Krios 5 Cryo-TEM

High data fidelity: fast, high-resolution structure determination of proteins and their cellular context

Cryo-electron microscopy (cryo-EM) is enabling a new era in structural biology. Single particle analysis (SPA) provides atomic resolution insights into the functions of proteins. Cryo-electron tomography (cryo-ET), meanwhile, reveals these protein interactions in their cellular context.

Designed for high-performance SPA and cryo-ET, the Thermo Scientific™ Krios™ 5 Cryo-Transmission Electron Microscope (Cryo-TEM) is capable of quickly producing high-quality structures thanks to a unique focus on data fidelity. Increased automation of daily alignments, improved optical precision, and advanced applications software make the Krios 5 Cryo-TEM a highly productive and "ready-to-use" system for all expertise levels across multiple scientific fields.

Paired with Thermo Scientific Tomography 5 Software, the Krios 5 Cryo-TEM can easily access signal beyond the Nyquist frequency of the Thermo Scientific™ Falcon™ 4i Direct Electron Detector, enabling higher resolution structures or larger fields of view. Addition of the new Thermo Scientific Vacuum Capsule, developed specifically for the cryo-ET workflow, allows robust, contamination-free transfer of cryo-lamellae to the Krios 5 Cryo-TEM after focused ion beam (FIB) milling with the Thermo Scientific™ Arctis™ Cryo Plasma-FIB.

Designed with sustainability in mind, the Krios 5 Cryo-TEM has the ability to reduce energy consumption while it is not in use. Additionally, the instrument's ACT (Accountability, Consistency, and Transparency) Ecolabel unambiguously documents its environmental footprint, including manufacturing practices, energy and water use, and end-of-life disposal. ACT Ecolabels are used to detail the environmental impact of laboratory products and equipment and help you to make sustainable purchasing decisions.

Key features
High data fidelity, with magnification calibration accuracy within 1% as well as automated daily alignments
Accelerated single particle and cryo-tomography workflows with software-powered setup, multigrid data collection, and Discovery Viewer integrated within CryoFlow Software
Tomography 5 Software provides robust, on-the-fly motion correction, automatically capturing data beyond the Nyquist frequency for crisp tomograms and the high image quality required for sub-tomogram averaging
Vacuum capsule provides robust and contamination-free transfer from the Arctis Cryo-Plasma FIB
Designed-in sustainability provides energy-saving Eco modes with fast "wake up" scheduling as well as traceability of the instrument's environmental footprint through an ACT Ecolabel

“Always-right” electron optics ensure reliable, high-quality data

With an increased demand for rapid, high-quality data, instrument productivity and precision are more important than ever. For routine sub-micrometer cryo-tomography and atomic-resolution single particle analysis, this corresponds to high optical and alignment stability, so that the focus can remain on the science rather than on tuning the instrument.

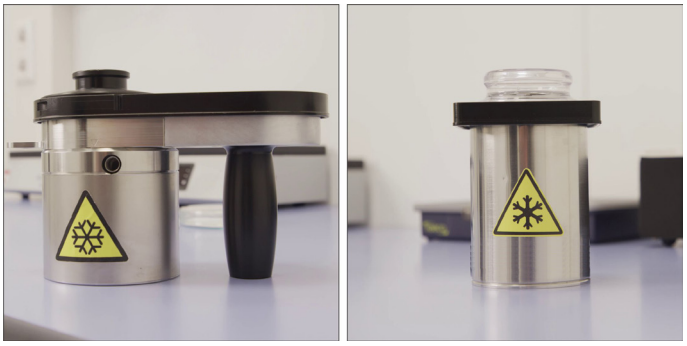
The Krios 5 Cryo-TEM features a precise magnification calibration accuracy within <1% for the selected area (SA) aperture and high magnification (Mh) range, which is most relevant for high-resolution data acquisition. This results in easier and more accurate data processing as well as simplified data analysis of multiple combined datasets.

“Daily” alignments, like centering of the beam and apertures, as well as coma and astigmatism correction, are also fully automated to ensure a streamlined acquisition experience.

