

New Scios Cryo™ - Dedicated FIB/SEM for Advanced Cryo-Lamella Preparation in Structural Biology

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We would like to introduce the new FEI Scios Cryo; a system which is built upon a successful prototype and other variants which have resulted already in significant publications [1,2,3]. This instrument offers customers a dedicated and reliable entry point to the world of '*in situ*' lamella preparation for cryo electron tomography (cryoET). Cryo-focused ion beam (cryo-FIB)-based sample preparation has recently been shown to extend structural biology from solely purified structures, to the dynamic complexity of the cell [4].

Cells typically need to be thinned to a thickness which is conducive for modern cryo-TEMs. To this end researchers have recently been exploring the use of DualBeam™ microscopes to enable thinning of frozen-hydrated biological cells. Such FIB-SEM instruments utilize a primary electron column for imaging and a secondary ion column for milling. Typically Gallium ions are focused onto the sample in order to facilitate removal of material [5].

The Scios Cryo consists of the following modules: A Scios 2 DualBeam™ platform, dedicated and equipped with a cryo loading system, as well as an innovative cryo stage with full rotation and tilt capabilities. Furthermore, a cryo cooling system involving a heat exchanger for cooling of nitrogen gas, a preparation station for loading and unloading samples under cryogenic conditions and finally, software which has dedicated functionality to assist the users in producing better quality lamellae. Figure 1 shows a schematic of the modules.

Plunge frozen cells which have been transferred to an FEI Autogrid can be loaded on to a shuttle under cryogenic conditions and transferred in the DualBeam™. Here the sample can be correlated to optical data using Maps™ software, allowing for identification of specific cells which have the desired phenotype. Cells can be identified and regions of interest can be selected. From here the user can navigate to the desired cell by merely clicking in an overview image.

Sputtered inorganic metal layers are used to facilitate conduction, which can be done inside the main chamber. Protective layers of organometallic platinum can be added to allow milling of straight edges without damage from stray ions. Typically a milling position, which is at a slight elevation with respect to the TEM grid, is chosen resulting in an *in situ* lamella which is as close to the plane with the grid as possible. Examples are shown in figure 2. These lamellae can be transferred out of the microscope into the loading station, where the grid can be transferred to the Talos Artica™ or Titan Krios™ TEM's allowing collection of tomographic data. The Scios Cryo complements our integrated product suite, providing a complete cryo-tomography workflow, by adding a dedicated preparation tool for vitreous sample thinning for structural biology.

References:

- [1] Chaikerasitak *et al*, *Science* **355** (2017), p. 194.
 [2] Mahamid *et al*, *Science* **351** (2016), p. 969.
 [3] Schaffer *et al*, *J Struct Biol.* **197** (2017), p. 73.
 [4] Lučić V *et al*, *J Cell Biol.* **202** (2013), p. 407.
 [5] Rigort & Plitzko JM., *Arch Biochem Biophys.* **581** (2015), p. 122.

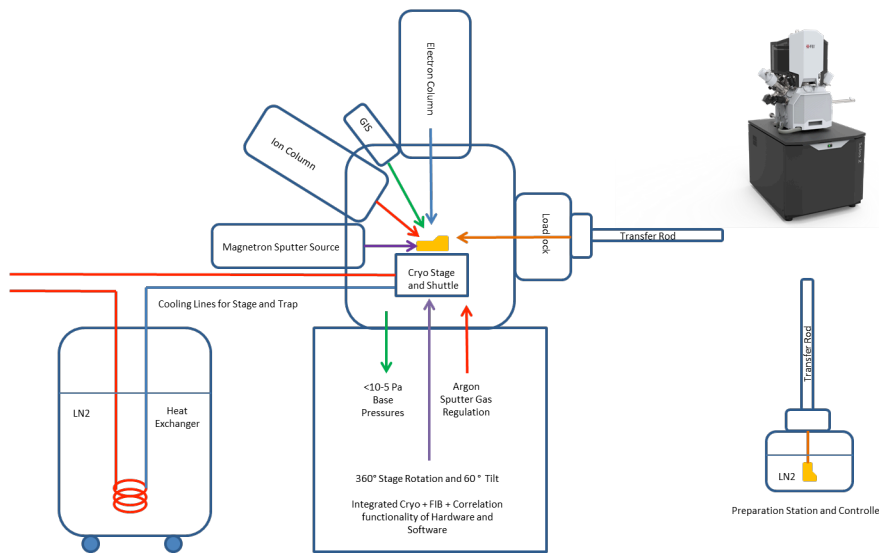


Figure 1. Schematic of the Scios Cryo showing the preparation station where a sample is loaded into the shuttle, then transferred via vacuum transfer to the load lock on the cryo-FIB. From here it can be loaded to the stage and then processed. The stage is cooled by a heat exchanger system immersed in liquid nitrogen. Inset is an image of the Scios 2 platform.

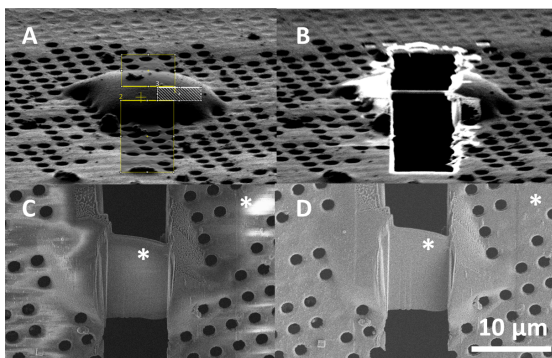


Figure 2. A. Vitrified cell marked with milling pattern, B. after cryo-lamella milling, C. Post-milling charging effects can be observed (asterisks) and D. Platinum sputter coated lamella exhibiting no charging effects. All images were recorded at 2kV, 13pA and 1 μs dwell time.