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Improving Cryo-Electron Tomography Data Quality and Throughput by Minimising Ice Contamination During Lamellae Fabrication using CERES Ice Shield

Caspar Jonker[1*], Stuart C. Howes[2], Katherine Lau[1], Friedrich Förster[2]

1. Delmic Cryo B.V., Delft, The Netherlands
2. Structural Biochemistry, Bijvoet Centre for Biomolecular Research, Utrecht University, 3584 CG Utrecht, The Netherlands

*Corresponding author: jonker@delmic.com

In situ cryo-electron tomography (cryo-ET) can reveal structural information on biomolecules and shed light on intermolecular interactions within the cell. Thin lamellae (150-300 nm thick) suitable for transmission electron microscopy (TEM) imaging are typically prepared from vitrified cells using a cryo-focused ion beam (FIB). Although the FIB chamber operates at high vacuum, residual water causes undesirable ice growth on the sample, increasing the overall thickness and resulting in lower TEM image contrast. A common strategy during lamellae preparation is to minimize the time between the final polishing and unloading by rough milling all lamellae then fine polishing each lamella. However, depending on the FIB model, the ice contamination rate can reach 50 nm/hour, setting limits on this strategy.

The CERES Ice Shield (Delmic Cryo, The Netherlands) was applied to minimise ice growth. Its design is improved upon the original design of the cryo shutter as seen in the Tacke et al publication [1]. It consists of a cryo shutter cooled by liquid nitrogen that is inserted between the scanning electron microscope pole piece and the sample, thereby reducing the partial pressure of water in the sample vicinity. A hole allows the ion beam access while protecting the sample.

Without CERES Ice Shield, the ice growth rate was 45±17 nm/hour over the first 2 hours. With the CERES IS the ice thickness after 3 hours was 5±7 nm, within the measurement error of the assay. Our results indicate that CERES Ice Shield reduces the ice growth on the cryo-ET samples during lamella preparation to a level near the detection limit. This increases the number of lamellae that can be prepared per session and improves the final TEM image quality and resolution.

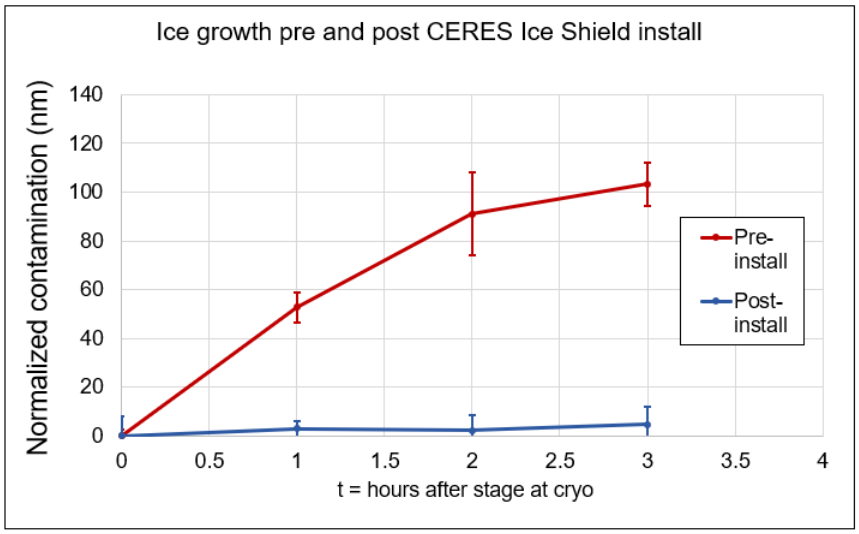

Figure 1. Scanning electron microscopy images were of the edges of the holes in carbon-coated TEM grids tilted at a 15 ° angle were taken at multiple time points over three hours. The thickness of the carbon film was measured. The normalized average thickness and error range at each time point are presented.

References:

[1]: S Tacke et al., Journal of Structural Biology **213**, 107743 (2021). doi.org/10.1016/j.jsb.2021.107743

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