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 methods 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 resin (EPON or DURCUPAN). The use of the densely acryl-based resins (e.g. LR White) on the other hand is very limited, although these resins can be beneficial in a range of life science studies. Major limiting factor is the poor stability of Acryl 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 (Ar, Xe, O, N) as an ion source. Oxygen provides clear improvement in cut face quality and image contrast1,2 and permits 30% faster data collection from the wide range of structures and the choice of correlative studies. Sample preparation for gallium-based FIB/SEM tomography has been established as a key technology for 3D volume analysis of life science samples.

Oxygen PFIB/SEM Tomography of Biological Samples
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Figure 1. Comparison of Gallium FIB and Oxygen PFIB milling of LR White resin embedded sample

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

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

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

Figure 5. Spin Mill technique

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

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

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 5).

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 6). A liberal area up to 1 mm in diameter can be milled with a plasma.

Preparation steps such as trenchless, fiducial marker, or protective layer depositions are not needed. A circular marker can be affected when the area of interest is smaller than the sample surface or its diameter is larger than 1 mm. This slices in the nanometer range can be automatically recorded and imaged later, like in a serial section 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 validation14 or for correlating light and electron microscopy15. 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).

CONCLUSIONS
Oxygen PFIB/SEM tomography extends the potential of FIB/SEM research in the life sciences. 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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