Hydra Bio Plasma-FIB

Breakthrough capabilities for cryogenic and room temperature volume EM, and highly versatile lamella preparation for the cryo-electron tomography workflow.

The Thermo Scientific™ Hydra Bio™ Plasma-FIB (PFIB) is a focused ion beam scanning electron microscope (FIB-SEM) designed for volumetric imaging of frozen-hydrated and plastic-embedded biological samples. The Hydra Bio Plasma-FIB also features proven automation and cryo-technologies for versatile cryo-electron tomography and lift-out.

Automated preparation of lamellae and Easy Lift workflow for cryo-electron tomography

The Hydra Bio Plasma-FIB offers a complete cryo infrastructure with a fully rotatable cryo-stage, integrated micro-sputterer, and a cryo-transfer system designed for frozen-hydrated biological samples. The seamless integration of all cryo-components in the instrument's software provides an improved user experience as well as enhanced productivity and efficiency.

Thermo Scientific™ AutoTEM™ Cryo Software is a dedicated tool for the automated preparation of cryo-ET lamellae. The guided workflow facilitates batch milling, where multiple points of interest can be selected for autonomous cryo-lamellae preparation in unattended runs.

Additionally, the Thermo Scientific™ EasyLift Nanomanipulator is designed for the preparation of lamellae from targeted regions within high-pressure-frozen bulk specimens.

Fluorescently labeled regions of interest are localized using the Thermo Scientific™ iFLM™ Correlative System and then extracted and thinned before being transferring to a cryo-transmission electron microscope (cryo-TEM) for data collection.

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Key features

Create high-quality lamellae for cryo-electron tomography. Versatile configurations enable lamella creation from cell or tissue samples using cryo-lift-out, or directly from bulk material with rapid PFIB milling.

Unlock high-resolution cryogenic volume EM. Gain new insights by studying frozen-hydrated specimens prepared by high-pressure or plunge freezing. Generate 3D datasets with automated serial milling and imaging in Thermo Scientific™ Cryo Auto Slice & View™ Software.

Streamline your volume EM workflows with AI. Exclusive AI-driven applications provide novel strategies for volume acquisition, enhancing reliability and throughput.

Access large sample areas and visualize multiple regions of interest with the Spin Mill Bio Method. This distinctive large-area planar milling technique generates microtome-sized areas with clean, smooth surfaces and slice thicknesses as small as 5 nm, facilitating precise localization and imaging of regions in 2D or 3D.

Identify, target, and confirm regions of interest in correlative workflows. Seamlessly correlate targets located with integrated fluorescence microscopy. Easily correlate data from external sources using Maps Software.

Optimize milling and imaging, regardless of your sample preparation protocol. The choice of four different plasma source ions helps you optimize milling and imaging of both cryogenic and resin-embedded samples.

