FIB Analysis of Fossils Plant Remains: Technical and Experimental Aspects

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We present a comprehensive study about the technical aspects of the application of focused ion beam (FIB) to the study of cuticles and compressions of fossil leaves.
The technique allowed us to cross section and image fossil coalified plant remains with a spatial resolution within the 10 nm range, far higher than any other method employed so far.
At various stages of the milling process, we observed significant gallium redeposition on the trench walls, particularly pronounced in the case of cavities. These highly unwanted artefacts can be greatly reduced, but not wholly eliminated, by lowering either or both the beam current and acceleration voltage; nevertheless, great care is needed when interpreting cross-sectional images.

Keywords: FIB, SEM, fossil plant cuticle, microstructures, milling damage

1. Introduction

The use of FIB in paleobotanical studies has been so far limited to imaging the internal layered disposition of Triassic spores embedded in metamorphic rocks [1] or the organization of Early Cretaceous spore masses [2]. However, its application to fossil cuticles and compressions could be particularly useful, as it allows the analysis of ultrastructural organization of fossil leaves and microstructures. Moreover, FIB represents a clear improvement compared to conventional techniques, such as light microscopy and scanning/transmission electron microscopy (S/TEM), both in terms of ease of sample preparation (none is required, except for Au deposition to make it conductive) and of spatial resolution. An additional advantage is the possibility of choosing a region of interest, sectioning it, observing it, and further sectioning it if necessary.

In a previous work, we have shown that this technique is an excellent tool for the analysis of internal three-dimensional structures of both micro- and nanostructural features in fossil plant coalified compressions [3].

However, during our analysis we encountered an extremely meaningful technical issue. We found that gallium redeposition inevitably occurs on the surface of the trenches, resulting in artefacts, which could be misinterpreted as real structures. Therefore, in this work, we investigate this effect and discuss how to minimize it.

2. Experimental Details

We applied the FIB–SEM technique to fragments of fossil conifer leafy twigs preserved as cutinized coalified compressions from the Early–Middle Jurassic deposits in central Argentina. In contrast with the standard SEM analysis, no previous chemical treatment or sample preparation was required. The fossil samples were mounted on a SEM stub and then Au-coated to make them conductive.

The use of a FEI Helios 600 Nanolab FIB, equipped with a field emission gun (FEG) and an Inca X-ray analysis system, allowed us to simultaneously slice and image the region of interest, with a spatial resolution within the 10 nm range. The first rough milling was performed at an accelerating voltage of 30 kV, with an ion current of 9.3 nA; the next step is a polishing of the surface of the cut at decreasing currents, starting at 2.8 nA and finishing at 93 pA. The whole process took approximately 8–10 h.

Since the sample surface is very irregular and rough, no platinum strip was deposited on the region of interest prior to ion milling, as it would result in a discontinuous strip that would not protect the surface from ion damage. The possible redeposition of gallium on the walls of the trench will be discussed in details in the next section.

3. Results and Discussion

The ion milling process induces some secondary effects on the specimen studied. Among them, redeposition of the milled material, particularly when high currents are used, is often a major issue. A possible solution is injecting water vapor (as magnesium sulfate heptahydrate) during ion milling, a technique known as selective carbon etching (SCE); in this way, the milling rate is considerably increased, consequently reducing the redeposition effects [2].

However, in the present case, the use of SCE did not produce the intended effect and some columnar structures appeared in front of the cut surface (Figure 1a). X-ray analysis revealed them to be composed essentially of gallium. A possible explanation for this counter-intuitive behavior could lie on the extremely inhomogenous composition of the specimens, where the carbon-based material composing the cuticle is usually mixed with clastic particles. This may result in a very fast milling of the soft matrix, leaving remains of much more resistant rocky material on which the gallium redeposits. We therefore carried out a systematic analysis to optimize the milling parameters and finally decided not to use the SCE system. This way, we obtained a much cleaner surface of the cut, at the price of a longer milling time (Figure 1b).

