### Arctis Cryo-Plasma FIB

# High-throughput, automated cryo-FIB-SEM designed for the cryo-electron tomography workflow

The Arctis Cryo-Plasma Focused Ion Beam is designed for automated cryotomography sample preparation. You can reliably produce cryo-lamellae *in situ* with a thickness of 200 nm or less while avoiding gallium-based ion implantation effects. The Arctis Cryo-PFIB offers significantly higher throughput rates compared to other cryo-FIB-SEM systems currently on the market.

## Direct interface to the cryo-TEM and tomography workflow

With the Autoloader system, the Thermo Scientific<sup>™</sup> Arctis<sup>™</sup> Cryo-PFIB offers robotic sample loading, automatic sample processing, and storage of up to twelve frozen specimens. The direct connection to any Autoloader-equipped cryo-TEM (e.g., Thermo Scientific Krios<sup>™</sup> or Glacios<sup>™</sup> Cryo-TEMs) eliminates manual grid handling and transfer steps between FIB-SEM and TEM. In order to meet the requirements of low-contamination cryo-EM applications, the Arctis Cryo-PFIB also features a completely new high-vacuum chamber and improved cooling/shielding.

#### **Key Benefits**

**Correlation to light microscopy and relocation in TEM.** "On-board" integrated fluorescence microscope (iFLM) allows the same area to be observed with light, ion, or electron beams. Specially designed TomoGrids always ensure correct lamella alignment to the tomographic tilt axis, from initial milling through high-resolution TEM imaging.

**High-quality lamellae with consistent thickness.** Plasma source provides multiple ion species (xenon, oxygen, argon) for high-quality lamella preparation without gallium implantation. An ultra-clean working environment is ensured for multiple days through the proven combination of a compact sample chamber and a dedicated cryo-box for shielding against water condensation.

Automated high throughput and connectivity for cryotomography. The Autoloader enables robotic sample handling for up to 12 grids, provides direct connectivity to the cryo-TEM and minimizes sample contamination risks. Set up milling runs from anywhere via a web-based user interface and perform autonomous, multi-day jobs for automated lamella preparation. Plasma source enables high milling rates for fast, large-volume material removal.



thermo scientific



Arctis Cryo-PFIB displaying enclosure (white) and electrical consoles (gray).

#### Key technologies integrated in the Arctis Cryo-PFIB Plasma focused ion beam (PFIB)

The Arctis Cryo-PFIB can switch between three ion species (xenon, argon, and oxygen) thanks to its fast switchable plasma ion source, offering outstanding performance for large volume material removal as well as precision milling. PFIB technology enables applications not covered by liquid metal ion source (LMIS) FIB systems. For example, it is possible to use the different milling properties of the three available ion beams to produce a high-quality lamella while avoiding gallium implantation effects.

#### Scanning electron microscope (SEM)

The SEM is optimized for high resolution and beam stability. It has a Schottky field emission gun and a dual objective that combines field-free magnetic and electrostatic lenses. In combination with the fluorescence microscope, the SEM enables sample navigation, targeting the region of interest, and precise end-pointing for cryo-lamella preparation.

#### Integrated fluorescence microscope (iFLM)

The Arctis Cryo-PFIB includes an integrated fluorescence light microscope (iFLM) correlative system, which enables fluorescence imaging at the electron/ion beam coincidence point. Fluorescence imaging for targeting, intermediate verification, and final target confirmation can easily be done before, in-between, and after the ion milling without moving the stage. The iFLM is set up for epifluorescent imaging and can be used in reflection and fluorescence mode; the 180° alpha tilt capability of the CompuStage allows imaging of the top and bottom surfaces of the sample, which can be helpful for thick samples.

#### Novel cryo-box in a compact sample chamber

The compact sample chamber of the Arctis Cryo-PFIB is specifically designed for cryo operation. Its reduced volume allows for an exceptionally clean operating environment that minimizes contamination. This designed-in cleanliness is further enhanced by sample cooling via braids and sample shielding in a dedicated cryo-box, ensuring a working environment that routinely allows for multi-day batch jobs.

### Efficient sample manipulation with Autoloader, CompuStage, and TomoGrids

The Arctis Cryo-PFIB is the first FIB to feature an Autoloader – the proven robotic sample-handling device that revolutionized cryo-electron microscopy by enabling easy and robust transfer of 12 cryogenically frozen grids at a time. Similar to Krios and Glacios Cryo-TEMs, the Arctis Cryo-PFIB uses a CompuStage for precise and stable sample manipulation.

TomoGrids have been specifically designed for the cryoelectron tomography workflow and ensure that the cryolamellae are always correctly aligned to the tomographic tilt axis in the cryo-TEM. TomoGrids are compatible with existing AutoGrid-based systems. The direct connection to the cryo-TEM via the Autoloader avoids risky sample-manipulation steps that could damage or contaminate the sample.