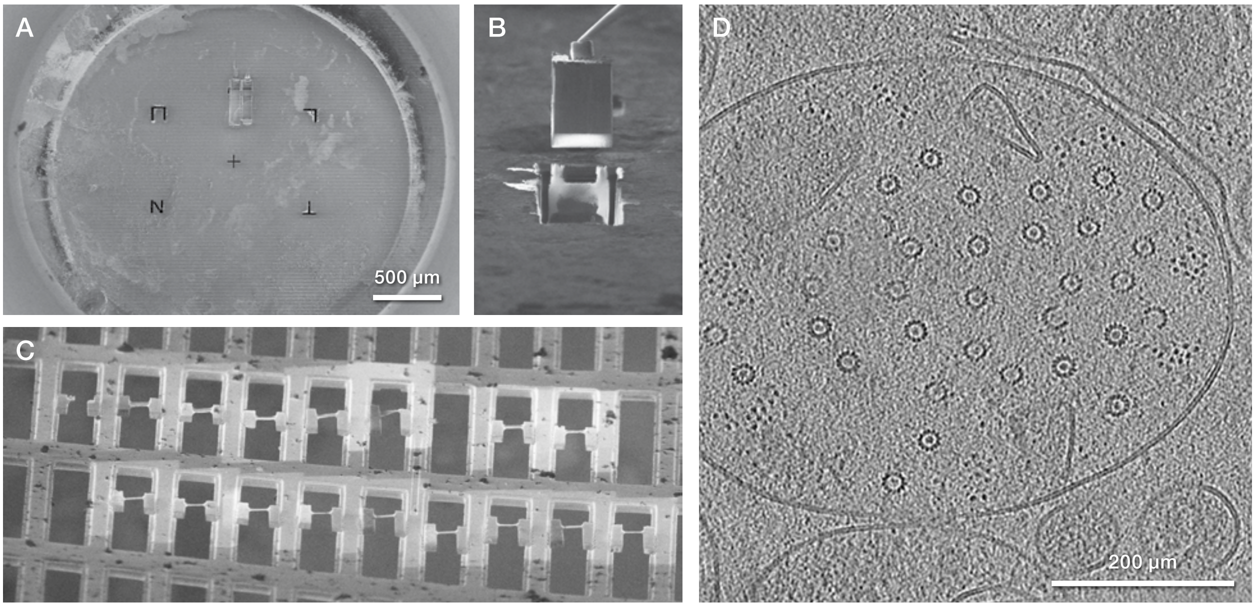

Figure 1: Serial lift-out of a high pressure frozen (mouse) hippocampal section. A) Overview of the HPF sample. B) Example lift-out from a targeted area. C) FIB-SEM image of the deposited serial lift-out sections. D) A slice-through tomogram revealing microtubules and actin filaments. Images courtesy of Calina Glynn, Jake L.R. Smith and Michael Grange, The Rosalind Franklin Institute.

Cryo-volume EM—study cells and tissues in their native-state

Cryo-fixation allows hydrated specimens to be imaged without the artifacts commonly associated with volume-EM sample-preparation steps such as heavy metal staining, dehydration, and resin embedding. The Hydra Bio Plasma-FIB makes it possible to view 3D volumes at cryogenic temperatures, revealing complex cellular structures in a near-native state.

The ability to quickly switch between plasma source ions allows for the seamless combination of xenon and argon milling. This dual-ion approach is essential for efficiently accessing regions of interest, joining the high-speed milling of xenon with the smooth serial cross-sectioning of argon for high-resolution imaging.

For cryo-volume acquisitions, AI-guided dynamic auto-functions significantly enhance the consistency and reliability of results. The AI-powered analysis adjusts the positions of the defined auto-functions to effectively mitigate the impact of sample variability and the challenges typically associated with cryo-prepared specimens. This is particularly valuable for unattended cryo-volume acquisition.