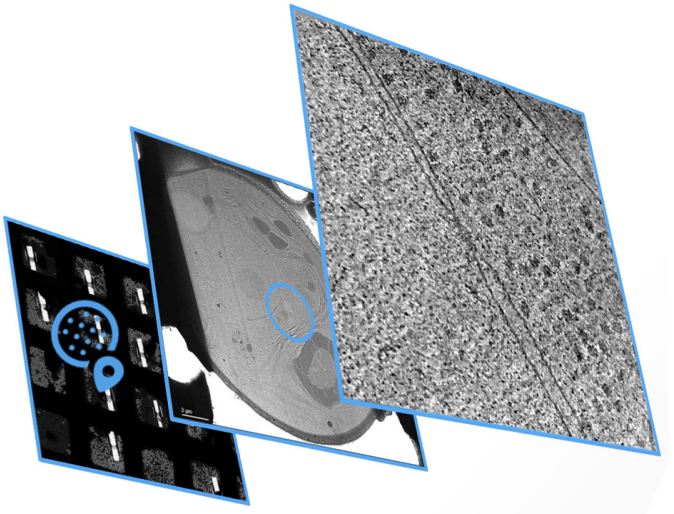
To further enhance imaging throughput, the range of aberration-free image shift (AFIS) has been extended to 20 µm, enabling the acquisition of large datasets with fewer mechanical stage movements.

High workflow efficiency for cryo-electron tomography and single particle analysis

All labs providing instrument access for researchers want to quickly generate relevant scientific results, so instruments must be optimally connected. For this reason, the Krios 5 Cryo-TEM supports sample transfer using the newly introduced Vacuum Capsule, which acts as a shuttle between the Arctis Cryo-PFIB and Krios 5 Cryo-TEM. It ensures a robust contamination-free environment for the transfer of FIB-prepared cryo-lamellae. The vacuum within the capsule eliminates ice formation, thereby preserving the integrity of the sample and enabling faster, more accurate, and higher-quality data acquisition.



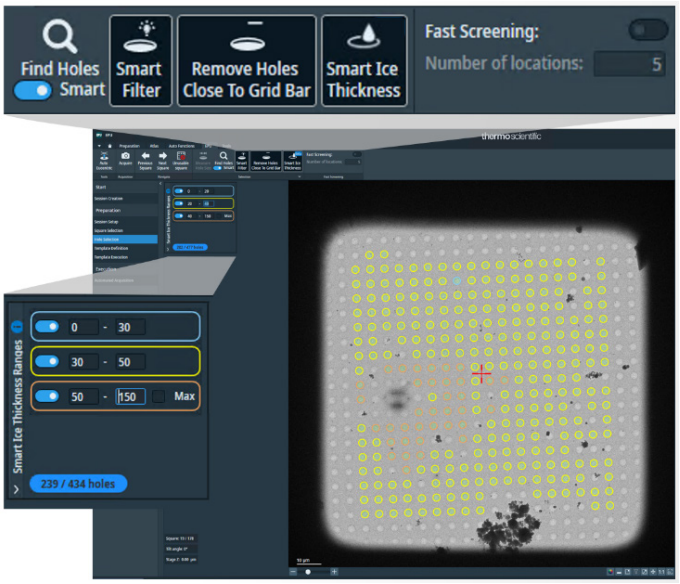
The Vacuum Capsule provides contamination-free transfer of prepared cryo-lamellae.



The Tomo Discovery Viewer allows you to navigate between associated atlases, search maps, and 3D-reconstructions. Access data quality metrics from trackable tilt-series and receive guidance for further data acquisition in collaborative work.

Additionally, the efficiency of the tomography data acquisition has been significantly enhanced through Tomography 5 Software with Multigrid Software along with task parallelization. Tomo Multigrid Software allows acquisitions to be set up across multiple grids in the Autoloader cassette for long (overnight) data collection sessions, similar to EPU Multigrid Software for single particle analysis. Thanks to improved parallelization routines, grid atlases can be collected while new acquisition schemes are set up, increasing overall productivity. Thermo Scientific™ CryoFlow™ Software features the Discovery Viewer which allows for easy offline navigation and analysis of large datasets without occupying the microscope.

For single particle analysis, the screening process is enhanced with new, AI-based Smart EPU plugins. Smart grid square selection, ice thickness estimation, and the ability to find foil holes allow high-quality data to be acquired efficiently and conveniently.



Smart plugins in EPU Software can be applied consecutively to the same grid square image to automatically identify foil holes, curate the initial hole selection, and classify the holes by their predicted thickness.

