Annual Report 01/01/10 – 12/31/10

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Title: Three-Dimensional Imaging and Quantification of Biomass and Biofilms in Porous Media

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Summary of second year research

A new method to resolve biofilms in three dimensions in porous media using high-resolution synchrotron-based x-ray computed microtomography (CMT) has been developed. Imaging biofilms in porous media without disturbing the natural spatial arrangement of the porous media and associated biofilm has been a challenging task, primarily because porous media generally precludes conventional imaging via optical microscopy; x-ray tomography offers a potential alternative. One challenge for using this method is that most conventional x-ray contrast agents are water-soluble and easily diffuse into biofilms. To overcome this problem, silver-coated microspheres were added to the fluid phase to create an x-ray contrast that does not diffuse into the biofilm mass. Using this approach, biofilm imaging in porous media was accomplished with sufficient contrast to differentiate between the biomass- and fluid-filled pore spaces. The method was validated by using a two-dimensional micro-model flow cell where both light microscopy and CMT imaging were used to im age the biofilm. We reported in detail on these experiments in last year's report, and the results are now accepted and in print with *Water Resources Research (Iltis et al., 2010)*. Additional work needs to be done to optimize this imaging approach, specifically, we find that the quality of the images are highly dependent on the coverage of the biofilm with Ag particles, - which means that we may have issues in dead-end pore space and for very low density (fluffy) biofilms. What we can image for certain with this technique is the biofilm surface that is well-connected to flow paths and thus well-supplied with nutrients etc.

In Nov 2009, we put this new method to work with experiments at the APS that focused on quantifying features of biofilms formed by different bacteria, and for different flow rates, see Figure 1a and 1b. We saw a clear effect of proximity to the inlet (located at the bottom of the column) where more biofilm grew and thus caused greater reduction in porosity. We are now conducting additional experiments to keep track of mass balances oxygen contents etc. to shed mor elight on the controlling mechanisms, but the image and data shows that we can begin to quantify thes type of features in a 3D opaque porous medium.

During 2010, we also started collaborating with Yohan Davit from Univ. of Toulouse in France on using a complementary tomographic imaging technique for visualizing and quantifying biofilms in porous media. The approach is based on use of a $BaSO_4$ suspension, which functions as a very good x-ray contrast agent, and is size-excluded from entering the biofilm. This work resulted in a co-authored paper in *Journal of Microscopy (Davit et al., 2010)*, however we felt that a verification study was warranted for this technique as well, so designed a round of fairly complex experiments in collaboration with Yohan Davit to test the Ag particle and the BaSO4 suspension approach on the same flow systems, and also included concurrent confocal imaging (with CLSM) of the biofilm carried out by James Connolly, a PhD student in Robin Gerlach's group at Montana State University (working on the SBR-funded project lead by Rick Colwell).