As for Ga redeposition, we found that the edges of sectioned cavities contain a relevant amount of gallium (Figure 2), suggesting that some caution is necessary when interpreting visible structures. It is worth noting that the gallium is concentrated in a clearly defined layer around the rim of the cavity. Redeposition may also occur inside the cavities, but in this case, very little or no gallium was detected by X-ray analysis, suggesting that both micro- and ultrastructures visible in these areas are not artefacts

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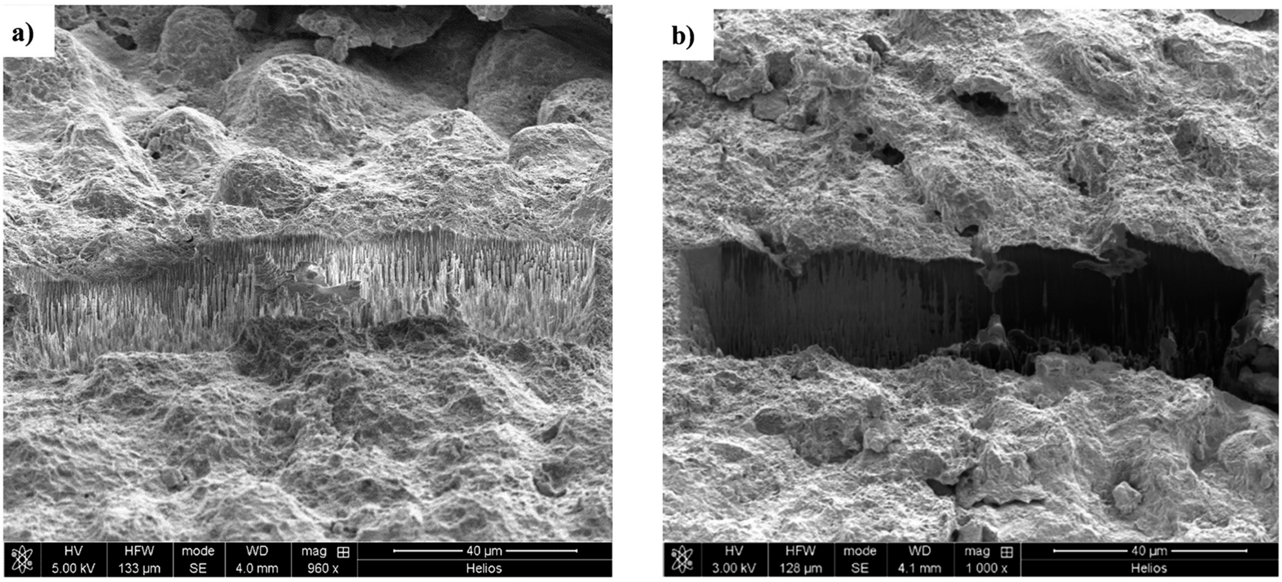

Figure 1. Trench milled with SCE (a) and without it (b), after milling parameters optimisation

