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Fluorescence in situ hybridisation coupled to ultra-small immunogold detection to identify prokaryotic cells on minerals by electron microscopy and synchrotron radiation

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Microbial life colonises most environments on Earth, being present even in the subsurface, and plays an important role in biogeochemical cycles. In this context, it is important to study the impact of microorganisms on the formation or dissolution of minerals. With this aim, we developed a technique allowing the visualisation and identification of microorganisms, together with the associated mineral characterization at the electron microscopy resolution level. This method is based on fluorescence *in situ* hybridisation (FISH) and immunogold detection. We hybridised universal and specific fluorescein-labelled oligonucleotide probes to the ribosomal RNA of prokaryotic microorganisms in a heterogeneous cell mixture. We then used antibodies against fluorescein coupled to subnanometer gold particles to label the hybridised probes in the ribosome. After increasing the diameter of the metal particles by silver enhancement, the specific gold-silver signal was visualized on various substrates by light microscopy, transmission electron microscopy (TEM), scanning electron microscopy (SEM) and X-ray microscopy (SXM). The possibility to couple phylogenetic identification of microorganisms by FISH to mineral analysis at micrometric (SXM, SEM) or nanometric (TEM) resolution has promising potential applications for unraveling microbe and mineral interactions in the deep biosphere.