

Latest developments in accurate and high-throughput correlative cryo-FIB milling for cryo-ET lamella production

Marit Smeets¹, Deniz Daviran¹, Wessel Teunisse¹

¹Delmic B.V., Delft, The Netherlands

Correlative cryo-FIB milling is a powerful sample preparation technique for in situ cryo electron tomography (cryo-ET). Using this technique, vitrified cells can be thinned down and cellular components can be directly visualized in their native context at near-atomic resolution. In recent years several solutions have been proposed to simplify the technique by integrating fluorescent microscopes (FLM) in the cryo-FIB. However, it remains challenging to quickly and accurately target the region of interest (ROI). Here we present a high-quality integrated fluorescent microscope, called METEOR, that allows quick and accurate targeting of the ROI.

To ensure accurate targeting the components of METEOR were carefully selected. We ensured it is compatible with objectives with high numerical apertures (NA), the highest being 0.9. The high-end camera brings background noise to an absolute minimum and provides an exceptional quantum efficiency allowing researchers to image challenging and dim samples. The system is equipped with a filter wheel which can be filled with single-bandpass filters avoiding fluorescent bleed-through. Additionally we developed software that allows FLM imaging, sample stage control and SEM and FLM correlation.

By measuring over a 100 sub-diffraction beads we found that the resolution of the METEOR provided 385 nm lateral resolution and 1.18 μm axial resolution when using a 50x/0.8 NA objective. This is in line with the theoretical resolution of such an objective. We also found that by integrating FLM and SEM correlation within the FLM acquisition software the SEM map could be used for navigation. This significantly speeds up the identification of suitable cells for milling..

The METEOR system is a high quality imaging platform that allows accurate identification of regions of interest within the challenging samples. The software provides additional benefits that speed up the workflow significantly. METEOR therefore provides an accurate and high-throughput correlative cryo-FIB milling workflow.

Keywords:

cryo-ET, Cryo-FIB, CLEM

Reference:

1. M. Smeets et al. (2021). *Micros Today*, vol. 29, no. 6, pp. 20–25.
2. D. Boltje et al. (2022). *eLife*, vol. 11
3. J. Yaeng et al. (2023) *Microscopy and Microanalysis*, vol. 29, no. 1, pp-1055-1057