Novel combination of integrated FLM with cryo-FIB allows targeted cryo-ET sample preparation from challenging samples

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Cryo-electron tomography (cryo-ET) has revolutionized structural biology by enabling the observation and characterization of biological samples at a molecular level in their near-native state [1]. This technique provides an unprecedented level of structural detail, but it necessitates thin specimens (typically below 200nm) for effective electron beam penetration. Presently, the prevailing method for achieving such thinning involves Cryogenic Focused Ion Beam (cryo-FIB) milling [2].

During this milling process, it is easy to miss the regions of interest (ROIs). Fluorescence labeling and fluorescence light microscopy (FLM) can improve targeting [3]. However, the conventional use of a stand-alone cryo-FLM invokes extra transfer steps, increasing the risk of ice contamination, devitrification, and mechanical damage [4]. Moreover, the correlation of the two imaging modalities is cumbersome and often inaccurate, leading to the loss of ROI inside the lamella, rendering it useless, reducing the overall efficiency of the whole workflow.

To address these challenges we present the novel combination of the TESCAN AMBER cryo- FIB/SEM with a fully integrated high quality FLM: Delmic's METEOR system [5]. Thanks to the software integration and position of FLM parallel to the FIB, this novel combination allows smooth and accurate 3D correlation of FLM, FIB and SEM data. Furthermore, the integration of the FLM significantly reduces handling and ice contamination. Additionally, a full collision model allows safe control of the microscope ensuring novel users can adapt the technique quickly. The high-end fluorescent light microscope provides excellent resolution and brings background noise to an absolute minimum allowing researchers to image and mill challenging samples.

In this talk we will delve into the practicalities of this accurate, easy-to-learn and high-throughput correlative cryo-FIB workflow. We will show that the workflow can be used for targeted fabrication of cryo-lamellae from thin specimen (on-grid lamella), larger volumes frozen by waffle method, and cryo lift-out from the most challenging samples. Additionally, we will present the latest results showing EM and FLM data of high-pressure frozen C. elegans and plunge frozen U2OS cells. We will also provide an outlook and highlight the potential of plasma FIB milling, enabling analyses of large biopsies, tissues and small organisms are also discussed.

The novel combination of METEOR with TESCAN AMBER cryo-FIB/SEM allows targeted preparation of cryo-TEM specimen from challenging samples, like high-pressure frozen samples and flat U2OS1 cells.

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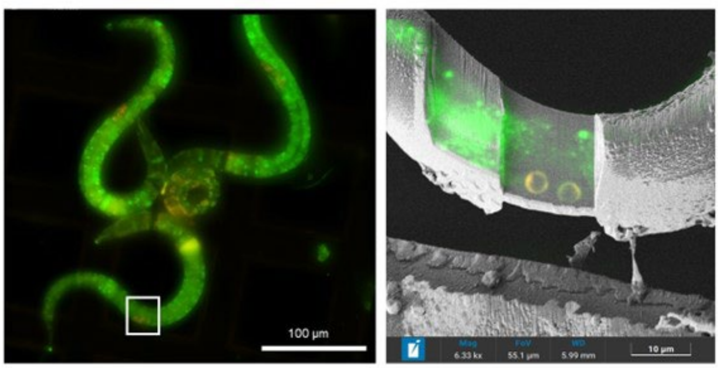
Graphic:



Keywords:

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