pathogen biofilm research

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