## Spectroscopy Reveals Biofilm-hosted Life in Deep Subsurface Sediments

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Though researchers have suggested much of the life in the continental subsurface may be contained within biofilms on rock surfaces, most studies to date have focused on the planktonic microbial communities in deep groundwaters. Important questions remain about whether biofilms are truly widespread in deep fractured rock networks and the degree to which microbes residing within them resemble the free-living organisms in the surrounding water. To better understand this aspect of subsurface life, we investigated sediments found within boreholes at a mine in northern Minnesota. The boreholes were drilled more than 700 m below the surface; they access 2.7 Ga rock formations and groundwaters known to be isolated from surface waters over long time scales. X-ray diffraction identified the sediments from one borehole as mackinawite, an iron sulfide mineral, and those in another borehole as a mixture of hematite and quartz. The sediments were brought to the Advanced Light Source beamline 5.3.2.2, a scanning transmission X-ray microscope (STXM) for characterization of the associated carbon and iron phases through elemental mapping and X-ray absorption near-edge spectroscopy (XANES). Elemental mapping showed that carbon was ubiquitous in all sediment samples in a tufted-mat morphology. Additionally, deeper sediments also contained a cell-like carbon morphology not seen in shallower samples. Analysis of the carbon XANES spectra showed that the tufted-mat carbon most closely resembled lipid, saccharide, and inorganic carbon standards. The cell-like carbon resembled protein standards, which is characteristic of XANES spectra of microbial cells. Iron XANES showed that the sediment particles contained Fe<sup>2+</sup>, while the film-like material had sorbed Fe<sup>3+</sup> ions. The presence of carbon resembling both extracellular polymeric substances and microbial cells suggests that biofilm-hosted life is an important component of the subsurface ecosystem. Additionally, the affinity of these film-like materials for Fe<sup>3+</sup> ions could provide an important energy source for iron reducing microbes residing within them. The discovery of these films within sediments opens up a new avenue for subsurface sampling, as drilling for hard-rock samples can destroy or contaminate fragile biological materials, while sediments can be more easily retrieved using non-destructive methods.