Electron microscopy reveals unique microfossil preservation in 1 billion-year-old lakes

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Abstract. Electron microscopy was applied to the study of 1 billion-year-old microfossils from northwest Scotland in order to investigate their 3D morphology and mode of fossilization. 3D-FIB-SEM revealed high quality preservation of organic cell walls with only minor amounts of post-mortem decomposition, followed by variable degrees of morphological alteration (folding and compression of cell walls) during sediment compaction. EFTEM mapping plus SAED revealed a diverse fossilizing mineral assemblage including K-rich clay, Fe-Mg-rich clay and calcium phosphate, with each mineral occupying specific microenvironments in proximity to carbonaceous microfossil cell walls.

1. Introduction

One billion-year-old rocks from the Torridon Group of northwest Scotland contain abundant microfossils that are a rare example of a Precambrian (>540 million years ago) non-marine biological community [1,2]. Traditional palaeontological methods, such as light microscopy lack the spatial resolution to reveal the true morphology of these microfossils in three dimensions, and accurately decode the chemical environment of fossilization. The combination of high-spatial-resolution, *in situ* techniques applied here, namely focused ion beam (FIB) sample preparation combined with scanning electron microscopy and transmission electron microscopy (FIB-SEM and FIB-TEM) allows the chemical composition of the microfossils and associated fossilizing minerals, plus cellular morphology, to be studied at the micrometer to nanometer scale.

2. Experimental methods

Study samples were standard uncovered polished geological thin sections, about 30 μm in thickness. A *FEI Helios NanoLab* dual beam FIB system was used to prepare TEM wafers from the polished thin sections. Electron beam imaging was used to identify microfossils of interest in the thin sections

allowing site-specific TEM samples to be prepared. The TEM sections were prepared using a series of steps involving different beam energies and currents [3], resulting in ultrathin wafers of ~100 nm thickness. The wafers were extracted using an ex-situ micromanipulator and deposited on continuous-carbon copper TEM grids.

TEM data were obtained using a JEOL 2100 LaB_6 TEM operating at 200 kV equipped with a Gatan Orius CCD camera and Tridiem energy filter. Energy-filtered (EFTEM) elemental maps were obtained using the conventional three-window technique [4], with energy windows selected to provide optimum signal-to-noise. Selected area electron diffraction (SAED) was performed using an aperture that selected a 200 nm diameter area of the sample.

Sequential FIB milling and SEM imaging was performed on *Zeiss Auriga Crossbeam* and *Zeiss Neon 40* dual-beam instruments, following the protocol outlined in [3]. Milling and imaging variables were optimized to suit the sample material (i.e. organic carbon in phosphate and clay), with ion beam currents of 2-3 nA for sequential slice milling, electron beam voltage of ~5 kV for imaging and an image capture time of ~30 seconds per frame.

FIB-SEM images were stacked, aligned and cropped using *SPIERSalign* [5]. The resultant stacks were imported into *SPIERSedit* [5] where a number of masks were added to segment individual components (e.g. mineral grains, cell walls) of the microfossil assemblage. The resulting files were exported and loaded into *SPIERSview* [5] to generate 3D volume renderings.

3. Results

3.1. Microfossil morphology

Over 500 serial slices through a cluster of small cells were milled and imaged using FIB-SEM to decode the true three-dimensional morphology of these microfossils (figure 1). The step size between images in z was 75 nm, the width of the milled area (x) was ~68 μm and the depth (y) was ~25 μm.

