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CERES Ice Shield specifications sheet

Ultimate protection against ice contamination during lamellae preparation

delmic

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CERES Ice Shield at a glance

Meet the new, updated version of the CERES Ice Shield: the ultimate protection against parasitic ice growth inside the cryo-focused ion beam (cryo-FIB) chamber during lamella milling.

In the cryo-electron tomography (cryo-ET) workflow, a cryo-FIB is routinely used for generating thin lamellae suitable for image acquisition in a cryo-transmission electron microscopy (cryo-TEM). The current typical 50 nm/hour in-chamber ice growth rate accepted by cryo-FIB providers means that your 150 nm lamella could become several times thicker at the end of a sample preparation session affecting the TEM image contrast and achievable resolution. With the patented, innovative CERES Ice Shield design, you can get better quality tomogram and resolution from your cryo sample. CERES Ice Shield protects your sample from the ice contamination originating from the electron column and prevents condensation on the sample. With the >10-fold decrease in ice contamination rate, you can process more samples per session before the first lamella becomes covered in ice, and truly benefit from your system's automated milling capability. The new, compact version of CERES Ice Shield is as highly effective and integrated in your cryo-ET workflow as before while occupying less space in your lab.

Key advantages

Get better quality cryo-electron tomography (cryo-ET) data

Get the best quality data from your cryo sample by minimising ice growth during lamella-milling

Achieve better resolution

Make more lamellae in one session without worrying about ice contamination. Achieve higher resolution by increasing your sample throughput and lamella quality.

A small adjustment with a big impact

Obtain better results more effortlessly with the easily implemented and highly effective CERES Ice Shield.

Optimize the whole cryo-electron microscopy workflow

Combine CERES Ice shield with the Delmic METEOR integrated fluorescence microscope to reduce transfer steps and streamline the Cryo-ET sample preparation workflow.

Working principle

The CERES Ice Shield consists of a high precision cryo shutter that inserts between the scanning electron microscopy (SEM) pole piece and the sample. This shields the sample from ice contamination originating from the SEM column while also increasing the local vacuum through cryo-pumping (Tacke *et al.*, 2021). The innovative shutter design comes with a hole that allows FIB milling while the shutter is inserted, fully protecting the sample during lamellae fabrication.

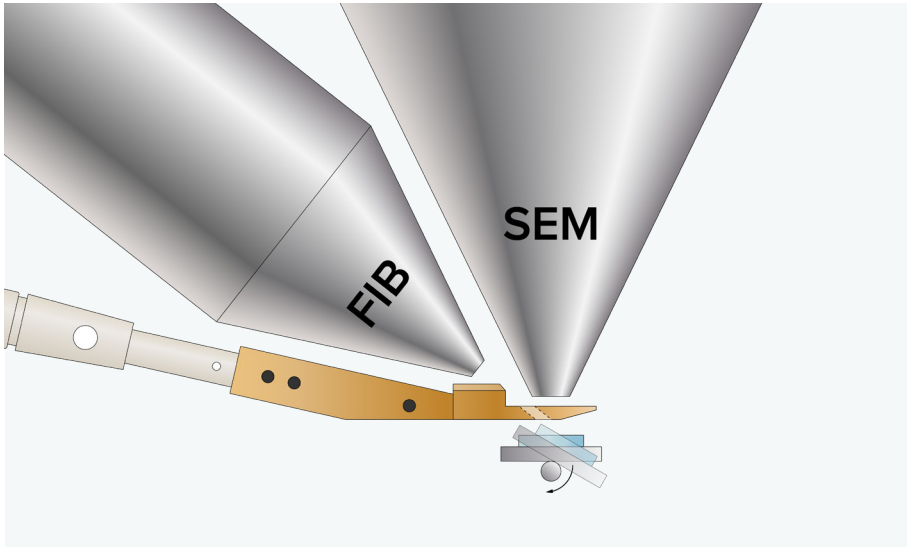

Figure 1 The CERES Ice Shield consists of a liquid nitrogen cooled cryo shutter that has a hole for the FIB beam. It blocks the line of sight from the SEM pole piece and protects the sample from ice contamination during FIB-milling.

