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Correlative Cryo-FIB Milling using METEOR, an Integrated Fluorescent Light Microscope

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Cryogenic electron tomography (cryo-ET) is emerging as a powerful technique to acquire high-resolution 3D structures such as intracellular organelles and protein complexes in their near-native cellular environment. Most cellular samples require thinning through cryo focussed ion beam (FIB) milling to create an electron transparent cell section called a lamella. Cryo-correlative microscopy, which requires acquiring images prior to cryo-FIB milling on a separate cryo-fluorescent light microscope (FLM), has proven an excellent technique to target a specific regions of interest (ROI) [1,2]. However, this sample preparation workflow is laborious, time-consuming, and prone to sample contamination and damage, due to multiple transfer steps. To overcome this limitation, fluorescence imaging systems which are directly integrated in the FIB/SEM chamber have been developed in recent years [3,4].

Here we present METEOR, a commercially available integrated widefield FLM that enables correlated cryo-FIB-milling of biological samples in one device. Besides avoiding unnecessary transfer steps it allows the user to verify the presence of the targeted fluorescent signal in lamellae during and after the milling process.

We show that METEOR can be used to target ROI's for cryo-FIB milling in a variety of cell types including yeast and HeLa cells. We also present different workflows METEOR can be incorporated into.

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