Oxygen PFIB/SEM Tomography of Biological Samples

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INTRODUCTION

Over the past years, the Focused Ion Beam Scanning Electron Microscopy (FIB/SEM) tomography has been established as a key technology for 3D volume analysis of biological samples. However, the gallium FIB/SEM applications are limited to a selection of compatible sample preparation methods and accessible volumes.

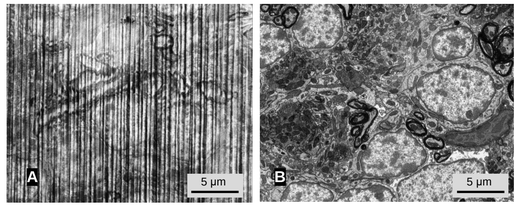
SAMPLE COMPATIBILITY

The sample preparation method is critical for the preservation of intricate subcellular structures and the choice of correlative studies. Sample preparation for gallium-based FIB/SEM tomography often includes heavy-metal contrast enhancement after fixation and samples are typically embedded in epoxy-based resins (EPON or DURCUPAN). The use of low-density acrylic-based resins (e.g LR White) on the other hand is very limited, although these resins can be beneficial for a range of life science studies. Major limiting factor is the poor stability of Acrylic resins under the gallium FIB resulting in severe milling artifacts (Figure 1A) compromising the image quality and the acquisition of relevant data.

These limitations can be addressed by the Thermo Scientific™ Helios Hydra™ DualBeam system featuring Inductively Coupled Plasma with four ion species (Xe, Ar, O, N) as an ion source. Oxygen provides clear improvement in cut face quality and image contrast[1] and permits 30% faster data collection from the wide range of biological samples[2] (tissues, cells and whole organisms) and resins, including acrylic resin (Figure 1B).

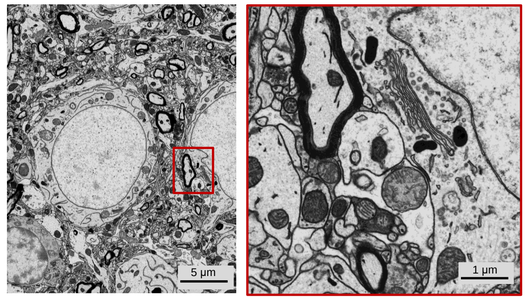
In combination with automated data collection, oxygen PFIB/SEM tomography results in excellent quality images revealing features of interest at nanoscale resolution (Figure 2) that can be combined into a 3D representation of the sample (Figure 3).

Figure 1. Comparison of Gallium FIB and Oxygen PFIB milling of LR White resin embedded sample



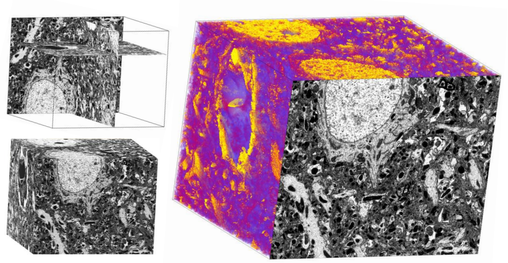
Chemically fixed mouse brain tissue embedded in LR White resin. (A) Gallium FIB milling at 30 kV, 65 nA. (B) Oxygen PFIB milling at 30 kV, 45 nA. Image taken with in-column detector at 2 kV.

Figure 2. Mouse brain tissue embedded in EPON resin showing subcellular details



Selected slice from oxygen PFIB/SEM tomography milling at 12 kV, 64 nA. Image taken with retractable backscattered detector at 2 kV.

Figure 3. 3D reconstruction of the LR White resin sample



Sample volume of region of interest using cross-section technique. Oxygen (30 kV, 0.61 nA) was used to mill 10-nm-thick slices. Image taken with thought lens backscattered detector at 2 kV. Selected slices along the XY, ZX, ZY planes. 3D reconstructed volume, segmented to reveal structural details. Total volume = 23.4 µm x 18.2 µm x 20.0 µm. Mouse brain tissue embedded in LR White resin

SPIN MILL TECHNIQUE

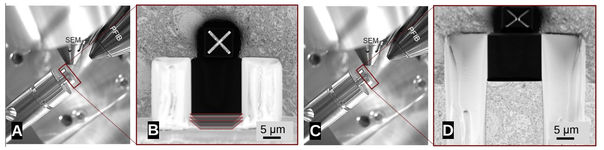
A conventional FIB/SEM Tomography approach is based on a sequential milling and imaging of the cross-section exposed by ion beam. In this case the sample surface is perpendicular to the ion beam during the milling (Figure 4).

