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

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## Purpose

Develop fundamental capabilities for enhanced visualization, compositional analysis, and functional characterization of biofilms.

Understanding how biofilms interact with substrates connects molecular and microbial scales and shows how organisms influence larger, pore-scale processes.

## Expected Outcomes

Understand chemical signals in a 3D biofilm as a function of cell physiology and the local environment.

Ability to visualize a biofilm in its nearest-to-native state.

Develop cryo X-ray imaging techniques and other technologies at synchrotron facilities.

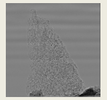
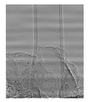
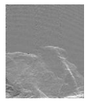
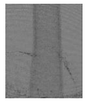
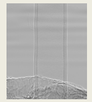
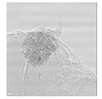
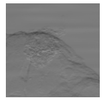
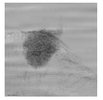
## Task 1: Established reproducible model systems for *Shewanella* biofilm growth and correlative imaging and chemical analysis.

Produced on hollow-fiber membrane bioreactor

Produced on/in Bio-Sep bead system

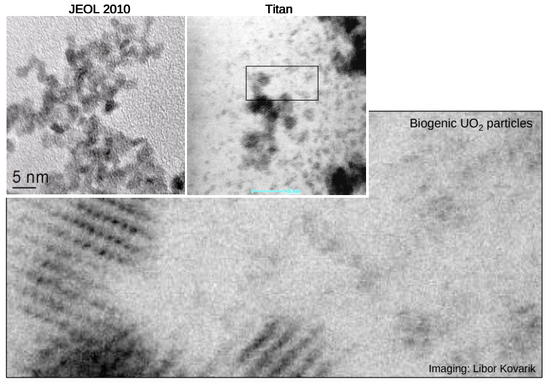
Produced on/in soil aggregate

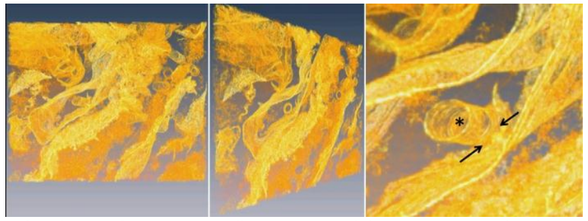
## Task 2: Investigated grating interferometry imaging of biofilms on complex substrates.

| Technique<br>All images are 1.4 mm high (0.7 μm/pixel) and taken at 18.3 KeV. | Absorption | Phase<br>Grating lines are horizontal. | Scatter |
|---|---|---|---|
| Absorption/Propagation[1]. BioSep bead segment mounted in wax is approximately 150 mm from scintillator. |  <br>Edge enhancements are clearly visible, and dominate the intensity scale. | | |
| Single grating (Fourier X-ray scattering radiography)[2]. Fiber segment mounted in wax imaged using a phase grating with 4 μm period (2 μm interference pattern) segment is approximately 150 mm from scintillator. |  <br>Edge effects due to propagation still present. |  <br>Differential phase image sensitive to gradients in vertical direction; low-Z materials. |  <br>Scatter highlights stronger texture present in fiber as compared to wax. |
| Double grating (Talbot interferometry)[3]. Fiber segment mounted in wax imaged using a phase grating with 4 μm period (2 μm interference pattern), modulated by a 2 μm period absorption grating at the first fractional Talbot distance (24 phase steps). |  | <br>Features similar to single grating, but improved signal to noise with higher fringe visibility. | <br>Texture highlighted. Improved signal to noise due to fringe visibility. |
| Double grating (Talbot interferometry)[3]. Soil aggregate mounted in wax; imaged as described in previous panel. |  |  <br>Note that the wax has a strong phase signal, even relative to the soil aggregate. |  <br>Highly textured soil aggregate is much more strongly scattering than the wax. |

[1]R. Fitzgerald, *Physics Today* v53 p23-26 (2000); [2]Pfeiffer et al., *Nature Physics* 2(4) 258-261 (2006); [3]Wen et al., *IEEE Transactions on Medical Imaging* 27(8) 997-1002 (2008).

## Task 3: Imaged biogenic $UO_2$ particles, reconstructed $UO_2$-EPS interaction.




***(Top)*** Using JEOL TEM, smallest nanoparticle was assumed to be 2.5 nm; with Titan S/TEM visualized nanoparticles smaller than 1 nm.

***(Bottom)*** Amira 3D reconstruction of *Shewanella* cells and EPS matrix associated with $UO_2$-EPS. Cell (*) can easily be seen in close proximity with $UO_2$-EPS (→).

## Key S&T Achievements

**MJ Marshall:** Attended ANL complexity workshop; IRENI workshop; presented data at 2011 Microscopy and Microanalysis conference

**AC Dohnalkova:** Attended ALS information sharing, collaborative meeting

**EA Miller:** Presented data at 2011 APS Users meeting

**MJ Marshall:** DOE Office of Science Early Career Research Program Award (5 yr, $2.5M)

## S&T Accomplishments: Beam Time Awards

| Facility | Beamline | Proposal Status | Comments |
|---|---|---|---|
| APS (ANL) | 2-BM-B | Accepted (GUP_# 23434) | 27 shifts |
| ALS (LBNL) | BL5.3.2.2 | Accepted (ALS-04551) | 9 shifts |
| ALS (LBNL) | BL 11.0.2 | not awarded | -- |
| ALS (LBNL) | BL2.1 | Accepted (ALS-04551) | 15 shifts |
| SRC (Univ. of WI) | IRENI | Accepted | 10 shifts |

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