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 lamella preparation.

Cryo-volume EM: study cells and tissues without preparation artifacts

Cryo-fixation avoids the artifacts that are usually associated with classic volume EM sample preparation steps like heavy metal staining, dehydration, and resin embedding. The Hydra Bio PFIB makes it possible to view 3D volumes at cryogenic temperatures with excellent contrast and minimal charging artifacts.

The quick switching between plasma source ions makes it possible to easily combine the high speed of xenon milling with the smooth surface quality of argon milling. This is important for both efficiently accessing regions of interest (through large, xenon-milled trenches), and for the preparation of smooth serial cross-sections for high-resolution imaging.

Key features

Avoid curtaining and charging in cryo-volume EM. Acquire high-resolution 3D data through automated serial milling and imaging in Cryo Auto Slice & View Software, enabling new studies of frozen-hydrated specimens (prepared by high-pressure or plunge freezing).

Optimized milling and imaging, regardless of the sample preparation protocol. The choice of four different plasma source ions can optimize milling and imaging for both cryogenic and plastic-embedded samples.

Uncover large sample areas and visualize regions of interest with the Spin Mill Bio Method. This unique large-area planar-milling technique generates areas of similar size as microtome slicing, but at slice thicknesses as small as 5 nm. Prepare clean, smooth surfaces used to localize regions of interest and subsequently image them in 2D or 3D.

Create high-quality cryo-lamellae for the cryo-electron tomography workflow. Versatile configurations allow for lamellae to be created from cell or tissue samples using cryo-lift-out, or directly from the bulk with the fast PFIB milling

Identify, target, and confirm regions of interest on the same instrument with the CLEM workflow. Identify and navigate to targets of interest with fluorescent light microscopy without transferring to another instrument. Maps Software is available for correlation of data from external sources.

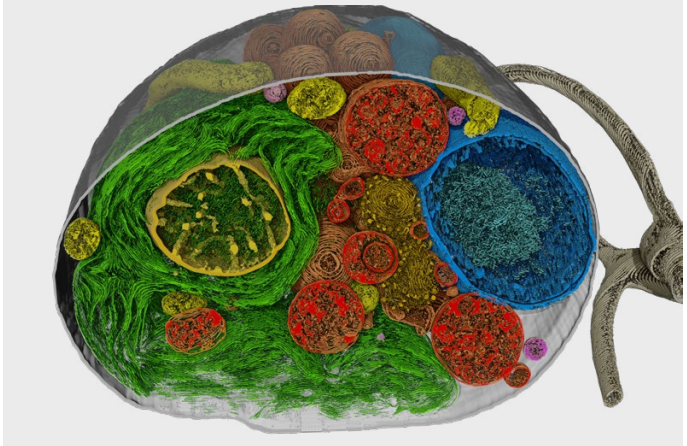


Figure 1. Hydra Bio Plasma-FIB images of unicellular algae *Chlamydomonas reinhardtii*. A) A single 2D cross section captured by Cryo Auto Slice & View Software. B) 3D volume was segmented and visualized using Amira Software.. The sample was prepared by plunge freezing, without added stains. Slice thickness is 20 nm. The 3D volume was segmented and visualized using Amira Software.

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.