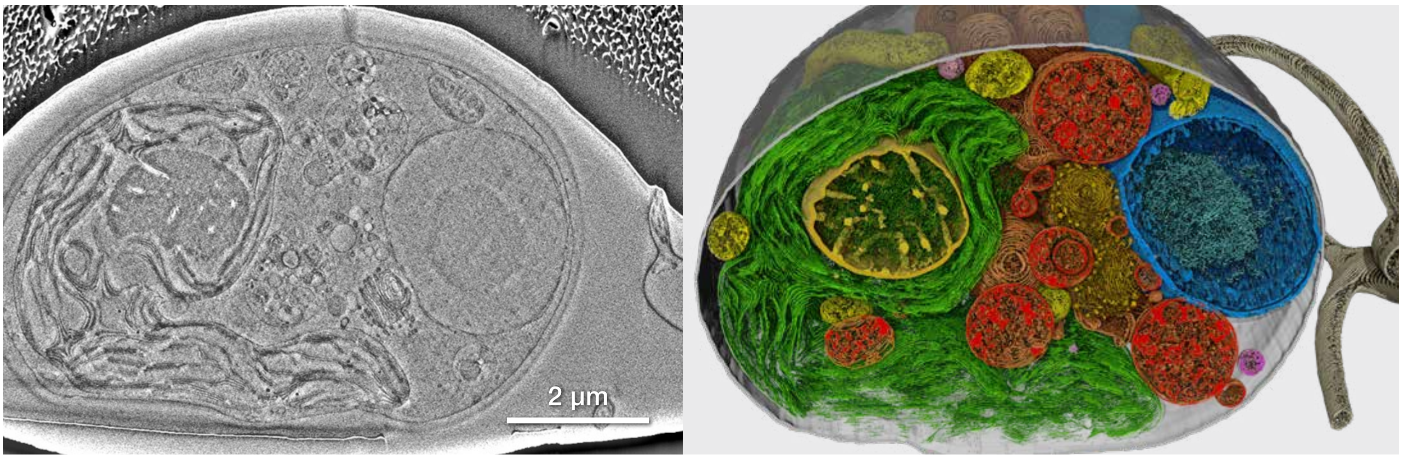

Figure 2. Hydra Bio Plasma-FIB images of unicellular algae *Chlamydomonas reinhardtii*. A) A single 2D cross section captured with Cryo Auto Slice & View Software. B) 3D volume visualized using Thermo Scientific™ Amira™ Software. The sample was prepared by plunge freezing, without added stains. Slice thickness = 20 nm.

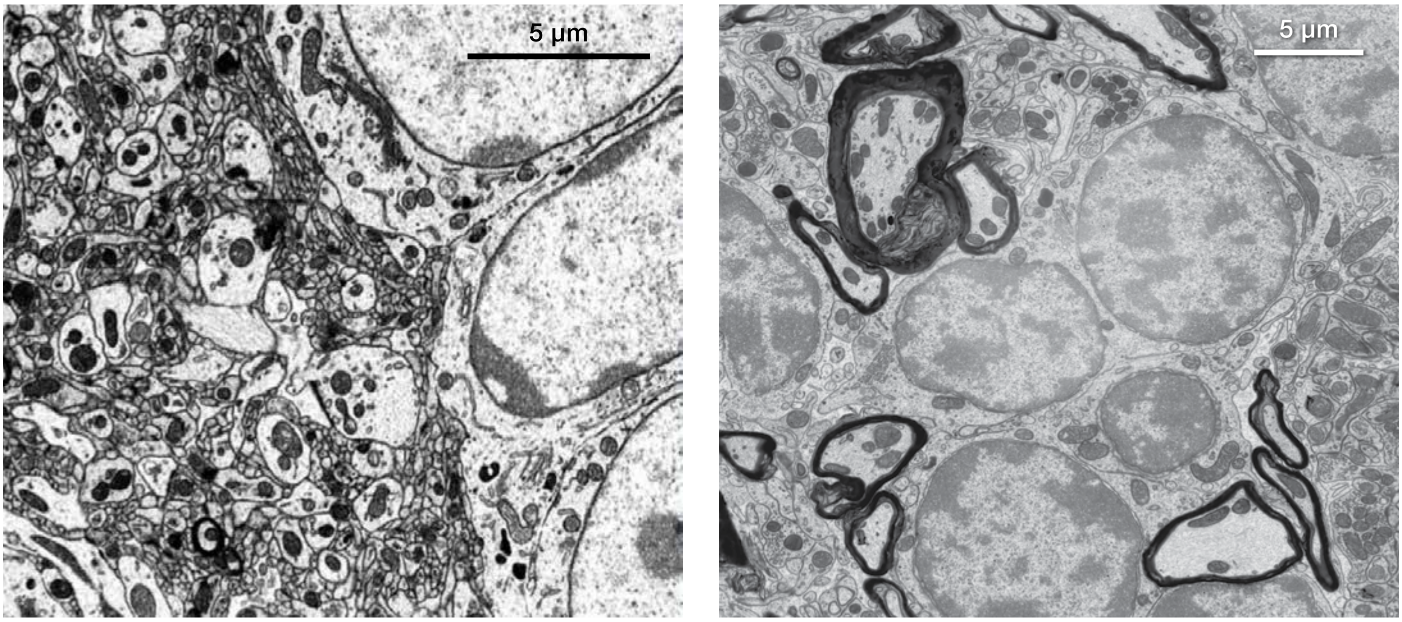

Figure 3. Selected PFIB slices from mouse brain tissue, chemically fixed with LR-White (left) and EPON (right) resin. Imaging with oxygen rather than gallium reduces charging and avoids curtaining artifacts that would otherwise be experienced with these resins.

