

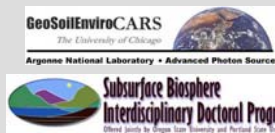
# Spatial Distribution of Biofilms in Porous Media

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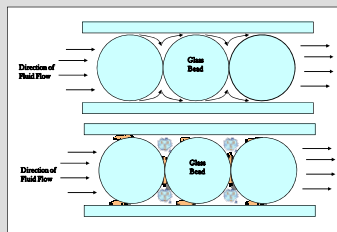


## Introduction

Current understanding of subsurface microbial biofilm formation and their impact on fluid hydrodynamics is limited by our ability to observe the *in situ* microscale geometry of developed biofilms. Biomass distribution in porous media has been observed previously in only two dimensional systems; currently, no high-resolution 3-dimensional datasets exist that give sufficient information about microbial distribution such that the impact on flow and transport at the microscale can be directly computed. Three dimensional biofilms can significantly alter pore flow velocities and overall mass transfer between the aqueous and biological phases. We are currently developing new methods to resolve high-resolution 3-dimensional tomographic images of biofilms in porous media using synchrotron based x-ray microtomography. Imaging biofilms without disturbing their natural spatial arrangement is a challenging task, primarily because most conventional dopants that dissolve in water also easily diffuse into biofilms. One method that we have developed to overcome this problem is the addition of silver nanoparticles to the fluid phase. Using this approach, we have been able to differentiate between the biomass-filled pore space and fluid-filled pore space. To date, the images that we have collected have yielded representations of the geometry and qualitative information about structures of biomass. Ultimately, we intend to combine this kind of experimental measurement with upscaling (via volume averaging) to determine how biofilms alter the physical properties of the porous media. By quantifying the spatial distribution of biofilms we will gain a greater understanding of how changes in physical parameters may impact the rate at which microbes degrade contaminants or produce products, and therefore this research has applications to bioremediation and bioprocessing.

## Motivation:

Changes in physical soil properties will not only impact bulk soil properties but also microscale flow within the pore space. Understanding the distribution of flow in void space is necessary in the modeling of substrate removal and mass transfer. Information acquired through nondestructive imaging is required for developing a full understanding of the multi-phase system. Results from experiments will later be used for development and validation of a hydrodynamic model that includes biofilm growth, cell attachment, and detachment as a function of substrate concentration and fluid forces.



Schematic diagram of flow around glass beads with and without biofilms

## X-Ray Microtomography:

Used in collection of three dimensional flow cell images

### Advantages

- Nondestructive
- High resolution
- Imaging above and below photoelectric edge
- Well developed quantitative image analysis techniques

### Disadvantages

- Traditionally used dopants diffuse into biomass
- Restricted to radio resistant microorganisms



Advanced Photon Source, Argonne National Lab

## 3-Dimensional Flow Cell

### Objective:

To determine dopant that will provide the clear contrast between biofilms, water, and porous media.

### Experiment Set Up:

- TGY growth media (1.0% tryptobactone, 0.1% glucose, 0.5% yeast extract)
- Packed with 0.8-1.2 mm glass beads or 0.6-1.0 mm polystyrene beads
- Each flow cell was inoculated with 1.0 mL concentrated solution of *D. radiodurans*
- Approximate flow rate of 1.4 mL/hr
- Allowed microbes to grow for extended period of time (5 to 21 days)
- Attempted to image biomass in flow cell using phase contrast x-ray tomography, and imaging solutions with x-ray tomography



Flow cells during biofilm growth at APS

### Column dimensions:

Inner Diameter	0.6 cm
Column Height	7.0 cm
Pore Volume	0.5 mL

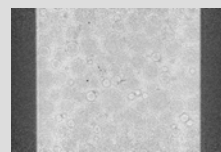
### Imaging Solutions:

Imaging Solution	Contrast Agent	Energy Edge
Fenestra	Iodine	33.2-36 KeV
Isovue	Iodine	33.2-36 KeV
Metallic Nanoparticles	Silver	25.5 KeV
Silver Coated Glass Spheres	Silver	25.5 KeV

### Images:



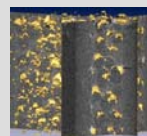
Horizontal slice of Fenestra Imaging Solution and Polystyrene Beads  
Resolution 13 µm



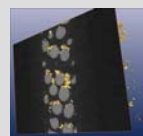
Vertical slice of Fenestra Imaging Solution and Glass Beads  
Resolution 13 µm



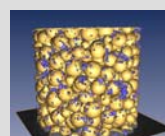
Horizontal slice of Silver Nanoparticles and Polystyrene Beads  
Resolution 13 µm



Processed Image of Polystyrene Beads without biofilm present  
Contrast Agent: Silver Nanoparticles  
Diameter - 200 nm  
Resolution 13 µm



Processed Image of Glass Beads with Biofilm Present - Vertical Slice  
Contrast Agent: Silver Coated Glass Spheres  
Diameter - 10-20 microns. Silver coating 150 nm  
Resolution 13 µm



Processed Images of Glass Beads (0.8-1.2 micron) with Biofilm Present  
Contrast Agent: Hollow Silver Coated Glass Spheres  
Diameter - 10-20 microns. Silver coating 150 nm  
Resolution - 13 µm



### Observations:

- Note the difference in distribution and morphology of the contrast agent particles in the column with biofilm and without biofilm present (see bottom row, first two images from the left).

### Future Work:

- Though silver coated hollow glass spheres appear to provide qualitative information about biofilm structure we are now exploring the use of gold antibodies, that adsorb to cell walls, as a method to resolve biofilm structure with greater resolution

## 2-Dimensional Flow Cell

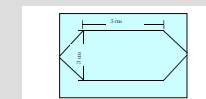
### Objective:

To determine distribution of particles in and around biofilms in a 2-Dimensional flow cell under conditions similar to those in the 3-D columns. Information will be used to qualitatively interpret distribution of particles in reference to biofilm structure and dopants in 3-D columns.

### Approach:

- Constructed 2-D acrylic flow cell
- Packed with 1 mm glass beads
- Sealed with epoxy
- Imaged biofilm in flow cell using digital microscopy

### Flow Cell Dimensions:



2-D flow cell top-down



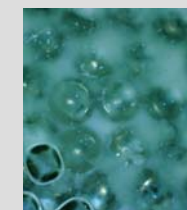
2-D flow cell under microscope

### Experiment Set Up:

- TGY growth media (1.0% tryptobactone, 0.1% glucose, 0.5% yeast extract)
- Flow cell inoculated with 0.5 ml concentrated solution of *D. radiodurans*
- Flow rate 2.8 ml/hr
- Allowed biofilm to grow for two weeks
- Imaged biofilm in flow cell using digital microscopy
- Injected (via syringe) with 0.5 ml 43 µm latex beads



Experiment set up in Flow and Transport Lab



1 mm glass beads with biofilms and 43 µm latex beads

### Preliminary Results:

With dense biofilm, the 43 µm latex beads congregate in the outer layer of the biofilm and at the water/bead interface. These observations support interpretation of nanoparticle congregation in and around biofilms in the 3-D tomographic images.

### Future Work:

- Additional experiments are required to determine the full range of particle sizes that can be used as contrast agents
- To obtain a more obvious contrast between particles and biofilm, fluorescent particles may be used

### Acknowledgements:

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