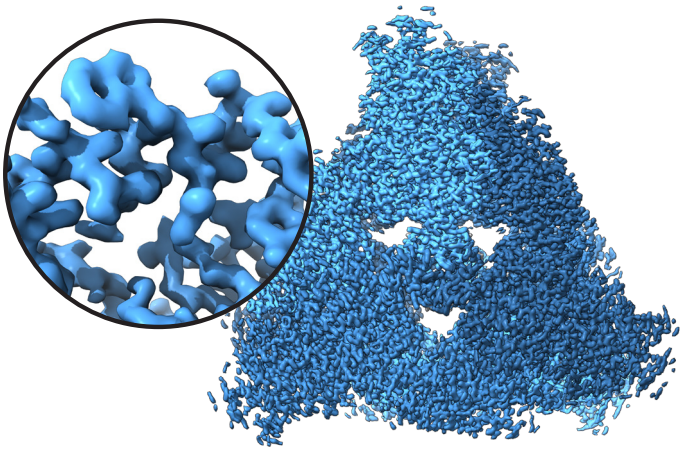
Enhancing productivity with the Falcon Direct Electron Detector

Direct electron detectors boost the productivity and performance of cryo-EM, enabling observations down to atomic resolution by combining the Thermo Scientific™ Selectris™ X Imaging Filter with the Falcon 4i Direct Electron Detector.

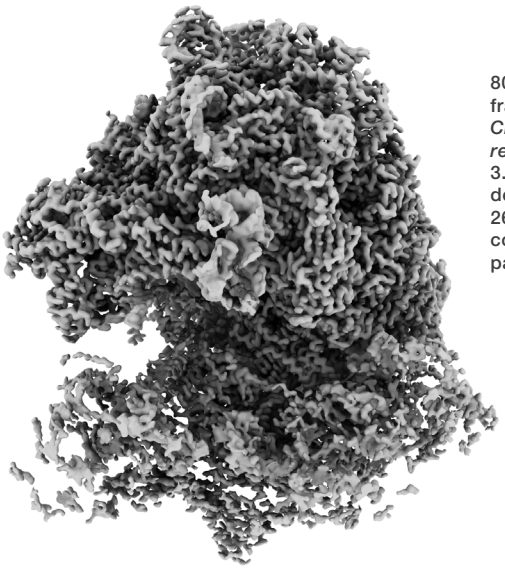
With its internal frame rate of 320 fps and low camera overhead times (0.5 s), the Falcon 4i enhances productivity by providing both high image quality and throughput.

Compensation for the increased drift induced by tilting is a key factor for image quality in tomography. Efficient, robust, on-the-fly motion correction is available through Tomography 5 Software. Optimized motion correction with fine fractionation, enabled by the proprietary Electron Event Representation (EER) image format, helps recover tomograms with relatively large amounts of drift, leading to fewer tomograms being discarded due to blurring. For tomography experiments with large fields of view, low magnification and large pixel size can be limiting for sub-tomogram averaging. The precise sub-pixel localization (DQE at 1.5Nq = 0.27) of the Falcon 4i Direct Electron Detector, as well as optional 8k x 8k image output, removes this limitation, as it allows information to be recovered beyond the physical Nyquist frequency.

Finally, designed-in configuration management, acquisition tracking, and calibration/validation ensure that the handedness of tomography structures is correctly represented via the metadata, making it more reliable.



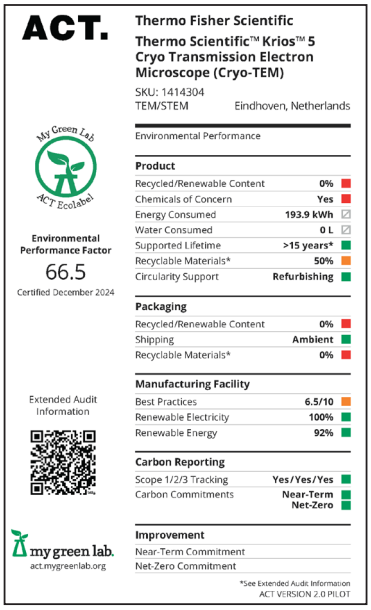
SPA reconstruction of the 190 kDa L-arabinose isomerase (LAI protein) at 1.52Å. The high-resolution cryo-EM map shows clearly resolved side chain densities. Data was acquired with a Krios 5 Cryo-TEM utilizing the Selectris Energy Filter and Falcon 4i Detector, as well as Smart EPU Software. Reconstruction was performed in CryoSPARC Live Software. Sample courtesy of BRIN Indonesia.



80S ribosome fragment from *Chlamydomonas reinhardtii* at 3.0 Å resolution, determined from 262 tomograms containing 130,000 particles.