Large-volume serial sectioning with AI guidance

The high-current capability of plasma-FIB allows large sample volumes to be investigated with high throughput. Access to four plasma source ions (xenon, oxygen, argon, and nitrogen) ensures compatibility with commonly used sample-embedding media and preparation protocols. Switching between these four source ions is quick and easy, so they can be used independently for site-specific, large-volume material removal, as well as for top-down and cross-section analysis in 2D or 3D.

For example, oxygen PFIB provides superior data acquisition efficiency and image quality for samples embedded in either epoxy- or acrylic-based resins. Unlike gallium-based FIBs, curtain-free surfaces are easily generated for a wide range of resins including LR-White, HM20, EPON, and Quetol. Furthermore, oxygen plasma mitigates charging at the resin surface, further improving image quality.

Combined with AI-driven acquisition protocols, the Hydra Bio Plasma-FIB enables improved throughput and reduced beam exposure for volume acquisitions of resin-embedded samples. Adaptive Scanning, powered by these protocols, tracks defined features of interest throughout the volume and images those regions of interest at a high resolution while contextual information is preserved at a lower resolution. This scanning approach reduces total acquisition time by up to 50% by only scanning the structures of interest at high resolution; it can also reduce beam dosage and charging. This accelerates high-resolution volume EM throughput while also enhancing 3D comparative studies and statistical analysis in room-temperature FIB-SEM experiments.

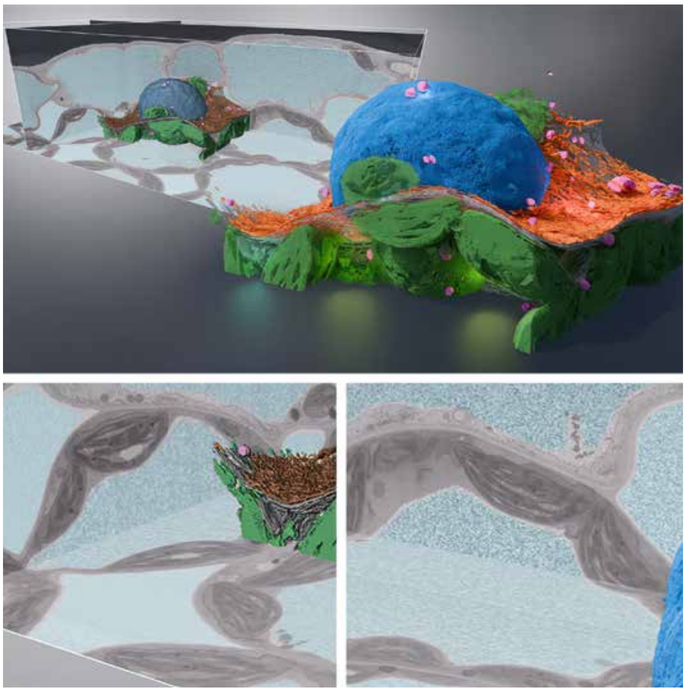

Figure 4. Volume reconstruction of a *Nicotiana benthamiana* (tobacco) epidermal cell. The dataset was acquired using the Adaptive Scanning application in Auto Slice & View Software. Areas marked with light blue were scanned at low-resolution while regions of interest were acquired at high-resolution. Stack alignment, post-processing, and segmentation illustrating detail in the selected sub-volume were performed with Amira Software. Horizontal field of view = 61.25 µm. Sample courtesy of Tessa Burch-Smith, Kirk Czymmek and Lolita Rotkina, Donald Danforth Plant Science Center.

