AUTOMATED CRYO-PLUNGING FOR CLEM AND CRYO-FLUORESCENCE

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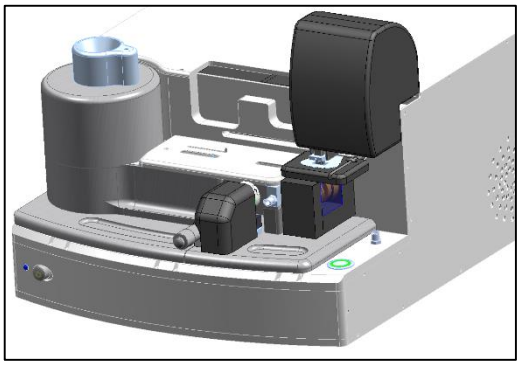
Imaging of biological samples embedded in vitrified ice has become of great interest in recent years as it provides several advantages: the biological sample is in a fully hydrated state with superior preservation down to ultra-structural level, a vitrified sample is naturally compatible with the vacuum required for EM / CLEM (Correlative Light and Electron Microscopy) [1,2] and cryo-fluorescence provides very low photo-bleaching [3] and high signal to noise imaging.

Preparation and handling of vitrified samples normally requires special skills and techniques. Here we discuss a novel design that simplifies and automates plunge-freezing and sample transfer, thus making this technique accessible for a wider audience.

There are several common issues in a conventional plunge-freezing setup. Typically the sample is prepared on an Electron Microscopy grid and excess liquid is removed inside a humidity chamber using blotting paper to control the ice thickness of the frozen sample. The blotting paper usually needs alignment and blotting force calibration, while the paper does not work consistently over time because it soaks up moisture in the humidity chamber, also preventing real-time optical transmission imaging and monitoring during blotting.



In our robotic plunger unit we substitute the common blotting mechanism to improve process stability. We combine real-time optical observation of the sample with control of the liquid film thickness. Film thickness is controlled by adjusting the speed at which the sample is drawn from the fluid as well as thinning of the film thickness by controlled suction. When the correct conditions are confirmed by the optical system, the user triggers plunging into a temperature controlled bath of liquid ethane. Plunging is followed by automated loading into a special cryo-holder and a cryo-transfer container. Fully automated, programmed operation is also possible. Transfer of the sample to a cryo-fluorescence imaging system is feasible while the EM sample grid remains in the safe cryo-cartridge, avoiding the risk of manual handling and sample contamination.

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