

FIB slicing and imaging of palaeopalynological remains: a 3D inside look into the past

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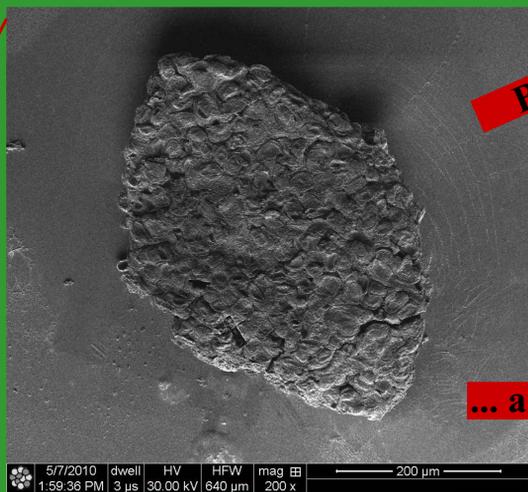
The Universities

Introduction

- FIB quickly growing applications in life science imply a whole new set of practical aspects totally different from material science
- Palaeobotany is a rapidly expanding branch of palaeontology, which provides valuable information on species long extinguished today
- Characterization has been so far severely hindered by the limitations of mechanical slicing and SEM imaging of fossil material

The Aim

- To be able to choose the single spore to be sectioned by the ion beam
- To cross section, analyze and image the internal structure of the chosen spore
- Finally, to reconstruct a 3D image of the inside of the spore

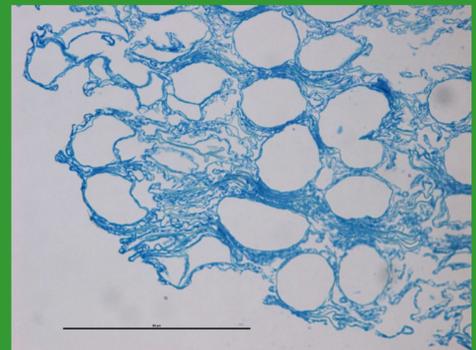


FIB ion image of a whole Sporangium

Before...

The past..

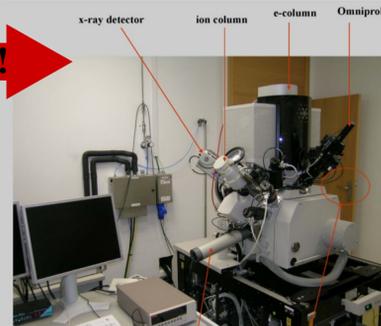
- Ultramicrotomo Reichert Ultracut



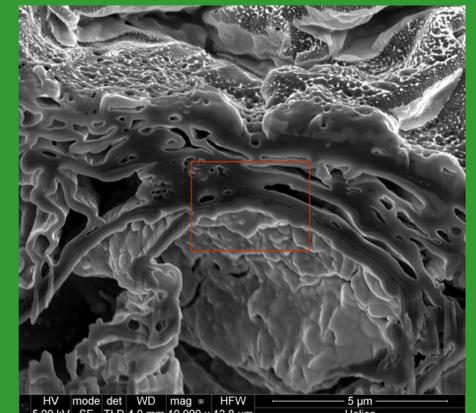
Optical microscope image of a ultramicrotome sectioned *sporangium*. Only purely 2D imaging is possible, with poor contrast and little structural details

... and the future!

- Helios 600 Nanolab
- Equipped with Cathodoluminescence (CL) system, EDS, Electron Back Scattered Detection (EBSD) system, Omniprobe
- Nanometre scale resolution for both electron and ion images



... and after!

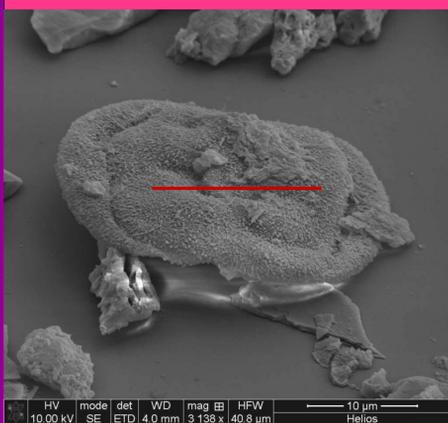


3D SEM image of the surface of the cut after low current ($I = 28 \text{ pA}$) polishing

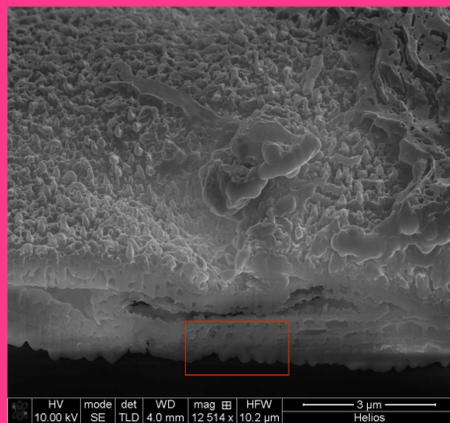
Experimental

- Sample preparation: samples prepared by critical point dryer
- No need for Pt deposition, as the material is very resistant to ion bombardment