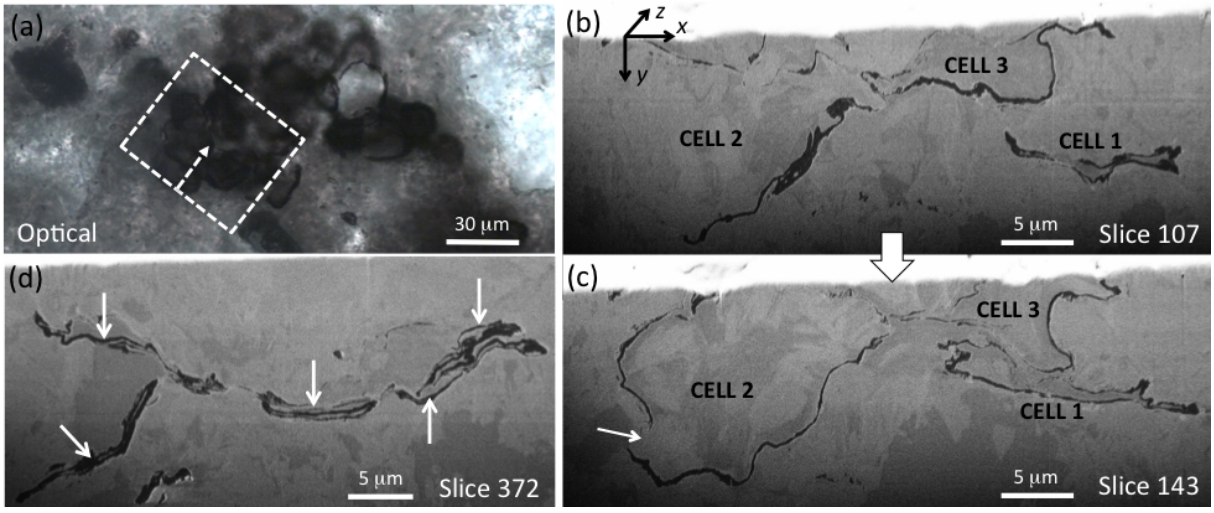

Figure 1. Morphology of microfossils from the 1 billion-year-old Torridon Group investigated in three dimensions. a) Optical microscopy image of a cluster of microfossils of unknown morphology. Dashed box shows region of interest (ROI) milled for 3D-FIB-SEM, where slicing proceeded in the direction of the arrow. b-c) FIB-SEM slices showing how the morphology of three cells changes over a distance of ~3 μm in the z direction. The increment between two adjacent slices is 75 nm, hence slice 107 is ~8 μm into the ROI, and slice 143 is ~11 μm into the ROI. Compression and folding of the microfossils is common. d) FIB-SEM slice 372 (~28 μm into the ROI) showing numerous examples (arrows) of more heavily folded and compressed cells.

Individual FIB-SEM slices (figure 1b-d) show the detailed morphology of several of these cells below the surface of the thin section. The cell walls (dark grey to black) have remained largely intact with only occasional minor degradation (e.g., arrow on slice 143), presumably allowing both the cell contents to escape and the fossilizing fluid to then permeate the interior of the cells. Mineral grains are rarely seen *within* the microfossil walls indicating a largely non-mineralized, purely carbonaceous cell wall composition.

All cell walls are folded, indicating that there was some degradation, dehydration and contraction of the walls prior to fossilization. Many microfossils are heavily compressed. However, some of these compressed microfossils occur in close proximity to those that have experienced little compaction (compare for example 'cell 1' with 'cell 2' in slice 143). This suggests that either the fossilizing fluids penetrated the interior of some of the microfossils earlier and to a greater extent than others affording them greater protection from later compaction, or that the fossilizing fluids crystallized at different rates within different fossil microenvironments.

The morphology of the cells can change rapidly over just a few micrometers. For example, in figure 1c slice 143 three cells are clearly visible and few would argue with an interpretation as microfossils. In contrast, figure 1d slice 372 shows at least five microfossils (arrows) but they are so compressed that they resemble simple carbonaceous laminations. It is only the high-spatial-resolution nature of these images that allow distinct microfossils to be visualized. In lower resolution microscopy, including standard light microscopy, the very narrow interiors (only ~100-300 nm wide) of these cells would not be visible. Instead, the microfossils would likely be mistaken for interwoven carbonaceous laminae and subsequent demonstration of their biological nature may be problematical.

3D volume renderings of the raw data from multiple FIB-SEM slices (figure 2) emphasize the highly compressed and folded nature of some of these small cells, which would have been spheroidal to ellipsoidal before death. They also show the extent to which the wall structure has been broken down during fossilization; in this case, 'cell 2' is significantly more degraded (higher volume of gaps within the red wall structure) than 'cell 1' despite the two cells being only a few micrometers apart.

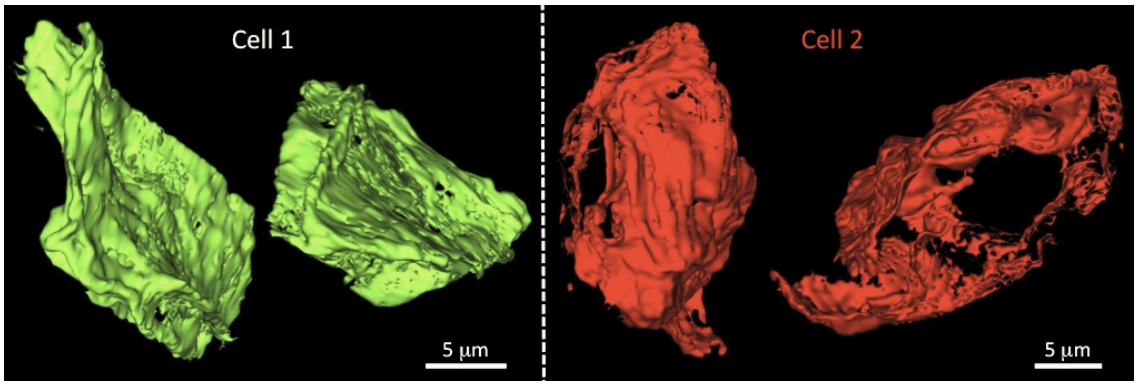

Figure 2. 3D reconstructions of the organic walls of cells 1 and 2 from figure 1, each viewed from two different perspectives. Cell 1 is very compressed but has a high percentage of its cell wall still intact. Cell 2 retains much of its original ellipsoidal shape but part of the cell wall is highly degraded.

