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**IT-13-O-2039 FIB-SEM Tomography of biological samples: Strategy of preparation to resolve high-resolution 3-D volumes**

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Elucidating the three-dimensional (3-D) spatial distribution of organelles within cells is essential for investigating numerous cellular processes. Tomography in the transmission electron microscope (TEM) is the method of choice for 3-D imaging of cellular structures down to 3nm resolution [1]. However, TEM tomography is typically limited to 500 nm thick sections making the reconstruction of an entire eukaryotic cell very challenging [2]. There is a need for a technology that can be used for rapid 3-D imaging of large mammalian cells to provide information at nanometre resolution. The most promising technology at the moment is the FIB-SEM tomography of fixed biological samples embedded in resin [3-7]. A FIB-SEM microscope is a scanning electron microscope combined with a focused ion beam (FIB) such that both beams coincide at their focal points. This combination enables bulk resin samples to be locally sectioned by ion milling, producing new block face imaged with the electron beam. This process can be repeated allowing 3-D analysis of relatively large volumes with a field of view of several micrometres.

Any fixed and embedded resin samples prepared for TEM examination can be used for FIB-SEM tomography. However, considerations have to be given to artefacts and surface damages induced by FIB milling and imaging [8]. In this study, different protocols of sample fixation and staining were explored in order to improve the signal/noise ratio, preserve the ultra-structure and reduce charging effects of biological samples. In addition, the behaviour of specific resin formulations [9] was investigated in the FIB-SEM microscope. The milling rate was measured and the damages caused by the ion impact on the resin were analysed. The most stable resin was used to improve the milling conditions. Finally, the geometry of the sample was optimized to improve the imaging conditions using detection of the backscattered electrons with the through-the-lens detector (BSE-TLD).

In conclusion, we propose a sample preparation and imaging strategy for high-resolution FIB-SEM tomography (Figure 1).

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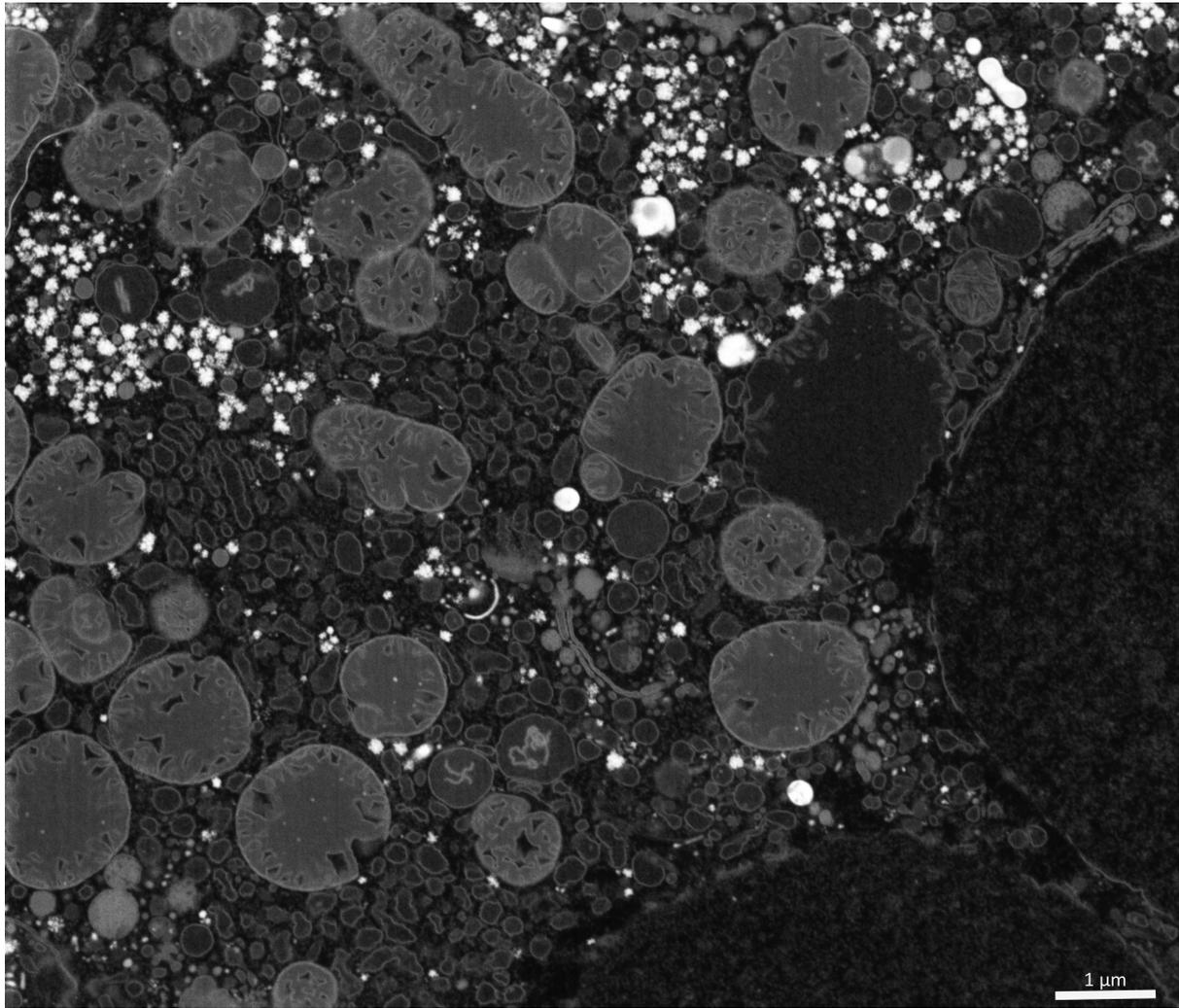


Fig. 1: FIB-SEM cross section of liver cell imaged at 2kV in backscatter electron mode. This image (4096 x 3536 pixels) comes from a series of 430 images with a voxel resolution of  $3 \times 3 \times 10 \text{ nm}^3$ .