

# Aquilos Cryo-FIB

## A dedicated cryo-FIB microscope to prepare cryo-lamellas for cryo-electron tomography

The Thermo Scientific Aquilos Cryo-FIB is a cryo-dedicated DualBeam system that delivers optimal sample preparation for high-end cryo-TEM tomography.

### Cryo-EM dedication

The Thermo Scientific™ Aquilos™ Cryo-Focused Ion Beam (Cryo-FIB) allows users to actively gain control over their sample thickness and prepare thin *in situ* cryo-lamellas from cellular specimens grown on EM specimen supports. The microscope has an integrated, fully rotatable cryo-stage and adjacent cryo-hardware that protects the frozen-hydrated samples from contamination, ensuring that delicate life science cryo-samples will be kept at vitrified temperatures.

### Connectivity to autoloader TEMs

The Aquilos Cryo-FIB is specifically designed to enable the cryo-tomography workflow. This workflow comprises a plunge-freezing Thermo Scientific Vitrobot™ device, correlative Thermo Scientific Maps™ software to import cryo-light microscopy data for targeting, calculation of milling positions, and subsequent transfer to autoloader cryo-transmission electron microscope (cryo-TEM) systems such as the Thermo Scientific Krios™ cryo-TEM.

### Prevention of sample charging

A retractable sputter coater is integrated within the Thermo Scientific DualBeam™ system chamber to allow for deposition of a nanometer-thin inorganic Platinum layer onto the milled cryo-lamellas. The integrated device ensures efficient coating operations and the vitrified sample does not need to be transferred to an external sputter coating device. The coating renders the cryo-lamellas conductive and prevents charge-up during cryo-tomography. In particular, charging can obstruct tomographic image acquisition when using a Volta phase plate. All sputter coater controls are embedded in the cryo-dedicated Aquilos user software.

### Correlation over different imaging modalities

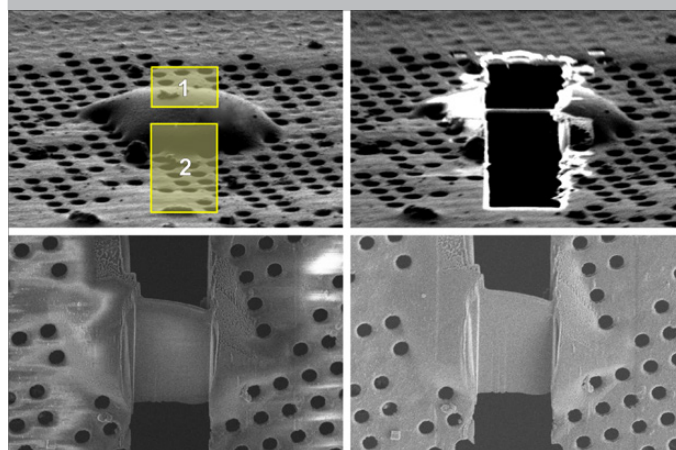
The Aquilos Cryo-FIB utilizes Maps software to enable correlative overlay of cryo-light microscopic data with cryo-FIB and cryo-TEM data. Maps software facilitates correlative identification of features of interest and subsequent

### Key Benefits

**Produce cryo-lamellas for cryo-tomography using autoloader TEM systems.** Cryo-dedicated hardware inside the chamber ensures minimal sample contamination, sample damage and loss of correlation accuracy.

**Create thinner cryo-lamella without cutting artifacts.** Ion beam milling enables the creation of compression-free cryo-lamella samples for TEM tomography imaging. The method avoids artifacts such as mechanical compression inevitable for mechanical cryo-sectioning, using a cryo-ultramicrotome.

**Improve sample preparation precision.** Using the guided user software and Maps correlation software makes the Aquilos Cryo-FIB easy to use, even for inexperienced users.



targeting by cryo-FIB milling. The software can import over 120 different image formats into an active project, thereby allowing light microscopic data to be further investigated using a Focused Ion Beam/Scanning Electron Microscope (FIB/SEM) or TEM.

### Flexibility and ease of use

The Aquilos Cryo-FIB supports the user in the preparation of cryo-lamellas by providing dedicated software guidance and easy-to-use setup and alignment procedures. This support enables users to quickly familiarize themselves with the system and get up to speed in cryo-sample preparation and lamella milling.

