Advanced Photon Source Activity Report 2003: Microscale Imaging of Pore Structure and Microbial Habitats in Hydrothermal Sulfide Chimneys Using X-Ray Micro-computed Tomography

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Introduction

Microorganism metabolism and reproduction are tightly coupled to the local geochemistry of the aqueous and solid phases in and on which they live. However, little quantitative information about the physical structure and chemical composition of microbial habitats actually exists at a scale relevant to bacteria and bacterial community size (~1-100 μm). This is especially true of extreme thermophilic environments such as deep-sea hydrothermal sulfide chimneys where novel microorganisms thrive at steep temperature and composition gradients that are difficult to study. A significant limitation to probing the physical and chemical structure of microbial habitats is the destructive nature of most characterization methods. Typical light or electron microscopy methods require cutting, grinding, polishing, freezing, drying, or other types of preparation methods that impact physical and biological materials and their spatial relationships. These methods also create opportunities for biological and chemical contamination. Molecular biology studies of real systems typically extract genomic material from natural samples and characterize RNA or DNA content with little knowledge of the physical-chemical environment from which the organism originated. As a result, we have the capability for complete characterization an organism's genome, while simultaneously having no knowledge of where it came from, what it does, or how it lives.

The development of data collection and analysis methods for synchrotron X-ray micro-computed tomography (μ-CT) has provided a powerful tool for non-destructive, three-dimensional characterization of microporous materials [e.g., 1-3]. We believe that this capability can be applied and extended to the study of geologic microhabitats and the microorganisms within them. Based on our initial feasibility study conducted in 1999, we conducted another set of μ-CT experiments to further quantify the physical microenvironments of seafloor hydrothermal sulfide chimneys at high spatial resolution (hopefully near to 1-5 μm voxel resolution) and attempted to extend the capability of this technique to the imaging of biological materials.

Methods and Materials

Natural blacker smoker chimney samples from 9 N° East Pacific Rise (EPR) and laboratory-prepared test samples were examined by μ-CT. Natural samples were broken or sectioned into mm-sized pieces. Friable samples were broken into small with a razor blade. In order to determine optimum incident energy, to maximize density contrast, and to experiment with filtering methods, data was collected on solid test samples. These included microporous ceramic aluminosilicate (Bolt Technical Ceramics) of known porosity and permeability, and standard sulfide and sulfate minerals. Selected solid samples were treated with propidium iodide and osmium trioxide biological stains to attempt to detect density contrasts. In order to determine the feasibility of imaging microbiological communities within pores, different cell concentrations of *E. coli* cells were grown and mounted in semi-solid agarose gel. Cells were treated with several different biological stains and tags, including ethidium bromide, propidium iodide, nanogold, silver-enhanced nanogold, and Brdu. Samples were mounted on silica pipette tips or metal needles with modeling clay.

Data was collected at GeoSoilEnviroCARS (GSECARS) Sector 13 bending magnet beamline (13-BM-D) with a Si(220) channel cut monochromator. Data were collected at different incident energies in order to compare grayscale contrast below and above the absorption edges of heavy elements such as iodine or gold for treated samples. Visible light was generated by a phosphorescent screen downstream of the sample and imaged with a microscope (10x objective and 25 mm tube) onto a high-speed 12-bit CCD camera. The CCD readout was binned by a factor of two for most samples; a few data sets were unbinned. Data was collected at pixel sizes from 1.14 to 4.78 μm. Data was analyzed using IDL software and routines supplied by GSECARS and by in-house software [4].

Results and Discussion

Reconstructed tomographic images of natural chimney samples from the EPR show a very large amount of porosity (>50% of the total volume) in very young samples, with visible pores ranging from about 50 μm to 1 mm (Fig. 1). Pore structure is very irregular and non-channelized, with patterns suggesting diffuse flow of variable rates. Collection of tomographic volumes down to a pixel size of almost 1 μm, which is probably about 3-4 μm spatial resolution, indicates that this method can achieve resolutions applicable to the scale of microbial communities. We are currently analyzing the EPR data to quantify porosity and pore structure, and to compare them to the more channelized pore structure characteristic of older, well-established chimneys [4].

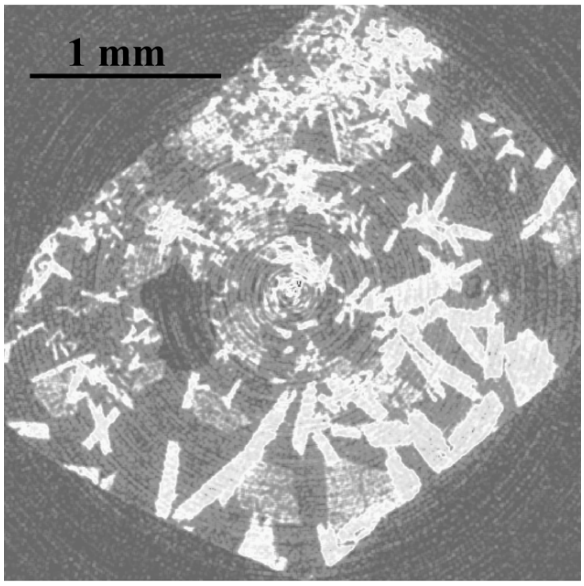

Fig. 1. Cross-section through a 3-D synchrotron X-ray μ-CT volume of a young chimney collected from 9°N EPR. Sample was impregnated with epoxy; bright minerals are sulfides, darker minerals are sulfates; interstitial gray is pore space.

Our attempts to image biological communities within media have not yet proven successful. Grayscale contrast between cells and the agarose matrix was less than expected, and distinct cells or cell communities are not obvious. We are working with different image processing approaches and volumes collected at different energies to improve contrast. We are also investigating better methods for introducing dyes and molecular tags into cells in order to get a larger concentration of heavy element into or on the cell. Characterizations with scanning electron microscopy and EDS elemental mapping are underway to improve our sample methodology before collecting more tomographic data.

Acknowledgments

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