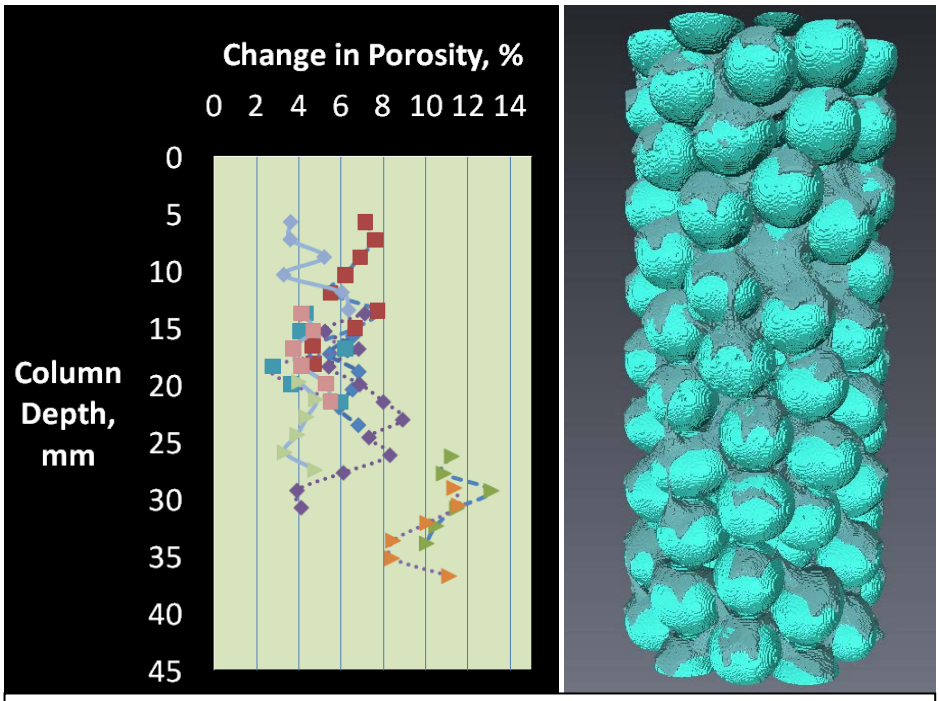

Figure 1. (a) glass bead pack (light green) with biofilm (darker green) identified by strained Ag particles for a longer section of column. **(b)** change in porosity along the column height due to biofilm growth (S. *oneidensis*).

The work was carried out in Nov 2010, and the preliminary analysis has resulted in some very nice tomographic images of biofilm in 3D flow cells (see Figure 2), as well as confocal images of the 2D flow cells that were also imaged with tomography for verification (see Figure 3). The left-hand image in Figure 2 shows biofilm as resolved using BaSO4, and the right-hand image is the same sample/biofilm as resolved with Ag particles (illustrated in darker blue behind the transparent point-wrapped surface rendering). On initial inspection, the BaSO4 approach appears advantageous, but we have yet to compare the 2D images using tomography and both contrast agents to the confocal images of the same flow cell to verify that the biofilm is not affected (compressed) by the relatively dense BaSO4 suspension. The confocal images in Figure 3 show both the Ag particles in the 2D flow cell (lower image), and the fluorescent-stained biofilm (upper image), and we see a very nice delineation of the outer biofilm surface with the Ag particles. Again, we have more work to do as we have not yet analyzed the images that contain both Ag particles and BaSO4 suspension.