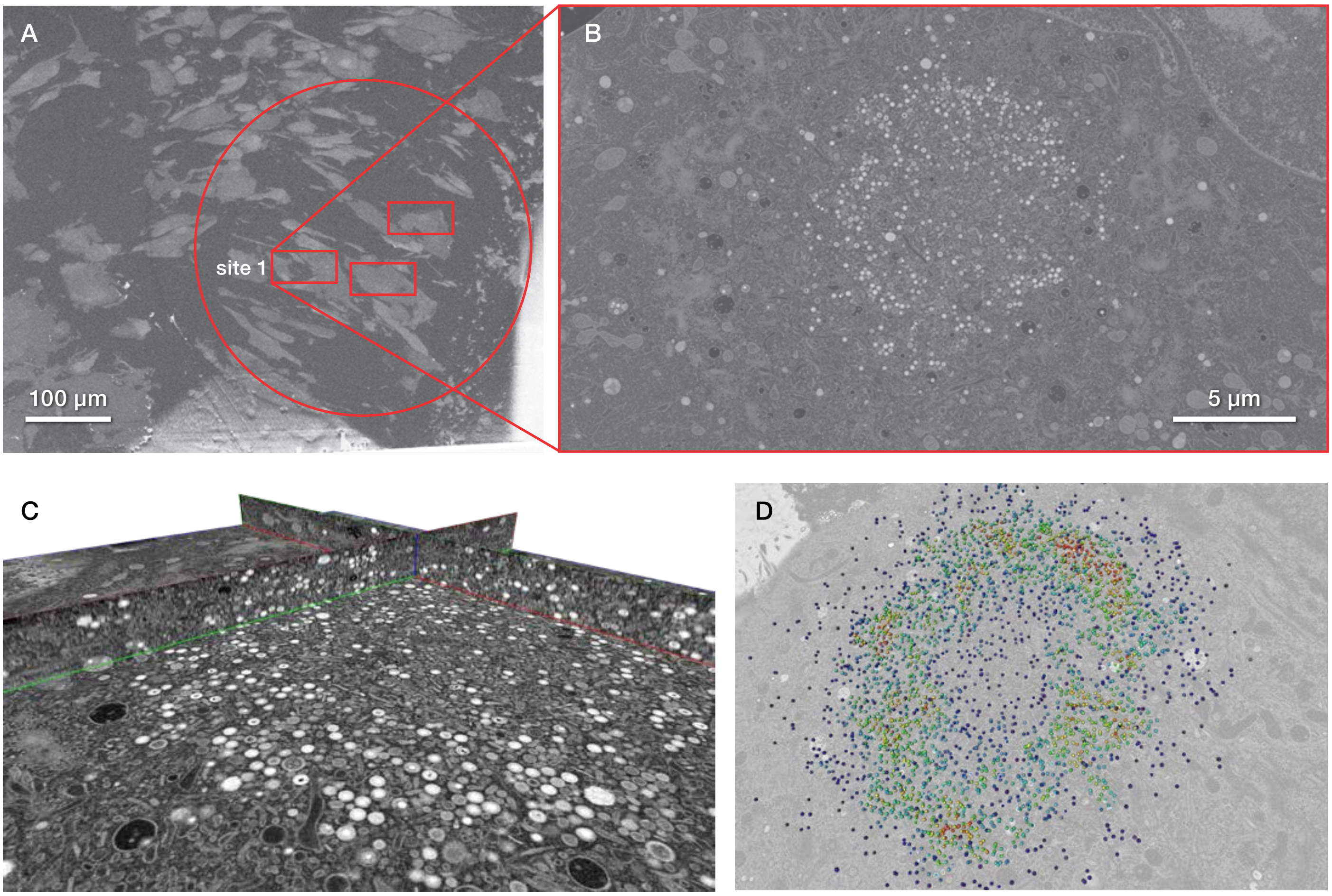

Figure 5. Fibroblast monolayer infected with human cytomegalovirus (HCMV), embedded in EPON epoxy resin. A) Top surface of the sample exposed using the Spin Mill Bio Method, allowing identification of infected cells and regions for subsequent 3D acquisition. B) Selected image from the SEM volume dataset acquired at Site 1 using Auto Slice & View Software. The image reveals a cluster of viral particles in the cytoplasm of the cell. C) Selected ortho-view slice from the volume reconstruction (Site 1). Stack alignment, denoising, and post-processing were performed with Amira Software. D) Segmentation and classification of the viral particles using AI deep-learning based segmentation in Amira Software. The size, number, and relative position of the viral particles was analyzed, and the presence of the capsid envelope was differentiated. The relative density of enveloped viral particles per volume unit is color-coded. Sample courtesy of Clarissa Read, Central Facility of Electron Microscopy at Ulm University, and Jens von Einem, Institute of Virology, Ulm University Medical Center.