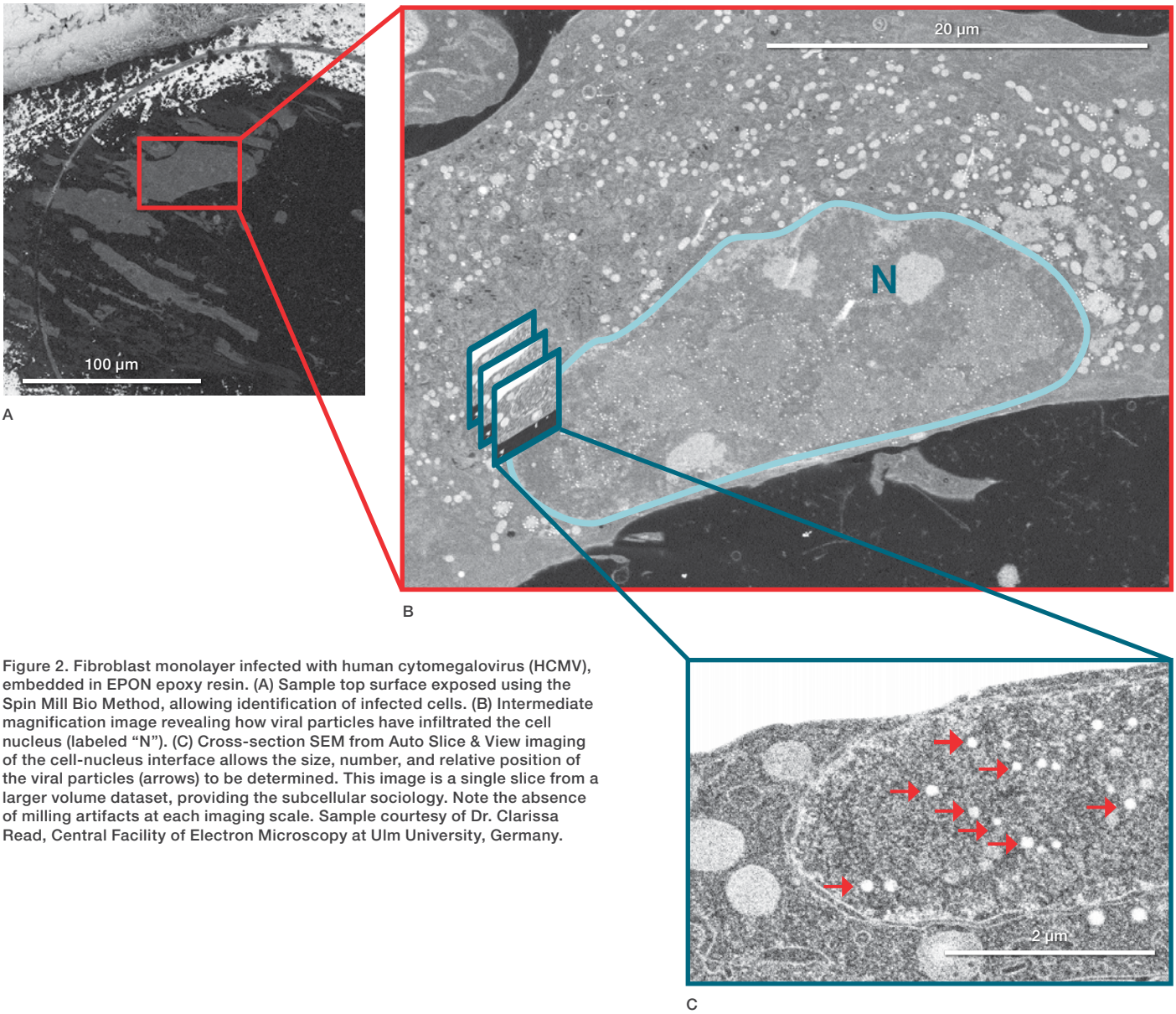


Figure 2. Fibroblast monolayer infected with human cytomegalovirus (HCMV), embedded in EPON epoxy resin. (A) Sample top surface exposed using the Spin Mill Bio Method, allowing identification of infected cells. (B) Intermediate magnification image revealing how viral particles have infiltrated the cell nucleus (labeled "N"). (C) Cross-section SEM from Auto Slice & View imaging of the cell-nucleus interface allows the size, number, and relative position of the viral particles (arrows) to be determined. This image is a single slice from a larger volume dataset, providing the subcellular sociology. Note the absence of milling artifacts at each imaging scale. Sample courtesy of Dr. Clarissa Read, Central Facility of Electron Microscopy at Ulm University, Germany.