3.2. Microfossil chemistry

FIB-SEM images suggest that the cells have been fossilized by more than one mineral phase (i.e., variable shades of grey within the cells in figure 1b-d likely correspond to different minerals). This was further investigated by analysis of FIB-milled ultrathin TEM wafers through several microfossils. EFTEM mapping (figure 3) confirms the carbonaceous nature of the cell walls and confirms that a variety of mineral phases have precipitated around the microfossil walls, probably subsequent to bursting of the cell and escape of the cell contents but prior to any further decay of the cell walls. In the example shown in figure 3, the cells have been fossilized by a mixture of alumino-silicates and

silica. Calcium phosphate is also present in the vicinity of most cells. SAED and HRTEM show that the alumino-silicates are layered clay minerals, close in structure and composition to illite (K-rich varieties) and berthierine/chamosite (Fe-Mg-rich varieties). Different minerals are restricted to specific microenvironments with mineral composition often changing sharply across cell boundaries. Current and future work aims to relate these mineral phases to original cellular chemistry and/or sediment porewater chemistry.

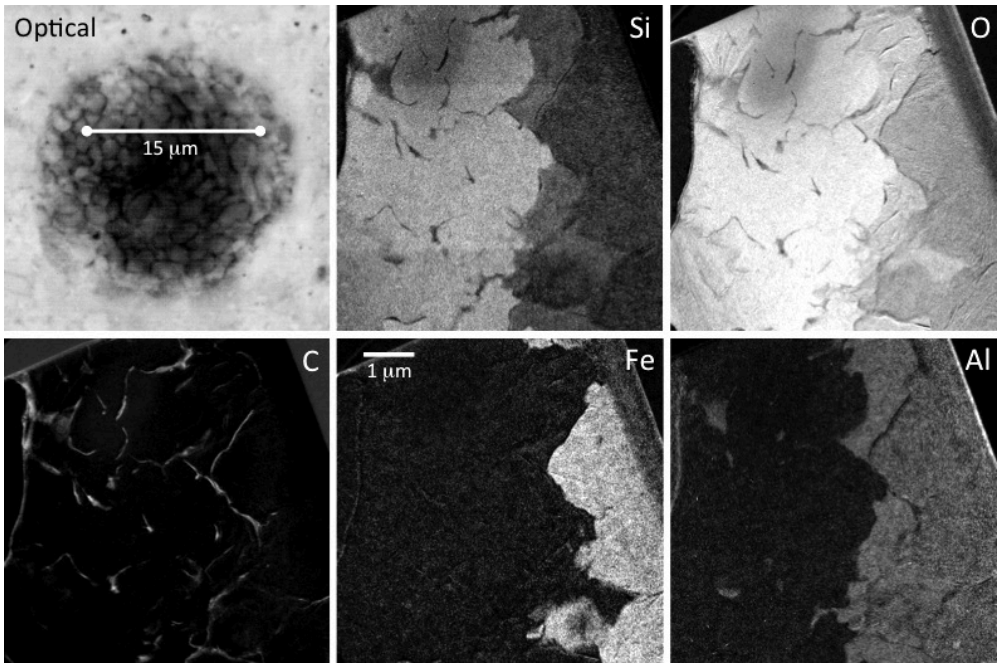

Figure 3. EFTEM elemental maps of a complex multicellular microfossil from the Torridon Group. The FIB wafer was extracted from the area indicated in the optical image. Cell walls appear white/light grey in the carbon map. Three distinct zones of minerals are seen and these change sharply across cell wall boundaries. The right hand zone is an iron-rich alumino-silicate approximating chamosite in composition, the narrow central zone is a potassium-rich alumino-silicate approximating illite in composition, and the left hand zone is silica.

4. Conclusion

The combination of high-resolution, *in situ* techniques used here allows the morphology and chemistry of Precambrian microfossils to be studied without the necessity to chemically extract the fossils from their rock matrix. Changes in cell wall structure, degree of wall degradation and deformation, together with mineralogy can be detected over a range of spatial scales from nanometers to millimeters within a single thin section. These data enhance our understanding of the composition of the preserved biological community and the environmental conditions that led to its fossilization.

References

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