Spin Mill Bio Method – millimeter-scale analysis of resin-embedded samples

The Thermo Scientific™ Spin Mill Bio Method is a unique approach for the preparation and analysis of large horizontal surface areas up to 1 mm in diameter, offering a geometry similar to microtome-based serial block-face imaging. Using the high-speed milling capability of plasma FIB, the sample surface is milled at a near-glancing angle, allowing planarization to reveal regions of interest along with layer-by-layer imaging, with slice thicknesses as small as 5 nm. On each milled layer, multiple regions can be selected and imaged. Sparse features can easily be identified, and statistically relevant 3D data can be collected from multiple areas.

Technology highlights

High-resolution imaging across a wide range of working conditions

The Hydra Bio Plasma-FIB features an ultra-high-brightness electron source with next-generation UC+ monochromator technology, giving access to nanoscale details across a wide range of working conditions. Fast, accurate, and reproducible results are obtained thanks to the unique design of the SEM column, which includes advanced auto alignments, constant power lenses for high thermal stability, and electrostatic scanning for high deflection linearity and speed.

High-throughput plasma-FIB milling of large volumes

The Hydra Bio Plasma-FIB enables efficient, large-volume analysis thanks to improved sputtering efficiency and reduced curtaining artifacts. The combination of high currents, high sputter rates, and reduced damage makes it possible to access volumes that are hundreds of micrometers in size while still observing nanoscale features. Optimize milling for each individual sample by quickly and easily switching between four available ion species (xenon, oxygen, argon, and nitrogen).

Proven automation and technologies for versatile cryo-workflows

The Hydra Bio Plasma-FIB is designed to deliver simple and dependable performance at cryogenic temperatures, enabling FIB-SEM volume EM and lamellae preparation for the cryo-electron tomography workflow.

Electron optics

- Extreme high-resolution field emission Thermo Scientific™ Elstar™ SEM Column with:
  - Magnetic immersion objective lens
  - High-stability Schottky field emission gun for stable, high-resolution analytical currents
  - UC+ monochromator technology
- Thermo Scientific SmartAlign Technology for automated alignments
- 60-degree dual objective lens with pole piece protection allows safe tilting of larger samples
- Automated, heated apertures ensures cleanliness and touch-free aperture exchange
- Electrostatic scanning for high deflection linearity and speed
- Thermo Scientific™ ConstantPower™ Lens Technology for high thermal stability
- Beam deceleration with stage bias from 0 V to -4 kV*
- Minimum source lifetime: 12 months

Electron beam resolution

- At optimum working distance (WD):
  - 0.7 nm at 1 kV
  - 1.0 nm at 500 V (ICD)
- At coincident point:
  - 0.6 nm at 15 kV
  - 1.2 nm at 1 kV

Electron beam parameter space

- Electron beam current range: 0.8 pA – 100 nA at all accelerating voltages
- Accelerating voltage range: 350 V – 30 kV
- Landing energy range: 20 eV* – 30 keV
- Maximum horizontal field width: 2.3 mm at 4 mm WD

Ion optics

- High-performance PFIB column with unique inductively coupled plasma source supporting four ion species with fast switching capability
- Ion species (primary ion beam): xenon, oxygen, argon, nitrogen
- Switching time <10 minutes, requiring only software operation
- Ion beam current range: 1.5 pA – 2.5 μA
- Accelerating voltage range: 500 V – 30 kV
- Maximum horizontal field width: 0.9 mm at beam coincidence point
- Xenon ion beam resolution at coincident point <20 nm at 30 kV (using preferred statistical method) and <10 nm at 30 kV (using selective edge method)

Detectors

- Elstar in-column SE/BSE detector (ICD)
- Everhart-Thornley SE detector (ETD)
- IR camera for viewing sample/column
- High-performance in-chamber electron and ion detector for secondary ions and electrons
- In-chamber Thermo Scientific™ Nav-Cam™ Sample Navigation Camera
- Retractable low-voltage, high-contrast directional solid-state backscatter electron detector (DBS)*

Stage and sample

CX configuration

Flexible, five-axis motorized stage

- XY range: 110 mm
- Z range: 65 mm
- Rotation: 360° (endless)
- Tilt range: -38° to +90°
- XY repeatability: 3 μm
- Max sample height: 85 mm clearance to eucentric point
- Max sample weight at 0° tilt: 5 kg (including sample holder)
- Max sample size: 110 mm with full rotation (larger samples possible with limited rotation, e.g., with the iFLM System, maximum sample size with full rotation is 3 inches)
- Compucentric rotation and tilt