### Aquilos Cryo-FIB comes with these key features:

- Complete cryo infrastructure comprising: large capacity liquid nitrogen Dewar for extended runtimes, heat exchanger, flow controller, load-lock system, sample preparation station, controller and transfer device
- Fully rotatable cryo-stage (<-170°C) keeping samples at vitreous temperatures
- Special sample holders customized for shallow-angle milling of EM grids: referred to as Cryo-FIB Autogrids (autoloader approved)
- Sample shuttle for Autogrids: Cryo-FIB shuttle with integrated shutter system during cryo-transfers
- In-chamber retractable sputter coater for applying conductive coatings
- GIS-system for applying protective coatings
- Includes cryo-FIB consumables kit: comprising of tweezers, clipping and grid box tools, Autogrid boxes, C-Clips and Cryo-FIB Autogrids
- MAPS Software including image correlation and lamella preparation functions
- Seamless compatibility and connectivity within our cryo-tomography workflow

### The Aquilos core instrument includes:

- NICol UHR non-immersion field emission-SEM column
- In-Lens Detection System: Segmented lower (T1) and upper detector (T2)
- Everhart-Thornley SE Detector (ETD)
- Sidewinder ion column
- Workstation with Windows® 7 + 2 x 24 inch LCD monitors
- Support Computer with Windows® 7 + 1 x 24 inch LCD monitor
- Large table top with support
- xT software
- 110 × 110 mm eucentric stage
- IR camera for viewing sample and chamber
- In-chamber Thermo Scientific Nav-Cam™ sample navigation camera
- Oil-free pumping system
- Integrated beam current measurement
- Automatic aperture system



### The Cryo package includes:

- Rotatable cryo-stage (Cooldown time: <30 min)
- Cryo-preparation station (including autogrid clipping insert)
- Controller for preparation station
- Sample transfer device (transfer rod)
- Cryo-loader (SEM load lock for transfer rod)
- 2 Cryo-FIB shuttles
- Standard Shuttle (for SEM alignment specimens and pin mount stubs)
- Large capacity liquid nitrogen Dewar (runtime: >10 hrs.)
- Nitrogen gas flow controller
- Heat exchanger (delivering cooling gas)
- Extra rotary pump for heat exchanger
- Dry diaphragm pump for cryo-preparation station
- Cryo-consumables kit with 20 Autogrid boxes, 100 Cryo-FIB Autogrids, 100 C-Clips, 2 clipping tools, 2 Autogrid tweezers, 2 grid box openers, 2 soft grip tweezers, forceps, crossover and stub tweezers
- Maps correlative workflow software for SEM
- Pt Deposition gas injection system (GIS) for deposition of protective layers
- In-chamber and automated retractable sputter coater (target: platinum) for deposition of conductive layers
- Sample bake-out box for cleaning the shuttles and samples
- Safety wear kit for maintenance
- Temperature logger
- Acoustic enclosure for heat exchanger rotary pump

## Accessories

- High-performance ion conversion and electron (ICE) detector for secondary ions (SI) and electrons (SE)
- Seismic restraint kit
- Acoustic enclosure for microscope scroll pump
- Additional preparation station
- Remote control and imaging
- Additional cryo-consumables and accessories including cryo-FIB Autogrids and hot plate

## Warranty and training

- 1-year warranty
- Choice of service maintenance contracts
- Choice of operation / application training contracts
- Standard Aquilos Cryo-FIB training for tool users
- Optional advanced DualBeam training course
- Customer witness acceptance test (CWAT)

## Documentation and support

- Online user guidance
- User operation manual
- Prepared for Thermo Scientific RAPID™ remote diagnostic support
- Free access to online resources

## Technical specifications

### Electron Optics

- High-stability Schottky field emission gun
- Minimum source lifetime: 12 months
- Easy gun installation and maintenance: auto bakeout, auto start and no mechanical alignments
- Continuous beam current control and optimized aperture angle
- Double stage scanning deflection
- Dual objective lens, combining electromagnetic and electrostatic lenses
- User guidance and column presets
- Beam current range: 1.5pA to 400 nA
- Accelerating voltage range: 200V - 30 kV
- Resolution (with cryo-stage): 1.6 nm at 30 kV, 2.6 nm at 2 kV (at room temperature), 6.0 nm\* at 2kV (at cryo-temperature)