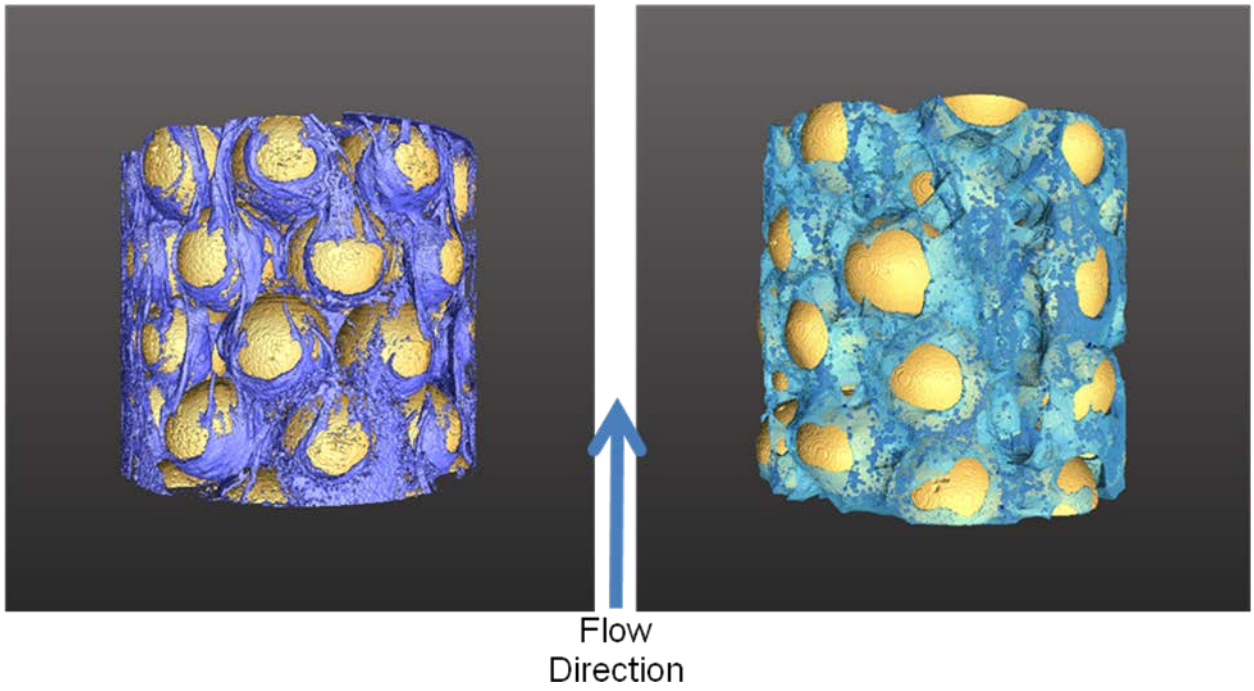

Figure 2. Glass bead pack (yellow) with biofilm (purple) identified using the BaSO4 suspension approach and (b) using the strained Ag particle approach.

