

# Glacios 2 Cryo-TEM with Smart EPU Software and Falcon 4i Direct Electron Detector

## High-resolution, high-throughput macromolecular structure determination

Cryo-electron microscopy is revolutionizing the field of structural biology. Using powerful tools like the new Thermo Scientific™ Glacios™ 2 Cryo-Transmission Electron Microscope (Cryo-TEM), you can work at near-native conditions and high resolutions.

Compared to the previous generation, the Glacios 2 Cryo-TEM delivers higher throughput and makes cryo-EM more accessible. In addition to an optional integrated Thermo Scientific™ Falcon™ 4i Direct Electron Detector, it features Thermo Scientific EPU Software, fringe-free imaging (FFI), improved optical specifications, and a new full enclosure, which combine to enhance image quality and system productivity, automate data acquisition, and simplify your work.

With superior productivity, performance, automation, and end-to-end support, the Glacios 2 Cryo-TEM offers structural biology labs a complete package for studying protein structures and delivers unique insights that cannot be achieved with any other technique. It also supports multiple applications, including single-particle analysis (SPA), cryo-electron tomography (cryo-ET), and micro-electron diffraction (MicroED), without any compromise in performance.

### Create images at near-atomic resolution

The Glacios 2 Cryo-TEM provides near atomic resolution and best-in-class productivity. It comes with a new enclosure that minimizes environmental influences. The information limit of Glacios 2 Cryo-TEM has been improved to 2.1 Å. Similarly, transmission loss due to ice growth has been reduced to 2% over 24 hours, helping you collect more high-resolution images with less effort. And you can further enhance productivity and performance with an optional Thermo Scientific™ Selectris™ Imaging Filter with a Falcon 4i Direct Electron Detector.

### Key features

System can perform SPA, MicroED, and cryo-ET

Best-in-class image quality with 2.1 Å information limit supported by a newly designed enclosure; less than 2% transmission loss over 24 hours; optional Falcon 4i Detector with highest-in-class DQE

Improved drift performance allows you to start your experiments faster and the combination of fringe-free imaging and the optional Falcon 4i Detector enhances imaging throughput and data quality

Simplified workflow with guided experimental setup supports non-expert microscopists

Connectivity with other Thermo Scientific instruments eases sample transfer

Accelerate Service Portfolio provides continuous expert support

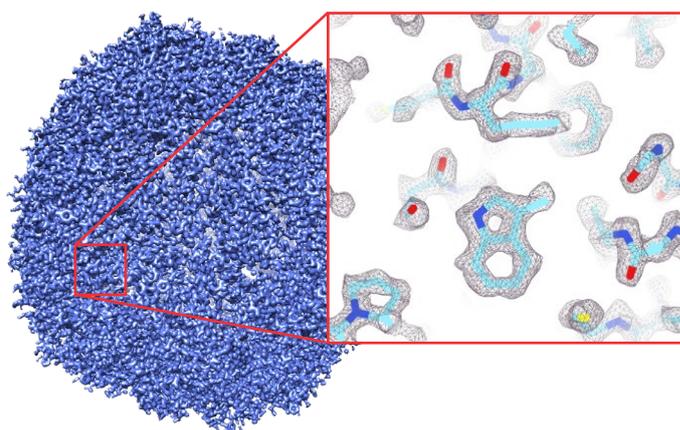


Figure 1. A reconstruction of apoferritin at 1.6 Å resolution from data collected over seven hours using the Glacios 2 Cryo-TEM with the Falcon 4i Direct Electron Detector, Selectris X Imaging Filter, and FFI.



## Take advantage of powerful software

The Glacios 2 Cryo-TEM is delivered with Smart EPU Software that improves user guidance and automates data acquisition. With the same easy-to-use interface as previous EPU Software iterations, it offers predefined and customizable imaging preferences that help you start sessions fast as well as a traffic light tool that monitors hardware subsystems and alignment status to support optimal imaging. Smart EPU also significantly reduces operator level of expertise and the amount of time needed for setting up a data collection session. Smart Filter, for example, is our first AI-driven algorithm. It deselects contaminated holes or holes close to edges or containing cracks in the ice, ensuring that only good quality data is collected. The software also features an open application programming interface (API) that allows you to develop solutions for your specific needs.