Proof of concept

Measuring in-chamber ice contamination can be performed by analysing the edge of grid holes of carbon-coated TEM grids under a shallow angle. Here, we used R2/2, 200 mesh grids and imaged grid holes under a 15° angle at multiple time points after stage cool down. We performed this analysis before and after the CERES Ice Shield installation. In the pre-installation condition, between image collections, the sample was unprotected at the eucentric height below the SEM pole piece with the beams off. In the post-installation condition, the CERES Ice Shield protected the sample between image collections. In **Figure 2**, representative images of grid holes can be seen before and after CERES Ice Shield installation. The images clearly show an increasingly thick layer of ice accumulating on the grid before the installation of the CERES Ice Shield, while the images post-installation show little to no ice accumulation. The thickness of the foil with ice was measured and plotted in **Figure 3**. This shows that the ice growth was approximately 45±17 nm/hour in the first 2 hours after stage cooldown before installation of the CERES Ice Shield. After installation, the increase in thickness is approximately 5±7 nm after 3 hours. The post-installation thickness at t=3 was not significantly different from the thickness at t=0 ($p=0.16$). Taken together, these results show that the CERES Ice Shield reduces the in-chamber ice contamination to near undetectable levels.

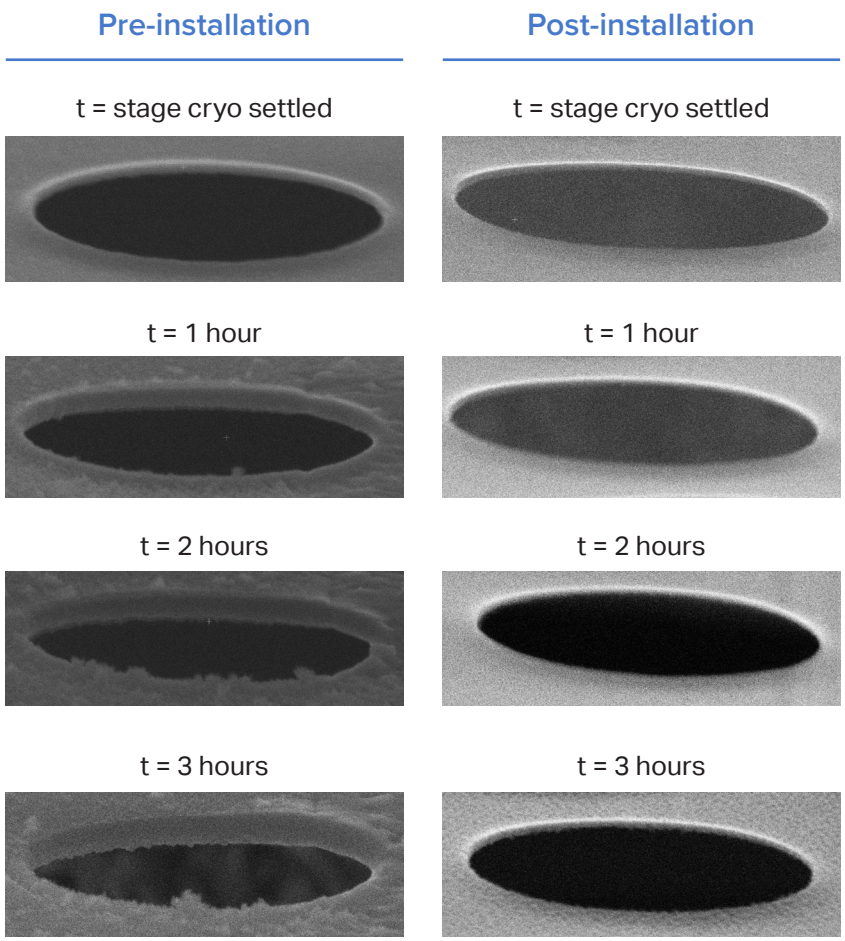

*Figure 2 Examples of 2 µm diametre grid holes of R2/2 200 mesh TEM grids imaged at a 15° angle at different time points. Pre-installation images show an increasingly thick layer of ice accumulating on the grid, while the images post-installation show little to no ice accumulation. **

