

## **Visualising individual microbial cells inside rock pores**

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Microbes play a fundamental role in regulating geochemical processes in our biosphere and they are immensely beneficial to our economy and society. For example, they degrade many forms of toxic waste, they induce mineral precipitation and dissolution which affects pore fluid dynamics, they produce organic molecules that change soil/rock surface properties and they help convert CO<sub>2</sub> into minerals. Quantifying these complex processes in soils and rocks has been very challenging however. Thus, we have simplified the system by switching to two-dimensional investigations or by focusing only on the physical and chemical processes, excluding the impact of biology. However, to make any accurate predictions we need to consider all processes that occur inside soil and rock pores.

With the continuous improvements in synchrotron radiation and recent advances in X-ray nanotomography, that allow faster image acquisition at higher spatial resolution, we embarked to test the possibility to monitor microbes directly and non-invasively, inside soil and rock pores, at nanometre scale, by synchrotron based X-ray nanotomography with phase contrast. When exposing microbes to high intensity X-rays, their viability is quickly at risk so at first we focused on visualising fixed microbial cells. Small pieces of sandstone and a silica bead pack that were 300 – 550 µm in diameter, were treated with fixed microbial cell suspensions and then imaged using 3D nanotomography at beamline ID16A-NI at ESRF, France. Visualising individual cells inside a soil or rock pore is challenging because of the strong X-ray adsorption by the mineral grains and because the microbes themselves are small (1 - 3 µm) and have weak absorption contrast against the empty pores. Preliminary results show that with a voxel size of 40 nm, single bacterial cells can be visualised within the bead pack, while this is less feasible in the more complex microstructure of sandstone.

Further work will improve image quality and find the optimal X-ray energy for resolving individual microbial cells in real rocks.