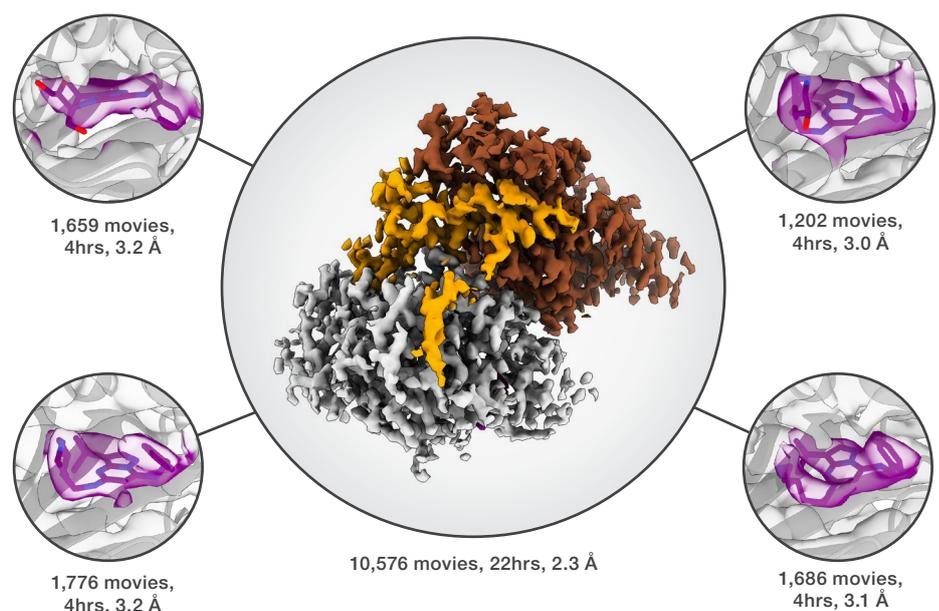
With the optional EPU Quality Monitor (EQM) or Embedded CryoSPARC Live components, Smart EPU Software can evaluate image quality in real time. These are separate components, with EQM providing motion correction and CTF estimations and Embedded CryoSPARC Live—a powerful image processing package—covering the complete pipeline of 2D and 3D image analysis and yielding high-resolution structures while still acquiring data.

Pairing Smart EPU Software with one of these monitoring options enables automated microscope adjustments on the fly via Smart Plugins, which are optimization feedback loops that increase data quality while reducing the need for manual intervention during data collection. As an example, a Smart Focus plugin assures the application of defocus in a desired range by monitoring image defocus on-the-fly and applying corrections when needed. You can easily follow the results of an ongoing session and share them via Thermo Scientific Athena Software.

The integrated Falcon 4i Direct Electron Detector provides better image quality than its predecessors in less time. To further enhance productivity and image quality, the Glacios 2 Cryo-TEM offers improved drift performance after sample insertion, which allows you to start your experiment faster, and fringe-free imaging (FFI), which eliminates Fresnel fringes at the edge of the beam. FFI implementation alone allows you to collect more images per foil hole and can accelerate SPA throughput significantly.

The combination of high productivity and image quality makes the Glacios 2 Cryo-TEM a powerful tool for studying biological samples. For example, cyclin-dependent kinases (CDKs) are a family of kinases first known for their role in controlling the cell cycle and were later found to regulate gene transcription. This led researchers to study CDK7 in complex with cyclin H and MAT (the CDK-activating kinase (CAK) complex) as a potential target for cancer treatment, which demonstrates the value of structure determination in developing therapeutics.

For this 85kDa CAK complex, the Glacios 2 Cryo-TEM with Selectris X Imaging Filter, Falcon 4i Detector, and FFI delivered a high-throughput, high-resolution screening and data collection workflow that can rapidly generate structures at approximately 3.0 to 3.2 Å resolution within four hours of data collection. The same system can be used to determine the occupancy of the drug binding pocket in as little as one to four hours of data collection with a throughput of approximately 500 movies per hour. Furthermore, overnight data collection on this sample produces a 2.3 Å structure sufficient for de-novo model building and accurate orientation of a small molecule inhibitor within the CAK complex to guide drug design and lead optimization. Those structures could then be improved to sub-2 Å resolution on an accompanying Thermo Scientific™ Krios™ Cryo-TEM.



**Figure 2.** CDK-activating kinase (CAK) complex structures solved on a Glacios 2 Cryo-TEM. Drug binding occupancy on this complex was analyzed following a fast-screening strategy of only a few hours of data collection per sample. A large dataset enabled a 2.3 Å structure in less than a day where an atomic model could be built unambiguously. This project was a collaboration with Basil Greber from Institute of Cancer Research, London, and Professors Simak Ali and Matthew J. Fuchter from Imperial College, London.

