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High resolution surface imaging of uncoated bacteria with Helium Ion Microscopy

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Helium Ion Microscopy (HIM) [1] offers increased lateral resolution, increased depth of field and new contrast mechanism compared to state of the art low voltage field emission scanning electron microscopy (LV-FESEM). In addition imaging of non-conductive samples using charge compensation with an electron flood gun is possible. This enables high resolution true surface imaging of uncoated samples. The He+ ion source consists of a tungsten needle with 3 atoms (trimer) at the very end and an electrode to extract ions. This gas field ion source delivers a bright He+ ion beam (>5 109 A/cm2sr), which can be focused with the final electrostatic objective lens in a sub 0.5nm probe.

Probe current can be defined with selectable apertures of 5, 10 and 20µm diameter. For high resolution imaging we use probe currents below 1pA. For lower magnification imaging, currents of several 10pA may be used. A range of ion energies can be selected between 15 and 35keV; in this work we used 30keV.

Due to the small surface interaction volume [2] and enhanced secondary electron signal (SE1) generation, the surface sensitivity and topographical contrast are well increased. For charge compensation a low energy electron flood gun is implemented (energy ranges from 100eV to 5000eV, typically 900eV in this work). The current density can be optimized for charge compensation by adjusting focus, x and y position as well as the flood time of the e-beam.

Possible drawbacks of HIM include sputter erosion of the sample (at high current densities on materials with high sputter rate, e.g. Au) and deposition of hydrocarbon contamination layers during imaging. Contamination can be reduced through sample cleaning with an integrated plasma cleaner.

In this work we present application of HIM for imaging of cell-mineral aggregates (CMAs) that contain both sensitive soft matter such as bacterial cells and bacteriogenic extracellular polymers and insensitive, inorganic Fe(III)-oxides. These heterogeneous CMAs were formed by nitrate-reducing bacteria under anoxic, circumneutral pH conditions as described elsewhere [3]. Samples were freeze-dried after plunge freezing in liquid propane. Samples for HIM were analyzed without surface coating whereas samples for FESEM were sputter-coated with a 4nm Pt layer.

HIM-SE images showed increased surface-sensitive material contrast (i.e. Fe(III)-oxides on the cell surface) (Figure 1) compared to FESEM that shows a rather homogeneous, topography-dominated contrast (Figure 2). Since no Pt-coating was applied, true fine surface structures without distortions became more clearly visible and can be analysed with more precision in the HIM as compared to FE-SEM.

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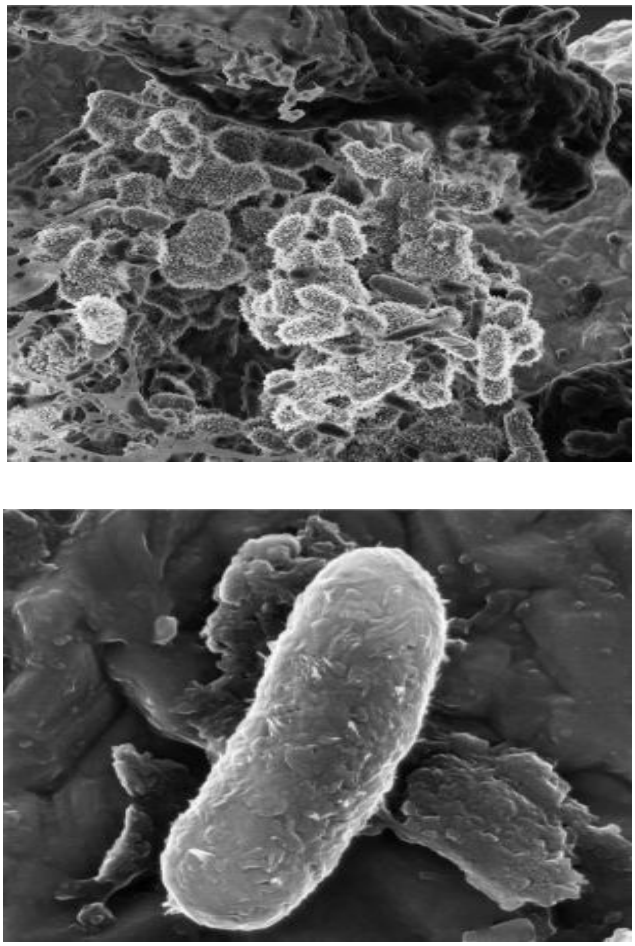

Figure 1. HIM secondary electron images of a CMA (left) and surface image of a bacteria with different level of mineral encrustation (right); uncoated samples.