UX configuration

High-precision, five-axis motorized stage with Piezo-driven XYR axis

- XY range: 150 mm
- Z range: 10 mm
- Rotation: 360° (endless)
- Tilt range: -38° to +60°
- XY repeatability: 1 μm
- Max sample height: 55 mm clearance to eucentric point
- Max sample weight at 0° tilt: 500 g (including sample holder)
- Max sample size: 150 mm with full rotation (larger samples possible with limited rotation, e.g., with the iFLM System, maximum sample size with full rotation is 3 inches)
- Compucentric rotation and tilt

Vacuum system

- Complete oil-free vacuum system
- Chamber vacuum: <2.6x10-6 mbar (after 24-hour pumping)
- Evacuation time: <5 minutes

Chamber

- Electron and ion beam coincidence point at analytical WD (4 mm SEM)
- Ports: 21
- Inside width: 379 mm
- Integrated plasma cleaner

Sample holders

- Multi-purpose specimen holder with adjustable height
- Additional holder(s) available based on configuration*

Image processor

- Dwell time range: 25 ns/pixel – 25 ms/pixel
- Up to 6144×4096 pixels
- File type: TIFF (8, 16, 24-bit), BMP, or JPEG standard
- SmartSCAN (256 frame average or integration, line integration and averaging, interlaced scanning)
- Drift-compensated frame integration (DCFI)

System control

- 64-bit GUI with Windows 10, keyboard, optical mouse
- Up to four live images showing independent beams and/or signals
- Live color signal mixing
- For local language support, check with your Thermo Fisher Scientific sales representatives
- Three 24-inch widescreen monitors (1920×1200 pixels) for system GUI and full-screen images
- Microscope control and support computers seamlessly share one keyboard, mouse, and monitors
- Multifunctional control panel (MUI)
- Remote control and imaging*

Supporting software

- Beam per view graphical user interface concept, with up to 4 simultaneous active quads
- SPI (simultaneous FIB patterning and SEM imaging), iSPI (intermittent SEM imaging and FIB patterning), iRTM (integrated real-time monitoring), and FIB immersion modes for advanced, real-time SEM and FIB process monitoring and endpointing
- Patterns supported: lines, rectangles, polygons, circles, donuts, cross-sections, and cleaning cross-sections
- Directly import BMP files or stream files for 3D milling and deposition
- Material file support for "minimum loop time," beam tuning, and independent overlaps
- Image registration for sample navigation in an imported image
- Sample navigation on an optical image

Cryo-workflow options

- EasyLift NanoManipulator for the preparation of lamellas from bulk-frozen sample
- Cryo-package includes:
  - Complete cryo-infrastructure: liquid nitrogen Dewar, cryo-loader, cryo-loading station, and cryo-transfer system
  - Fully rotatable cryo-stage
  - In-chamber micro-sputter coater (piezo-driven) for applying conductive coatings
  - Cryo-FIB consumables kit: tweezers, clipping and grid box tools, AutoGrid boxes, c-clips, and cryo-FIB AutoGrids
- iFLM Correlative System combines fluorescence, light, and electron microscopy in one system

Accessories*

- Gas injection system (GIS)
- FIB charge neutralizer
- Acoustic enclosure for pre-vacuum pump
- Electron beam deceleration

Software options*

- AutoTEM Cryo Software for automated batch milling of cryo-lamellae
- Thermo Scientific™ AutoScript™ Software, an advanced automation suite for Thermo Scientific FIB-SEMs
- Thermo Scientific Maps Software for automatic acquisition of large images and optional correlative work
- Auto Slice & View Software for automated, sequential milling and viewing of serial image slices for 3D reconstruction
- Spin Mill Bio Method for planner milling of large areas
- Adaptive scanning
- Amira Software for 3D reconstruction and analysis

Warranty and training

- 1 year warranty
- Choice of service maintenance contracts
- Choice of operation/application training contracts

Documentation and support

- Online user guidance
- User operation manual
- Prepared for RAPID support (remote diagnostics)

* Optional

Learn more at **thermofisher.com/HydraBio**