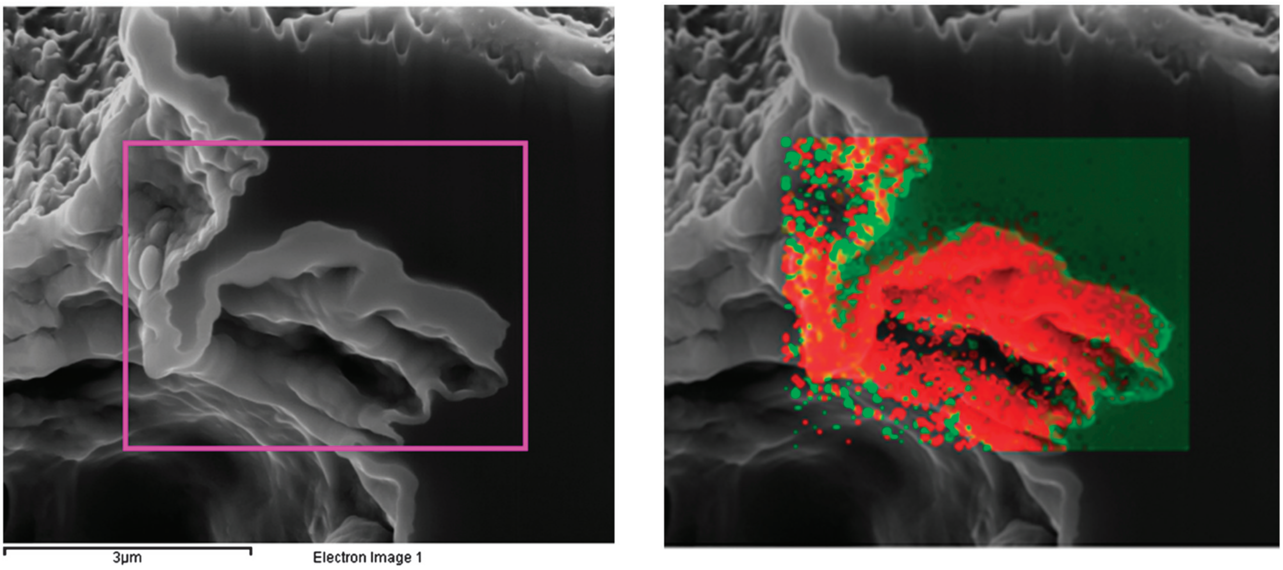

Figure 2. SEM image of a cavity (left) and elemental map of the framed region (right); red: Ga; green: C

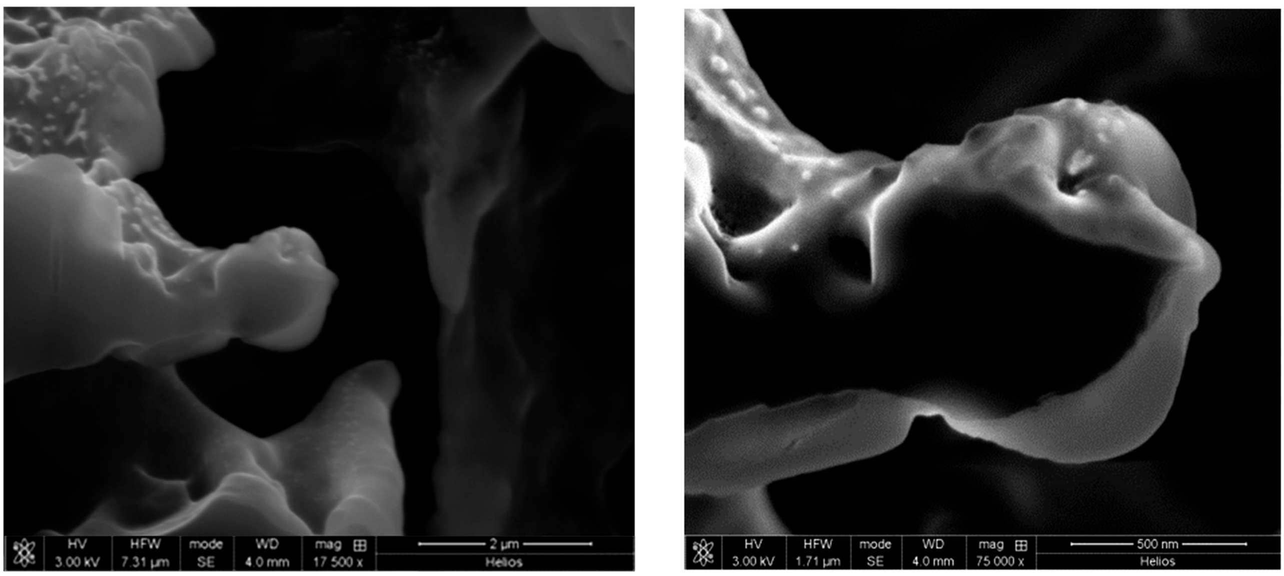

Figure 3. Same region before (left) and after (right) final cleaning at 16 kV, $I = 0.46$ pA

but real structures, at most merely covered by an extremely thin layer of redeposited material.

By lowering the ion current used for the final polishing, we were able to reduce the ion damage, as can be seen in Figure 3. Here, final polishing was performed at 16 kV and 46 pA ion current (left and right-hand image, respectively), and it can be seen as the Ga redeposited on the surface of the cut is considerably reduced, although not fully eliminated. However, a lower current implies a much longer milling time, which is not practical for large areas of interest.

4. Conclusions

FIB–SEM represents an extremely useful technique for the characterization of microstructures in fossil plant remains, due to its versatility, ease of sample preparation, and a spatial resolution as good as a few nanometers. We found that gallium redeposition invariably takes place on the surface of a cut, but can be greatly reduced, though not completely eliminated, by low voltage and ion current final cleaning. Moreover, no gallium was detected inside the sectioned cavities, suggesting that the visible microstructures do not represent a process-induced artifact.

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