X-ray computed nano-tomography for visualizing microbial cells and their surrounding environments

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Recent developments of the X-ray computed tomography (CT) reaches to the spatial resolution of down to few tens of nanometers scale, which is enough to visualize single microbial cells, together with those distributions as well as its surrounding environments. In this study, we tried to visualize microbial cells in the environmental samples by using nanometer-scale X-ray CT.
We used anaerobic granular sludge and subseafloor sediments for the test samples. The X-ray CT analyses were conducted at beamlines of BL20XU and BL47XU in synchrotron radiation facility of Spring-8. The soft samples were either freeze-dried or embedded in the epoxy resin for the analysis. Since the high-spatial resolution observation of the X-ray CT analysis requires a higher density of X-ray per field, the samples often deformed during the analysis, which results in the failure of the 3D reconstruction of the data. Optimization of the resin, its polymerization condition, together with the stain of the cells by osmium tetroxide to put heavy element onto the microbial cells, we could successfully visualize single microbial cells in the samples. Also, the observations of the sample at the pre- and post-edge energies of the element (such as Os) enabled specific signal enhancement of osmium-stained cells.
The investigations of subseafloor biosphere have often based on destructive measurements. Although these analyses revealed the large-scale distribution of microbes along the chemical gradients, no previous attempt explored the spatial distribution of microbial cells at their size range. The visualization of microbes with its surrounding environments will give basic but critical information to understand the microbial in-situ ecophysiology.

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