Using FIB/SEMs to study biological samples

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< FIB/SEMs, which combine a Scanning Electron Microscope (SEM) and a Focused Ion Beam (FIB) in a single device have become the "go to" tool in the materials sciences and semiconductor industry. FIB/SEMs can reveal a sample's subsurface structures at high resolution, prepare TEM-lamellas, as well as reconstruct 3D sample models at precisely selected points within the sample (slice and view process) which cannot be achieved by other technique. This tool has recently found increasing interest in the life sciences (1-5). However, appropriate milling and imaging parameters for soft materials like polymers and non-resin-embedded biological samples are not yet well known. Generic parameters which are commonly used for hard materials cause heat damage and produce undesired artefacts in the samples.

This study focuses on gallium ion-solid interactions and their simulations to derive suitable operational parameters for cutting/imaging soft materials. A technique, based on the simulations and heat transfer models is derived which prevents heat damage in soft materials. The technique is successfully demonstrated on non-resin embedded collagen, a biomaterial which serves as a case study for other soft tissues. Collagen was chosen as a suitable test sample as it loses its fibrillary structure when denaturated by heat, permitting damage to easily be recognized. Cross-sections and TEM lamellas were prepared from non-embedded collagen with conventional FIB processing parameters (see Figure 1 left) as well as heat reducing FIB parameters (see Figure 1 right).

The results also show that heat damage can be prevented by reducing the local dose rate and area underneath the ion beam. A TEM comparison of a microtome prepared lamella and a FIB prepared lamella (using heat reducing parameters) shows that the fibrillar structures can be maintained, and heat damage avoided. The approach described here can be used to determine suitable parameters for other soft materials.

References

[1] Drobne et al., 'Surface Damage Induced by FIB Milling and Imaging of Biological Samples is Controllable'; Microscopy Research and Technique 70; 895-903 (2007)

[2] Leser et al., 'Focused ion beam (FOB) / scanning electron microscopy (SEM) in tissue structural research'; Protoplasma 246 (1-4); 41-48 (2010)

[3] Earl et al., 'Characterization of dentine structure in three dimensions using FIB-SEM'; Journal of Microscopy 240, Pt 1, 1-5 (2010)

[4] Milani et al.; 'How to study biological samples by FIB/SEM?'; Modern Research and Educational Topics in Microscopy, FORMATEX; 148-154 (2007)

[5] Villinger et al.; 'FIB/SEM tomography with TEM-like resolution for 3D imaging of high-pressure frozen cells'; Histochem Cell Biol, 138; 549-556 (2012)

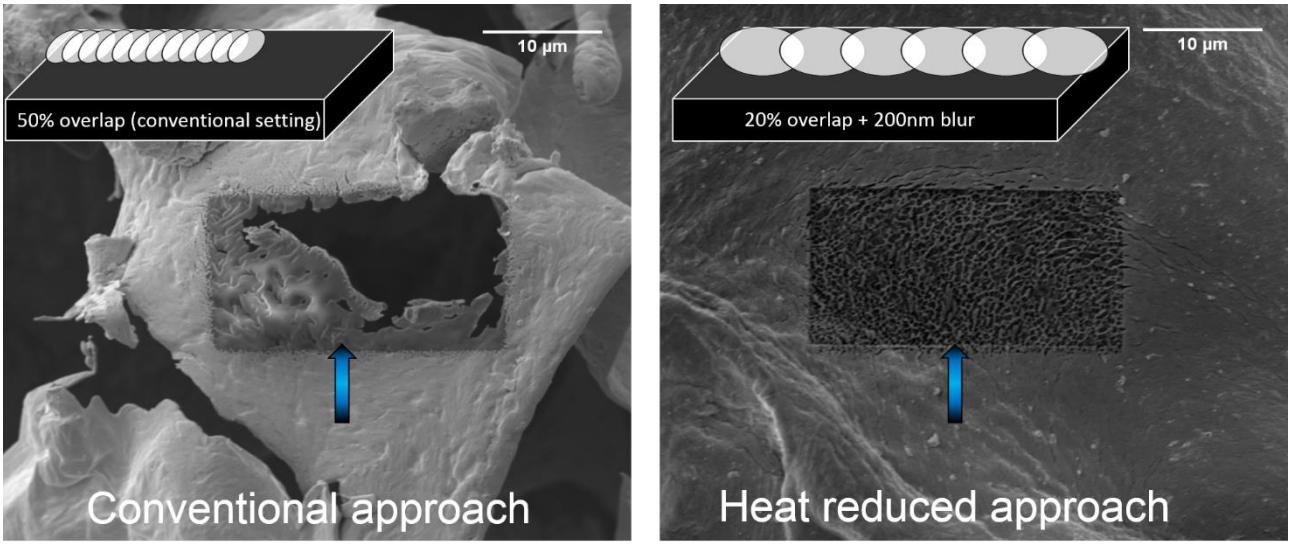

Figure 1. SEM images showing FIB prepared cross-sections in collagen with conventional parameters (left) and heat reducing parameters (left). The difference between the parameter sets is illustrated in the top part of the image.

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