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Helium ion microscopy and sectioning of Spider Silk

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Focused ion beams have recently emerged as a powerful tool for ultrastructural imaging of biological samples. In this presentation, we will show that Helium Ion Microscopy (HIM), combined with ion milling, can be used to visualize the inner structure of both Major and Minor Ampullate silk fibres of the orb-web weaving spider *Nephila madagascariensis*. The internal nanofibrils were imaged in pristine silk fibres, with little or no observed damage to the sample structure. Furthermore, a method to cut/rupture the fibres using He^+ ions combined with internal sample tension is presented.

This work showed that HIM is a valuable tool for visualizing biological samples. The inherent sputtering of the He^+ ions could be used to mill the samples without damaging the biological structures softly. This contrasted with the Ne^+ ions, which caused damage to the spider silks' internal structure.

It was also shown that combining He^+ ion milling and the inherent tension in the spider silk sample made it possible to cut the specimen and visualize the rupturing process and the internal structures in the silk. This method could be used for other fibrous structures or even with non-elastic samples if used with stretched adhesive carbon tape, which could be used to apply the rupturing force.

The HIM images of the spider silk revealed that the rupturing process was highly dynamic involving rearrangement of the material in the fibre and showing strong indications of an internal fibril structure in the silk fibres with typical dimensions of 100-200nm. We anticipate that HIM will significantly contribute to some of the most challenging imaging applications and may open new directions in future bioimaging when paired with other imaging modalities.

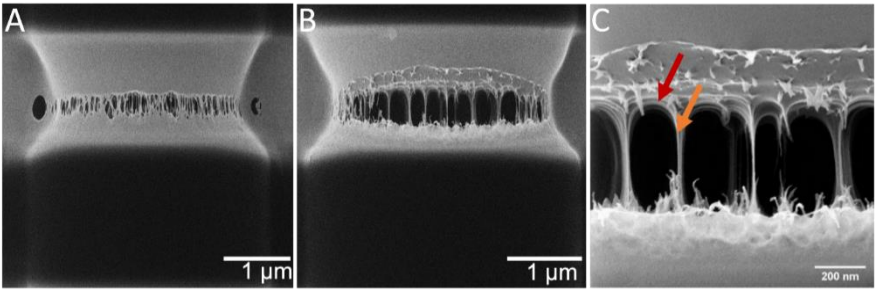

Fig. 1: HIM images of a MiS fiber that has been cut in half by He+ ion sputtering. A) HIM image of a MiS fiber before breaking. B) image of the fiber stretching. C) Further zoom in on B) showing the individual fibrils being stretched (orange arrow), causing deformation in the base of the fibril (red arrow).