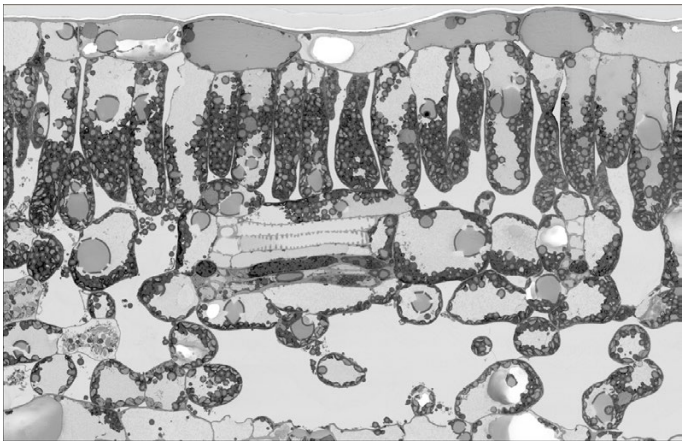


Figure 3. Cross section of a tobacco leaf, stained and embedded in Quetol resin. Sample courtesy of Kirk Czymmek, Donald Danforth Plant Science Center, USA

Large-volume FIB-SEM serial sectioning, compatible with all common sample-preparation protocols

The high-current capability of the plasma-FIB allows large sample volumes to be investigated with high throughput. The availability of four plasma source ions (Xe, O, Ar, and N) ensures compatibility with all 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, and for top-down and cross-section analysis in 2D/3D.

For example, O+ plasma-FIB 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 materials such as LR-White, HM20, EPON, or Quetol resins. Furthermore, O+ mitigates charging at the resin surface, further improving image quality.

Array tomography: automatic and precise imaging of serial sections

Array tomography provides rapid localization of cells and their interaction partners within tissues. With its large imaging field of up to 3 x 2 mm, array tomography is particularly well-suited for tissue histology.

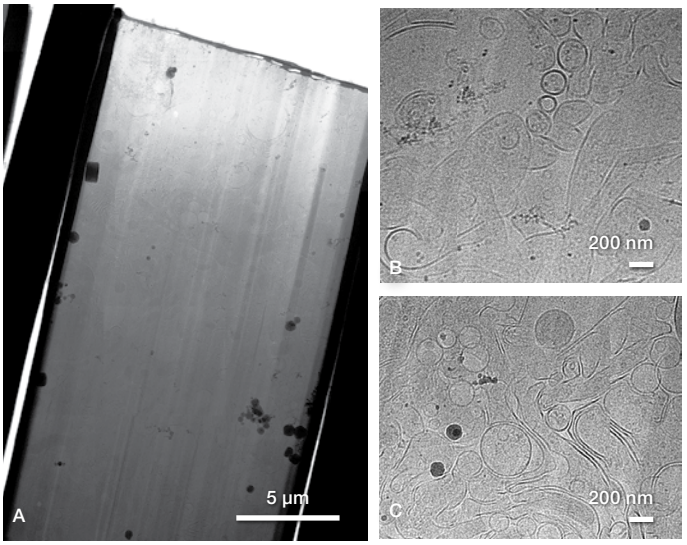
The Hydra Bio Plasma-FIB can be equipped with automated Thermo Scientific Maps Software for Array Tomography, a specialized version of Maps Software dedicated to this technique. Maps Software enables the recording of nanometer-resolution datasets from plastic-embedded tissue sections that have been laid out in series on supports. The array tomography workflow is compatible with a wide range of preparation methods, including ribbons of sections and sections collected on tape. The workflow is highly automated, so that imaging across the set of serial sections is extremely fast and easy.

Automated preparation of lamellae for cryo-electron tomography

The Hydra Bio Plasma-FIB supports automated preparation of cryo-electron tomography lamellae with a thickness of 150 nm or less, while avoiding the gallium-ion implantation of traditional Ga-FIB.

Thermo Scientific™ AutoTEM™ Cryo Software facilitates automated batch milling of cryo-lamellae, increasing productivity. The guided workflow allows selection of multiple points of interest and then autonomously prepare several lamellae in unattended runs.

Additionally, the Thermo Scientific™ EasyLift™ Cryo Lift-Out System 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-TEM for data collection.