### Ion Optics

- Source lifetime: 1,300 hrs
- Voltage: 500 V to 30 kV
- Beam current: 1.5 pA – 65 nA in 15 steps
- Drift suppression mode as standard for non-conductive samples
- Resolution (with cryo-stage): 7.0 nm\* at 30 kV

\*Cryo-imaging conditions subject to gas flow rates used

### Vacuum system

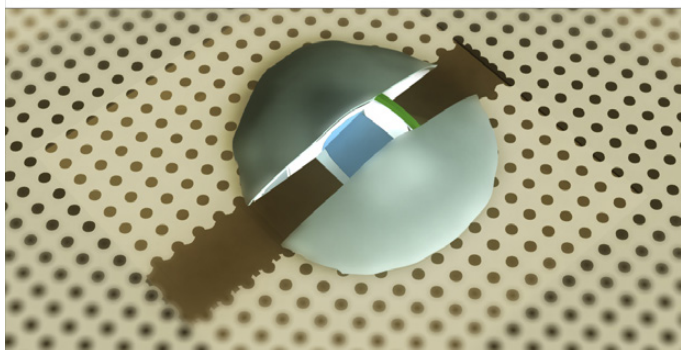
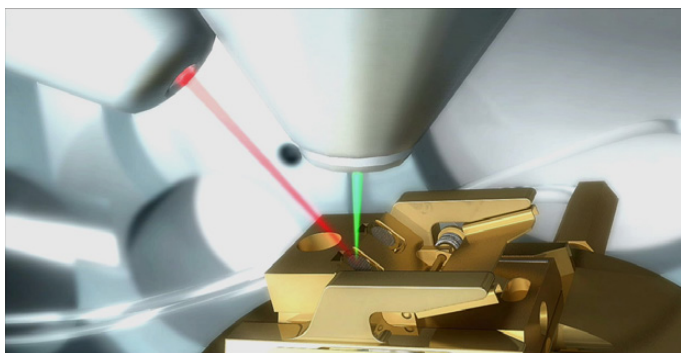
- Complete oil-free vacuum system in the core microscope
- 1x TMP with turbo drag section, 240 l/s
- 1x Scroll pump
- 3x IGP, 25 l/s
- Chamber vacuum at room temperature: <4e-4Pa
- Chamber vacuum at cryo-conditions: <8e-5Pa

### Cryo-stage

- Integrated rotatable cryo stage
- Rotation: 360° (endless)
- Compucentric rotation and tilt
- Cooldown time: <30 min
- XY range: 110 mm
- Z range: 65 mm
- Tilt range at cryo (eucentric WD): -15° to +55°

### Image processor

- Dwell time range from 25 ns – 25 ms/pixel
- Up to 6144 × 4096 pixels (up to 64k through Maps Software)
- File type: TIFF (8, 16, 24-bit), BMP or JPEG standard
- Electronic scanning rotation: 360° degrees
- Thermo Scientific SmartSCAN™ System (256 frame average or integration, line integration and averaging, interlaced scanning)
- DCFI (Drift Compensated Frame Integration)



## System control

- 64-bit GUI with Windows 7, keyboard, optical mouse
- Up to four live images showing independent beams and/or signals. Live color signal mixing
- Local language support: Check with your local Thermo Fisher Scientific sales representatives for available language packs
- 3 × 24-inch widescreen monitors with 1920 × 1200 pixels
- Multifunctional control panel (MUI)

## Supporting Software

- Maps 3 software for tiling and stitching (import of 120+ image data formats, up to 64k × 64k), correlation of LM and SEM data (software assists in computation of eucentric position for milling and fast retrieval of ROIs)
- XT software with dedicated cryo-controls

- “Beam per view” graphical user interface concept, with up to 4 simultaneously active quads
- Thermo Scientific SPI™ software (simultaneous FIB patterning and SEM imaging)
- Thermo Scientific iSPI™ software (intermittent SEM imaging and FIB patterning)
- Thermo Scientific iRTM™ software (integrated real time monitor) and FIB immersion modes for advanced, real-time SEM and FIB process monitoring and endpointing
- Patterns supported: rectangle, line, circle, cleaning, cross-section, regular cross-section, polygon, bitmap, stream file, exclusion zones, arrays
- Sample navigation on an optical image
- Undo / Redo functionality
- User Guidance for most common DualBeam system operations and applications



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