The new Spin Mill approach for large-area planar milling with oxygen PFIB represents a novel technique for accessing and investigating large areas of the biological samples. A thin layer of the sample surface is removed by ion beam at a nearly glancing angle while rotating sample stage (Figure 5). A broad area up to 1 mm in diameter can be milled with a plasma.

Preparation steps such as trenches, fiducial marker, or protective layer deposition are not needed. A circular marker can be utilized when the area of interest is smaller than the sample surface or its diameter is larger than 1 mm. Thin slices in the nanometer range can be automatically milled and imaged iteratively, like in a serial block-face imaging. High-resolution 2D-image stacks can be taken from multiple areas of interest imaged at different imaging settings within one Spin Mill experiment (Figure 6), combined for 3D visualization (Figure 7).

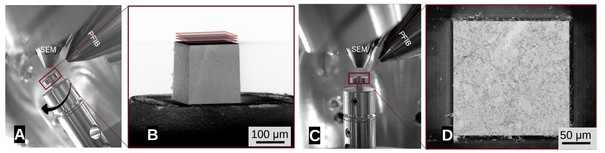
Spin Mill is especially beneficial for identifying and accessing sparse regions of interest easily, as well as generating quantitative data for statistical validation[3] or for correlative light and electron microscopy[1]. The sample preparation and Spin Milling process is fully automated and easy to set up using Thermo Scientific™ Auto Slice & View™ software (as shown figure 6).

Figure 4. The conventional PFIB/SEM tomography cross-section technique



Orientation of the sample surface with respect to the PFIB/SEM columns. (A) Position of the stage and sample during the milling process. Cross-section is milled with the ion beam perpendicular to the sample surface. (B)) Ion beam view with illustrated sequential milling of the selected prepared area (20 µm). (C) The stage at imaging position with the sample surface kept perpendicular to the ion beam. (D) Exposed cross-section area of the mouse brain tissue embedded in EPON resin.

Figure 5. Spin Mill technique



Orientation of the sample surface with respect to the PFIB/SEM columns. (A) Position of the stage and sample during the milling process. Sample surface is milled under the glancing angle and the stage continuously rotated by a defined number of steps. (B) Ion beam view under the glancing angle with illustrated sequential milling of the entire sample surface (189 x 187 µm). (C) The stage at imaging position with the sample surface being perpendicular to the electron beam. (D) Exposed surface of the mouse brain tissue embedded in EPON resin.

Figure 6. Spin Mill area with four areas of interest for image acquisition

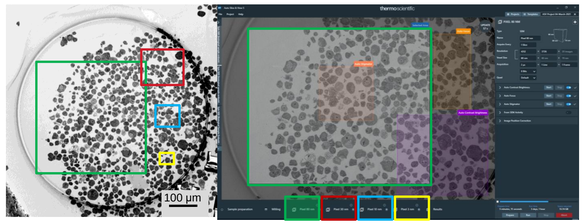
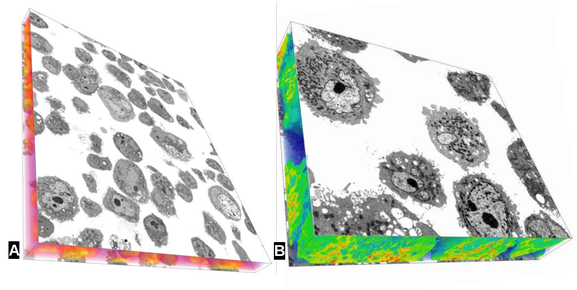


Figure 7. 3D reconstruction of the spin-milled sample



Selected sample volumes from one Spin Mill experiment. (A) Represents the region of interest marked by the red box in Fig. 6. 3D reconstructed volume is 138 x 119 x 12,8 um, pixel width: 30 nm. (B) Represents the region of interest marked by the blue box in Fig. 6. 3D reconstructed volume is~44 x 37 x 8 µm, pixel width: 10 nm. Oxygen (12 kV, 64 nA) PFIB was used to mill 70-nm-thick slices. Images taken with with retractable backscattered detector at 2kV. Chinese hamster ovarian cells embedded in DURCUPAN resin.

CONCLUSIONS

Oxygen PFIB/SEM tomography extends the potential of FIB/SEM research in life-science. It allows for sample preparation flexibility and higher milling throughput in a wide range of life-science samples. In addition, the possibility to access large areas up to 1 mm in the horizontal plane by using the Spin Mill technique can facilitate the design of new experiments to address challenging scientific question.

REFERENCES

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