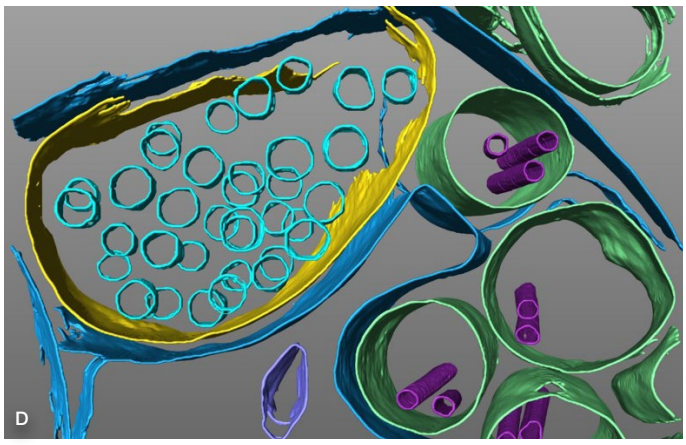


Figure 4. FIB-milled mouse brain tissue. A) TEM overview of a lamella. B–C) Higher magnification TEM images showing cellular features. D) The tomogram shows compartments filled with synaptic vesicles (cyan), microtubes (magenta), as well as several membranes (blue, green, and yellow). This work is part of a collaboration with Prof. Radu Aricescu, Laboratory of Molecular Biology, Cambridge, UK.

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 higher thermal stability, and electrostatic scanning for higher 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 the milling process for each individual sample by quickly and easily switching between four available ion species (Xe, O, Ar, and N).

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. Some of its key features include:

- A fully integrated, 360° rotation cryo-stage that maintains sample temperature below -170°C
- A loading system with dedicated shuttles that ensures samples remain vitreous during transfer in and out of the microscope
- The iFLM Correlative System and Maps Software, which combine fluorescence and reflection imaging, electron imaging, and ion milling for targeted lamella production as well as cryogenic/room temperature correlative workflows
- Dedicated AutoTEM Cryo Software that ensures fully automated, consistent, high-quality lamella preparation for the cryo-electron tomography workflow
- An integrated micro-sputter coater for deposition of thin conductive layers of platinum
- The EasyLift Cryo Lift-Out System, which supports lamella preparation “on the grid” and from bulk high-pressure-frozen (HPF) samples

Technical highlights

- Multi-ion plasma focused ion beam with 4 ion species: xenon, oxygen, argon, and nitrogen
- Ultra-high-resolution (UHR) monochromated field-emission gun scanning electron microscope (FEG-SEM) with immersion lens
- In-chamber, in-lens, and in-column secondary electron/backscattered electron (SE/BSE) detectors and (optional) directional backscatter (DBS) detector
- Manual user interface; the control console allows for direct beam adjustments
- 3 x 24’’ widescreen LCD monitor (with triple monitor stand)
- Oil-free pumping system

Configurable workflow options

- Eucentric stage: 110 x 110 mm (CX) or 150 x 150 mm piezo (UX)
- Cryo Package
- EasyLift Cryo Lift-Out System
- iFLM Correlative System (integrated fluorescent light microscope) for targeting and correlative light and electron microscopy (CLEM)
- GIS (gas injection system) for deposition of protective platinum layers on sample surfaces at both cryogenic and room temperatures in order to prevent milling artifacts
- Integrated Micro-Sputter Coater for ion-beam deposition of metallic platinum for increased conductivity
- Directional backscatter (DBS) detector
- Sample holders for cryogenic and room temperature workflows

Automation and data processing options

- Thermo Scientific™ Auto Slice & View™ Software
- Spin Mill Bio Method
- AutoTEM Cryo Software
- Thermo Scientific™ Autoscript™ Software
- Maps Software for Correlative Workflows
- Maps Software for Array Tomography
- Thermo Scientific™ Amira™ Software with various license options

Accessories and training

A range of additional accessories are available, including specialized sample holders and shuttles. Training is also available in a variety of formats and locations. Please enquire with your local account team for further details.

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