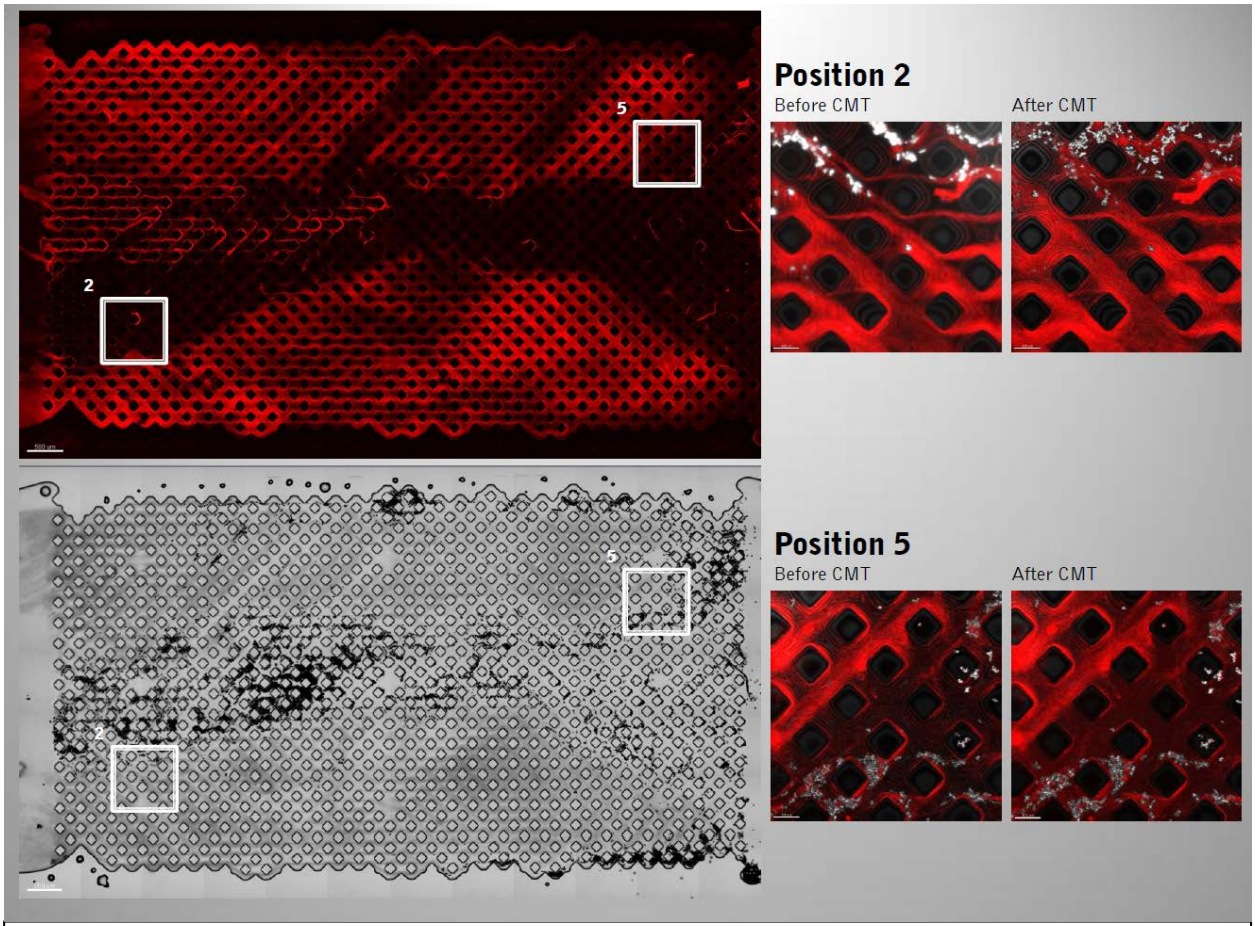

Figure 3. CLSM imaging performed by James Connelly (MSU) to compare confocal images (using a fluorescent stain to outline the biofilm) (top image) and comparison to the surface outlined by Ag particles (lower image) that are used in tomographic imaging. The right-hand images show the highlighted sections in more detail and verifiy that there was no change in biofilm structure due to tomographic (CMT) imaging.

Discussion

We continue to develop the technique based on x-ray tomography to image biofilms in opaque porous media, currently using two different agents for contrast between biofilm and the surrounding fluid and solid phases. The technique facilitates three-dimensional imaging of biofilm within porous media and we foresee it developing to the point where we can accurately represent the solid-biofilm-aqueous phase spatial arrangement. This method is particularly well-suited for pore-scale investigations were a triangulated mesh can be generated which would provide a convenient platform for additional analysis of fluid and solute transport via a finite element or finite volume numerical simulator.

To our knowledge, this is the first successful attempt using high resolution CMT to image biofilms in porous media, and likely using any method at this resolution and in three dimensions for opaque media. Iltis gave an invited talk at last week's AGU Fall Meeting which prompted significant interest from colleagues who want to start using the method in their research.

Work during the remaining 3 months of this award will focus on completion of all the image analysis tasks, and publication of the comparison study with Davit and Connelly. However, Iltis will continue to work on the technique and further publications throughout 2011 (likely with funding from the larger Colwell grant), possibly including a second trip to the APS in Summer to complete the confocal/Ag particle/BaSO4 suspension comparison study in collaboration with Gerlach's group at MSU. At this point we believe that the contrast agent approach is a more versatile route to pursue than the much more highly specialized anti-body approach outlined in the original proposal, which requires significant work for each new microbe, whereas this approach works for any bacterium that forms a sufficiently dense biofilm.

References

Iltis, G., Armstrong, R.T., D.P. Jansik, B.D. Wood, and D. Wildenschild, 2010. Imaging Biofilm Architecture within Porous Media using Synchrotron based X-Ray Computed Microtomography. In print, *Water Resour. Res.*.

Davit, Y., G. Iltis, G. Debenesta, S. Veran-Tissoiresa, D. Wildenschild, M. Gerinoc, M. Quintard, 2010. Imaging biofilms in porous media using X-ray computed microtomography, *Journal of Microscopy*, DOI: 10.1111/j.1365-2818.2010.03432.x.

Iltis, G., Y. Davit, J. Connelly, B. D. Wood, D. Wildenschild, 2010, In situ imaging of biofilm within opaque porous media (Invited). *Eos Trans. AGU,* Fall Meet. Suppl., Abstract NS33A-03.

Iltis, G., R.T. Armstrong, and D. Wildenschild, 2010. Visualization and quantification of biofilm architecture within porous media using synchrotron based x-ray computed microtomography. *Geox2010: 3rd International Workshop on X-Ray CT for Geomaterials*, New Orleans, LA, March 1-3. 2010.

Acknowledgements

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