Designed-in sustainability

Created with a focus on sustainability, the new, low-power Eco modes of the Krios 5 Cryo-TEM can be used to deactivate specific parts of the microscope when the instrument is not in use. This reduces power consumption by up to 80%, decreasing the instrument's carbon footprint and lowering running costs. Upon activating a low-power Eco mode, the microscope's LED lighting indicator turns green, clearly showing that the system is in an energy-saving idle mode.



Preview of the ACT Ecolabel for the Krios 5 Cryo-TEM.

Reactivation of the microscope can happen in less than 1 hour from an overnight stand-by mode, whereas a longer power down (i.e., sleep mode) requires up to 12 hours of recovery time. The microscope software can be programmed so that wake-up is initiated early and the system is ready to use as needed. The Krios 5 Cryo-TEM is also one of the first microscopes with an ACT Ecolabel that unambiguously documents the environmental footprint of the instrument, as assessed by My Green Lab, an independent non-profit organization. The label documents the instrument's environmental impact regarding manufacturing practices, energy and water use, and end-of-life disposal.

Technical highlights

Source

- Low-energy-spread cold field-emission gun (E-CFEG) (<0.3 eV)

Accelerating voltage

- 80–300 kV

Cryo-autoloader

- Automated and contamination-free loading of cassettes (up to 12 grids)
- Compatible with Vacuum Capsule for contamination-free transfer of grids

Temperature management software

- Features liquid nitrogen autofill and scheduling of cool down after cryo-cycles

Lenses

- Automatic condenser, objective, and SA apertures
- Three-condenser-lens system for automated, continuous, and parallel sample illumination
- Symmetric constant power C-TWIN objective lens with wide-gap pole piece (11 mm)
- Constant power lens design minimizes lens hysteresis and image aberrations during mode switching between imaging modes and diffraction

Stage

- Computerized 4-axis specimen stage with ±70-degree alpha tilt
- Cryo-stage with single axis holder for optimized stability and drift performance

Imaging

- Rotation-free imaging with changing magnification

Advanced performance monitoring

- Highly precise magnification calibration over the full SA range (<1%)
- Self-assessment of optical microscope status, combined with automated alignments, ensures ideal experimental conditions
- Automated user alignments including Center Beam, Center C2 Aperture, and Falcon 4i Detector gain reference collection through Microscope Companion Software
- Optimized optics including the center objective aperture, AutoComa, AutoStigmate, and filter tuning in Thermo Scientific EPU and Tomography Software

Room size requirements

- 17' × 22' × 10' (L × W × H)

AFIS (aberration-free image shift)

- Enhanced throughput with short relaxation times when moving coma-free between grid holes
- Extended AFIS range (up to 20 µm) allows for quick data collection without compromising quality

FFI (fringe-free imaging)

- Enhanced throughput with multiple image acquisitions per grid hole

Thermo Scientific EPU 3 Software

- Automated sample screening and data acquisition
- EPU Multigrid functionality

Additional components

- Three 24” monitors
- Hand panels to be placed within 15 meters of the column; can be extended up to 300 meters from the column (optional)
- Windows 11 Operating System

Energy savings mode

- Eco mode designed for sustainability, including scheduling capabilities for rapid wake up

Detectors (optional)

- Falcon 4i Direct Electron Detector
- EPU-D Camera package for microcrystal electron diffraction (MicroED)
- Thermo Scientific™ Ceta™ D Camera
- Thermo Scientific™ Ceta™ 16M Camera
- HAADF STEM detectors
- On-axis Thermo Scientific Panther BF/DF STEM Detectors

Energy filter (optional)

- Thermo Scientific™ Selectris™ X Energy Filter
- Thermo Scientific™ Selectris™ Energy Filter
- Gatan BioContinuum Energy Filter

Software (optional)

- Thermo Scientific Smart EPU Software with all available AI plugins
- Thermo Scientific Smart EPU Software with Embedded CryoSPARC Live
- Thermo Scientific Tomography 5 Software and Thermo Scientific™ Tomo Live™ Software
- CryoFlow Software for data management

Other options

- 60°C heat decontamination solution for installation in higher biosafety-level containment facilities (e.g., BSL-3)
- Cs Image Corrector
- Thermo Scientific Phase Plate Solution
- Accelerate and Advance Integrated Service and Applications Support Packages for Drug Discovery, Single Particle Analysis, and Cryo-Tomography

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

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