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High-Throughput 3d Visualization of Large Volumes at High Resolution by 3View™

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Recent development in high-resolution, 3d visualization by electron tomography and single particle reconstruction methods have brought immense steps forward in obtaining spatial ultrastructural information, and consequently advanced many areas of biological and material research. However, these methods are impractical when the object of interest extends for several tens or even hundreds of micrometers. Confocal laser scanning microscopy (CLSM) can provide excellent 3d visualization of large volumes; but the resolution given by its diffraction limits is not sufficient to follow structures on the sub-micron scale in great detail (Fig 1A). Conversely, TEM tomography provides excellent resolution (in the range of single nm), yet the reconstruction of large volumes requires immense effort. Previously, we combined serial TEM tomograms obtained from correlated regions of consecutive ultrathin sections (Fig 1B) to reconstruct a cyanobacterial cell of ~30 μm^3 volume with excellent ultrastructural detail [1]. However, to facilitate the investigation of a dynamic process such as bacteria interacting with mineral surfaces, a different approach for obtaining 3d volume on the order of several hundreds of cubic micrometers is required.

Microbe-metal interactions in the subsurface may play important roles for developing technologies to bioremediate soils contaminated with heavy metals and radionuclides. *Shewanella oneidensis* MR-1 are able to utilize soluble metals such as U(VI) as electron acceptors for anaerobic respiration, resulting in reduction and thus precipitation of less soluble UO_2 oxides. In our previous communication [2], we identified that *Shewanella* cells incubated anaerobically with U(VI) and lactate for 24 hr led to the precipitation of UO_2 biominerals within the cell periplasm and also associated with an extended (>100 μm) extracellular polymeric substances (EPS). Here we present a thorough 3d ultrastructural characterization of *Shewanella* cells and UO_2-EPS to generate high-resolution, accurate interpretations of how UO_2 nanoparticle immobilization by EPS may influence U mobility within the subsurface with implications on the fate and transport of U in subsurface waters.

Two novel methodologies were used for obtaining a 3d arrangement of *Shewanella* cells and UO_2-EPS: (a) 3View™ (Gatan) and (b) XuM™ (Gatan). (a) 3View™ is based on automated acquisition of 3d ultrastructure by sequentially imaging a freshly cut, resin-embedded block face using FEGSEM in a backscattered electron (BSE) mode. *Shewanella* cells were incubated with U(VI) as described above, processed for TEM [2] and embedded in a hard grade LR White resin. The cured blocks were sectioned with a 45 deg knife (Diatome) by an ultramicrotome positioned within the FEGSEM. For visualization, the sample did not require counterstaining with electron-dense stains since this sample provided excellent natural contrast due to the electron density of uranium (Fig 1C-F). The process was fully automated, and hundreds of ultrathin slices were removed and consecutive BSE images were generated from the block face (Fig 1C). The images obtained by 3View™ provided immense volume (1000 serial slices in 50 nm steps) of high linear resolution (less than 20 nm) ideal for

extracting bacterial associations with UO_2-EPS in 3d. In contrast, (b) XuM™, a non-destructive 3d visualization method based on reconstruction of rotational projections by X-ray imaging provided a "large picture" of the overall UO_2-EPS arrangement. When used in conjunction, areas of interest determined by XuM™ would be subsequently sectioned and visualized by 3View™ to obtain high-resolution 3d images.

In conclusion, 3View™ provided new perspectives on *Shewanella* and its UO_2-EPS in a high-resolution, large 3d volume that significantly contributes to our understanding of bacterial processes. In comparison with combining serial tomograms from semi-thin consecutive sections, several technical issues such as section-to-section alignment, uneven section thickness, missed section(s) and distortion were avoided by the fully automated process. 3View™ proved an elegant, highly-efficient, and powerful method for visualization of large volumes at high resolution.

[1] Dohnalkova AC et al., *Microsc Microanal* 13 (Suppl 2), 1338 (2007), [2] Marshall MJ et al., *PLoS Biology* 4:1324-33 (2006). This research was performed using EMSL, a national scientific user facility sponsored by the Department of Energy's Office of Biological and Environmental Research located at Pacific Northwest National Laboratory.

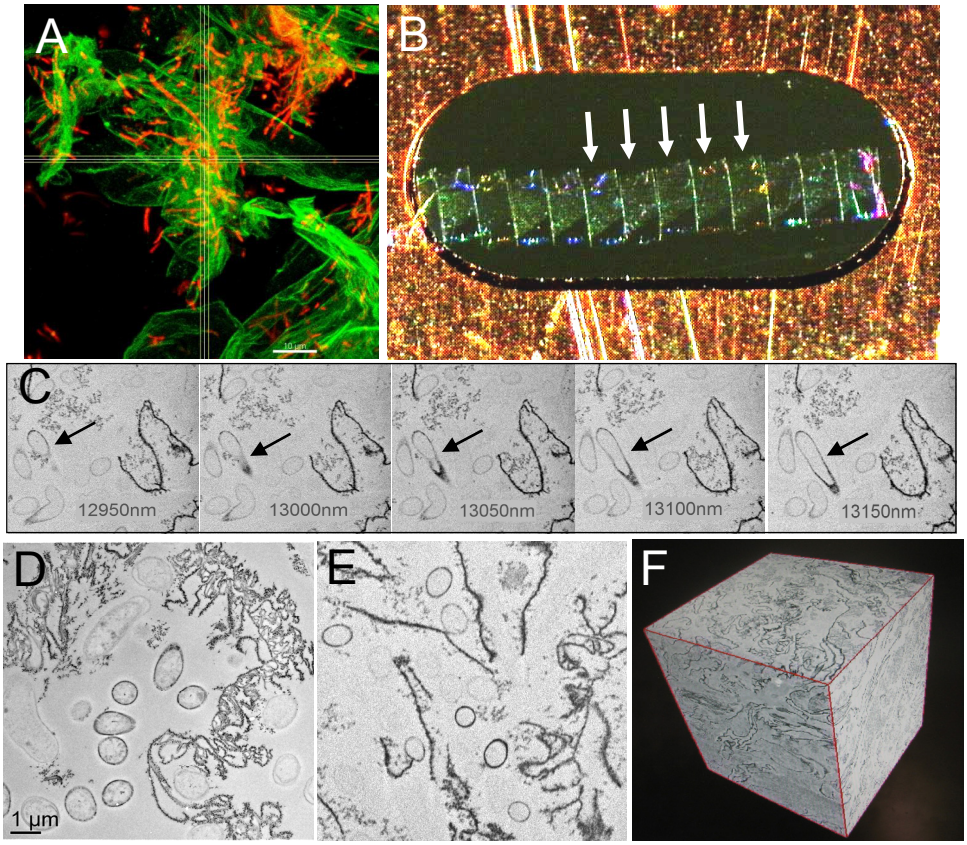

Fig 1. (A) CLSM image of *Shewanella* cells (red) and UO_2-EPS (green). (B) Consecutive plastic sections collected for reconstruction of serial tomography volumes. (C) Consecutive BSE images generated by 3View™ showing progression of material features in volume. (D) TEM image of a thin section of *Shewanella* with UO_2-EPS. (E) Images with practically identical contrast were obtained by imaging the block face by BSA during the 3View™ process. (F) Final reconstructed volume of stacked images from (E) (approx 50x50x50 µm).

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