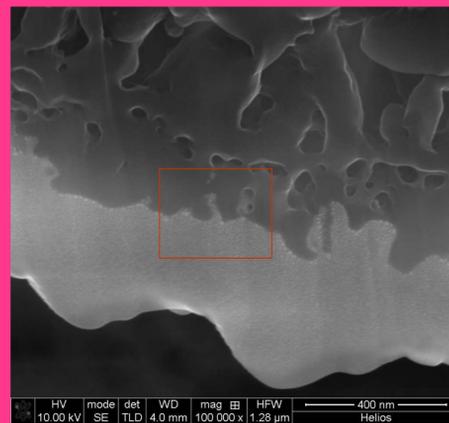
Another example: *Classopolis*



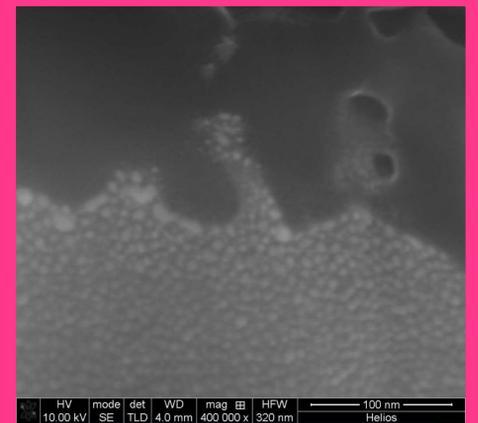
Low mag SEM image of a *Classopolis*. The red line indicates the position of the cut.



SEM image of the cut surface



Higher mag SEM image of the cut surface after polishing at $I = 93 \text{ pA}$



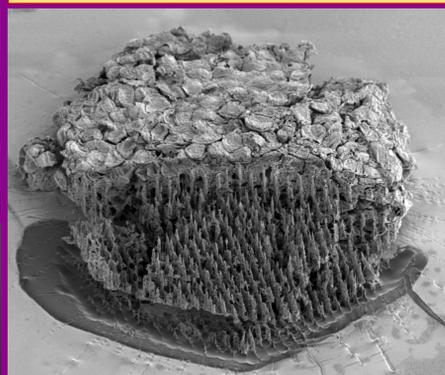
High resolution (TLD) SEM image of the cut surface; granular structures can be clearly

Practical issues

- Redeposition of material during milling; Long milling time

Possible solutions

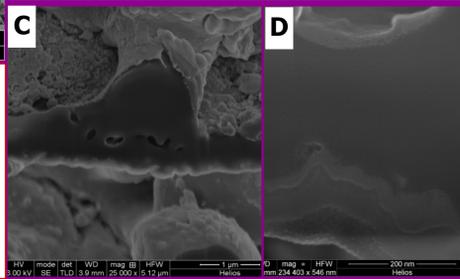
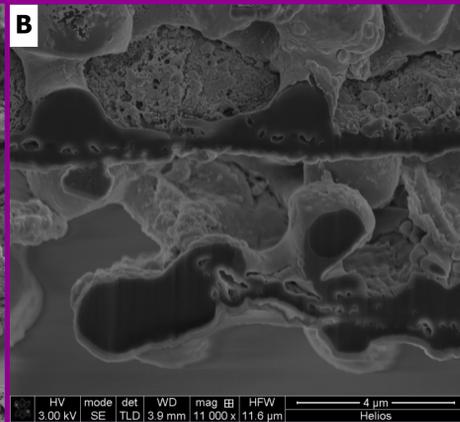
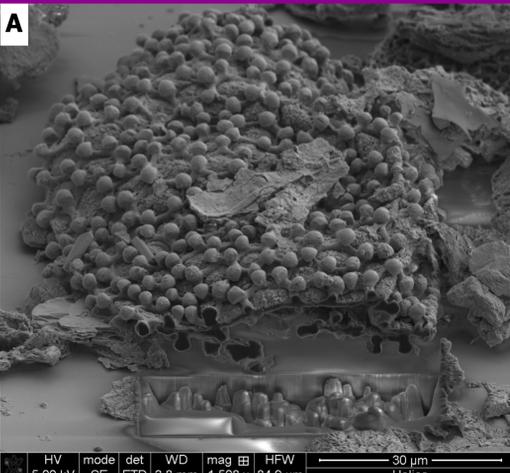
- Selective Carbon Etching (SCE) to enhance milling rate; Lower pitch and overlap to reduce redeposition



Cross sectioned *sporangium*; milling performed at current $I = 93 \text{ nA}$, milling time = 10 hours! Redeposited material is clearly visible

Conclusions

- The use of a DualBeam FIB allows a complete characterization of the internal structure of the analysed species
- The possibility of 3D imaging represents a giant step towards a better knowledge of the evolutionary behaviour of fossil plants
- The method can be easily applied to any taxonomic specie, accounting for the different milling rates



Other example: Stellatopollis

A) Cross section of the Albian pollen *Stellatopollis barghoornii*. Milling hole clearly visible; B) Detail of the cross section showing the wall structure of the pollen grain; C) High magnification of the supratectal ornamentation with laminated layers at its base; D) Laminated exine of the pollen with nanogranulate ornamentation.