New TomoGrid sample carrier ensures that the FIB-milled lamella is always correctly aligned relative to the TEM tilt axis.

#### Gas injection system

The Arctis Cryo-PFIB features a gas injection system (GIS) for deposition of a micrometer-thick protective layer of metal-organic platinum. This is essential for the production of thin, electrontransparent cryo-lamellae while avoiding beam erosion effects.

#### Integrated sputter deposition

An integrated, retractable platinum sputter target is incorporated into the Arctis Cryo-PFIB to render the final cryo-lamella electrically conductive. This is important to avoid charging in the cryo-TEM, and further improves the resolution of the cryo-tomography data.

#### System enclosure, designed with biosafety in mind

The Arctis Cryo-PFIB comes with an environmental enclosure and integrated service hoist. A system height of 2.6 meters allows the instrument to fit into standard laboratories. The enclosure surface is designed to be easily wiped down, and for higher biosafety level labs, a heat decontamination solution is available.



Key technologies purposely designed and chosen for the Arctis Cryo-PFIB, enabling an unprecedented productivity and connectivity within the cryoelectron tomography workflow.

#### Designed for automation and remote operation

The Arctis Cryo-PFIB is designed for fast, reliable and reproducible production of *in-situ* cryo-lamellae. Once the samples have been loaded, the lamella production process can be set up remotely from a web-based control interface. You



(a) *S. cerevisiae* cryo-lamella produced by xenon ion milling in the Arctis Cryo-PFIB. (b) Slice from a tomographic reconstruction. (c) Corresponding sub-tomogram averaging of 7,000 particles shows the 80S ribosome at a global resolution of 6.5 Å. The 60S large subunit reaches 4.5 Å local resolution.

can batch-screen grids, perform correlative imaging, and set up lamella preparation for multiple sites on multiple grids. This will then be executed autonomously via automated milling software. After automated lamellae preparation is completed, the samples are easily transferred to a cryo-TEM using the Autoloader capsule.

Technical highlights*	
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lon optics	
lon gun	High-performance PFIB column, with inductively coupled plasma (ICP) source for fast ion switching
lon species	Xenon, argon, oxygen
Switching time	<10 minutes, only software operation
Beam current range	1.5 pA to 2.5 µA
Accelerating voltage range	0.5–30 kV
Maximum horizontal field width	0.9 mm at beam coincidence point
Resolution (Xe+ beam)	<20 nm at 30 kV
Electron optics	
Electron gun	High-stability Schottky field emission gun
Column	UHR non-immersion field-emission SEM column
Source lifetime	Minimum 12 months
Gun maintenance	Auto bakeout, auto start and no mechanical alignments
Room ourront range	
Detectors	In-lens detection system: T1 (BSE) and T2 (SE) / In-chamber: ETD (SE)
Resolution (T2)	<2.6 nm at 2 kV
Fluorescence microscope	
Coincidence point	Triple beam coincidence at sample position for photons, electrons, and ions
Objective	100x Zeiss Epiplan Neofluar NA 0.75; Piezo-driven
Objective working distance	4.0 mm
Modes	Fluorescence and reflection (motorized filter changer)
Filters	4-channel fluorescence Semrock LED-DA/FI/TR/Cy5-B-000 (Quadband) BrightLine <sup>®</sup> full- multiband filter set, optimized for DAPI, FITC, TRITC, & Cy5 and other like fluorophores, illuminated with LED-based light engines

Camera	Basler a2A4504-18umPRO with Sony IMX541 CMOS sensor (20.2 MP resolution)
Imaging FOV	>150 µm (diagonal)
LED source	CoolLED, 4 channels (365 nm/450 nm/550 nm/635 nm)
Vacuum system	
Vacuum system	Completely oil-free pumping system
Vacuum chamber pressure (at cryo-conditions)	<5 × 10 <sup>-5</sup> Pa
Cooling and shielding	Nitrogen-cooled Autoloader and CompuStage with Thermo Scientific DualBeam <sup>™</sup> System cryo-box
Stage and sample holder	
Туре	CompuStage, computerized 4-axis specimen cryo-stage with $\pm 90$ -degree alpha tilt Single axis specimen carrier holder for optimized stability and drift performance
Eucentric point	10 mm (from electron column pole piece)
Compatible grid carriers	TomoGrids, AutoGrids, FIB-AutoGrids
Sample loader	Autoloader: automated loading of cassettes (up to 12 grid carriers) under cryo-conditions
Protective coating	
Gas injection system	Retractable platinum GIS for chemical vapor deposition
Conductive coating	
Sputtering	Retractable platinum-ion sputter target for conductive sputter coating
Environmental protection	
Enclosure	System fully enclosed
Enclosure height	2.6 m
Nitrogen cooling	
Nitrogen refilling	Automatic liquid nitrogen filling system / Software controlled
* Charifications subject to change without notice	

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