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Fluorescence-guided lamella fabrication with ENZEL, an integrated cryogenic CLEM solution for the cryo-electron tomography workflow

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Cryogenic electron tomography (Cryo-ET) is an imaging technique used to obtain high resolution 3D reconstructions of biomolecules in their near-native cellular environment. In Cryo-ET, a sample is flash frozen, thinned to the appropriate thickness (100-200 nm) and a tomogram is captured using a cryo transmission electron microscope (TEM). To create the thin section for the acquisition of the tomogram, using a Focused Ion Beam in a Scanning Electron Microscope (FIB/SEM) has become the gold standard. The FIB is used to mill away the surrounding material and create a lamella [1], [2]. Identifying the region of interest (ROI) to mill in the right location is a crucial step, since being off-target for this process could result in milling away your structure of interest. To overcome this, cryo-Fluorescence Microscopy (cryo-FM) is often used to identify the region of interest and avoid 'blind' milling. In cryogenic Correlative Light and Electron Microscopy (cryo-CLEM), fluorescent markers are used to label the structures or proteins of interest, which are then found back in the FIB/SEM.

Incorporation of a cryo-FM in the cryo-ET workflow brings many challenges. Firstly, transfer of the sample to the cryo-FM before milling (to identify ROIs) and again after milling (to ensure the ROI is not milled away) significantly increases the handling of the sample and thereby increases the risk of contaminating or damaging the sample [3], [4]. Secondly, the correlation of the fluorescence image with the image in the FIB/SEM is not trivial. It requires markers for navigating to the correct spot on the sample and if the overlay of the fluorescence and SEM images is not exactly correct, the ROI could still be missed or milled away [5].

We have developed ENZEL, an inverted widefield fluorescence microscope that can be integrated in dual beam systems that allows simultaneous, coincidence imaging of both FM and SEM. A custom sample stage and micro cooler setup allow the approach of an objective lens from below the sample, directly underneath the electron column polepiece. This setup allows imaging using SEM and FM without separate registration.

Fluorescence imaging is especially beneficial to the cryo-ET workflow when targeting specific intracellular compartments or distinctly localized proteins. We illustrate the benefits of this system with several example problem cases. We performed multi-channel fluorescence imaging of plunge-frozen

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HeLa cells and could distinctly localize TGN, mitochondria and peroxisomes. We also performed milling of lamellae that were analysed by cryo tomography, confirming the accuracy of the stage and performance of the cooler. In addition, we present fluorescent-guided milling and discuss and present subsequent transfer to a dedicated cryo-TEM microscope for high-resolution cryo-ET on the targeted lamella.

References

[1] M. Marko, C. Hsieh, R. Schalek, J. Frank, and C. Mannella, "Focused-ion-beam thinning of frozen-hydrated biological specimens for cryo-electron microscopy," *Nat. Methods*, vol. 4, no. 3, pp. 215–217, 2007, doi: 10.1038/nmeth1014.

[2] E. Villa, M. Schaffer, J. M. Plitzko, and W. Baumeister, "Opening windows into the cell: Focused-ion-beam milling for cryo-electron tomography," *Curr. Opin. Struct. Biol.*, vol. 23, no. 5, pp. 771–777, 2013, doi: 10.1016/j.sbi.2013.08.006.

[3] C. Hsieh, T. Schmelzer, G. Kishchenko, T. Wagenknecht, and M. Marko, "Practical workflow for cryo focused-ion-beam milling of tissues and cells for cryo-TEM tomography," *J. Struct. Biol.*, vol. 185, no. 1, pp. 32–41, Jan. 2014, doi: 10.1016/j.jsb.2013.10.019.

[4] C. M. Hampton *et al.*, "Correlated fluorescence microscopy and cryo-electron tomography of virus-infected or transfected mammalian cells," *Nat. Protoc.*, vol. 12, no. 1, pp. 150–167, Jan. 2017, doi: 10.1038/nprot.2016.168.

[5] M. Turk and W. Baumeister, "The promise and the challenges of cryo-electron tomography," *FEBS Lett.*, vol. 594, no. 20, pp. 3243–3261, Oct. 2020, doi: 10.1002/1873-3468.13948.

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