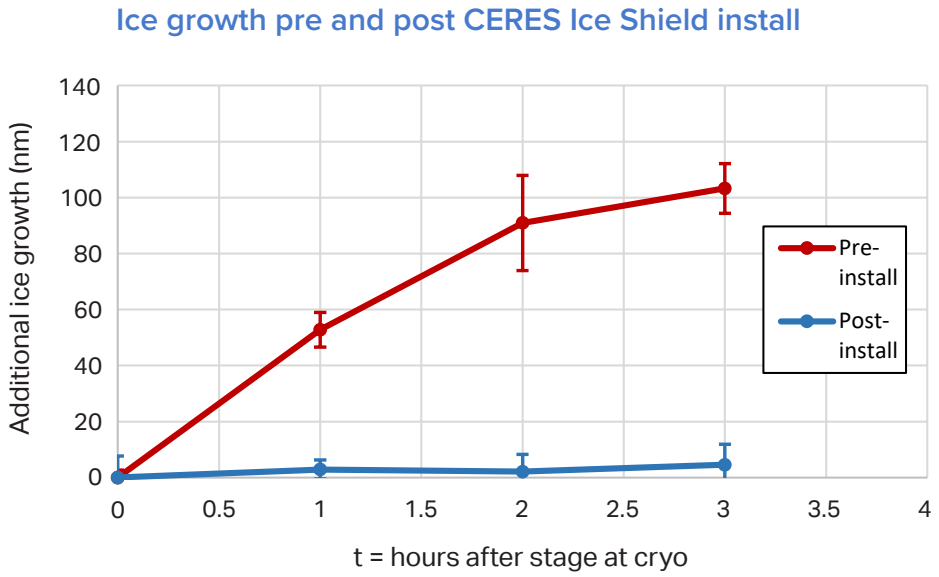

*Figure 3 The thickness of the foil and ice was measured at set time intervals after settling of the cryo stage (t=0). The data was normalized to the thickness at t=0 to allow better comparison of the growth over time. The graph shows that the contamination was approximately 45 nm/hour in the first 2 hours after stage cooldown before the CERES Ice Shield installation (red curve). After the installation (blue curve), the increase in thickness is significantly reduced to approximately 5±7 nm after 3 hours. **

* *Data courtesy of Dr S.C. Howes and Prof F. Förster, Structural Biochemistry, Bijvoet Centre for Biomolecular Research, Utrecht University.*

System specifications

	Specifications
Compatible cryo-FIB/SEM models	ThermoFisher Scios, Aquilos and Aquilos 2; Please discuss the compatibility with other cryo-FIB/SEM models with your Delmic sales presentative. Compatible with EasyLift.
Compatible cryo-ET workflows	Cryo-ET lamella preparation from plunge frozen and high pressure frozen samples. CERES Ice Shield cryo shutter is not inserted between the SEM pole piece and the sample during the lift-out process. It is most importantly inserted during lamella thinning.
CERES Ice Shield components	CERES Ice Shield consists of a cryo shutter, bracket and braid. The shutter can be inserted between the SEM pole piece and the sample, protecting the sample from contamination originating from the pole piece and increasing the local vacuum quality. All CERES Ice Shield components are made of high vacuum compatible materials.
FIB milling compatibility	The FIB has unrestricted access to the sample when the Ice Shield is inserted. It is optimised for lamella milling. Compatible with ThermoFisher Auto-TEM.
Control	The CERES Ice Shield can be inserted and retracted through software control.
Run time	Equal to the SEM cryo stage run time.
Cryo shutter temperature	Equal to the SEM cold shield temperature.

Reference

1. Tacke, S. *et al.* (2021) 'A streamlined workflow for automated cryo focused ion beam milling', *Journal of Structural Biology.* Academic Press, **213**(3), p. 107743. doi: 10.1016/J.JSB.2021.107743.

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About

Delmic is a passionate high-tech company based in Delft, the Netherlands that develops powerful and user-friendly solutions for light and electron microscopy. Our systems are used by researchers and companies all over the world in fields ranging from life sciences, geology, material sciences to nanophotonics.

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