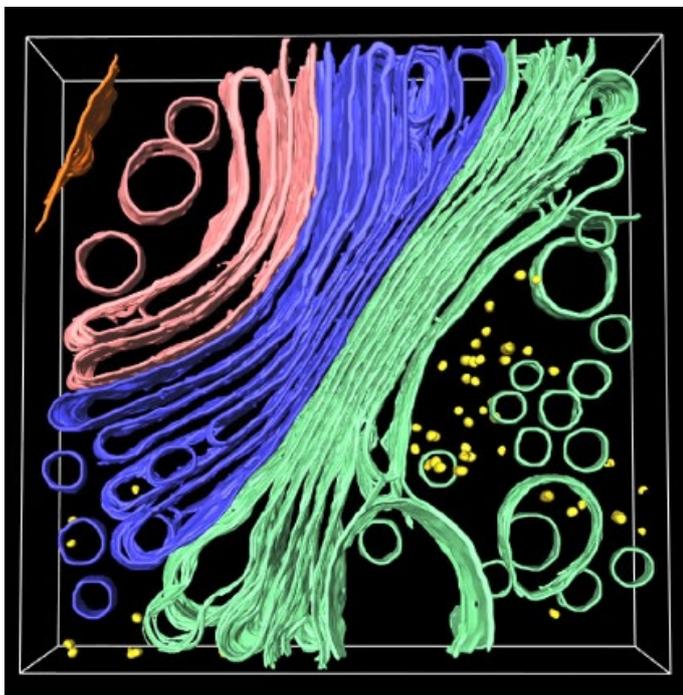


Figure 3. A 3D visualization of a Golgi apparatus from *Chlamydomonas reinhardtii* created on the Glacios 2 Cryo-TEM. The unicellular alga was flash-frozen without artificial stains or fixatives. Prior to imaging, a thin cryo-lamella sample was prepared from the vitrified cell by cryo-focused ion beam (cryo-FIB) milling with the Thermo Scientific™ Aquilos™ Cryo-FIB. Data segmentation and visualization was performed in Thermo Scientific™ Amira™ Software.

You can further optimize productivity and microscope time with optional EPU Multigrad Software. It allows you to queue multiple automatic EPU sessions and execute multiple unattended sample screening or high-resolution data acquisition sessions for grids available in the Autoloader.

The Glacios 2 Cryo-TEM includes optional Thermo Scientific Tomography 5 Software, which automates acquisition of tilt-tomograms. This can be paired with optional Thermo Scientific Tomo Live Software, which reconstructs tomograms into 3D volumes, to help you easily assess sample quality and obtain 3D data stacks for cell biology and structural biology workflows.

### Connect with other Thermo Scientific instruments

The Glacios 2 Cryo-TEM offers robust and contamination-free sample connectivity with the Thermo Scientific™ Talos™ Arctica™ Cryo-TEM and the Thermo Scientific™ Krios™ Cryo-TEM, allowing you to exchange AutoGrid cassettes and capsules between Autoloader-equipped instruments. This connectivity and the automated sample screening from the Glacios 2 Cryo-TEM enable direct sample transfer for analysis on the 300 kV Krios Cryo-TEM if higher resolution data collection is needed. Furthermore, samples can be loaded directly from a Thermo Scientific™ Tundra™ Cryo-TEM, Thermo Scientific™ Aquilos™ 2 Cryo-FIB, and Thermo Scientific™ Arctis Cryo-Plasma-FIB. With this connectivity, the Glacios 2 Cryo-TEM ensures that you can maximize data acquisition for most samples.

In addition to optimal mechanical connectivity, the Glacios 2 Cryo-TEM uses EPU Data Management Software to ensure the best connectivity for reproducible operation and storage of acquired data. With this robust connectivity, the system fits seamlessly into SPA and cryo-ET workflows.

### Get expert support

State-of-the-art hardware requires proper instrument operation and data interpretation. That's why we designed our Accelerate Service Portfolio to fully support cryo-EM labs. From initial installation to generating viable results, we'll provide the technical expertise and resources you need to be successful.

Tailored to your needs and coordinated by a dedicated customer success manager (CSM), your service program includes frequent contact with application experts and a unique combination of support elements that help enhance productivity. We provide consistent remote monitoring of your instrument's overall health to ensure optimal performance and conduct quarterly reviews with you to evaluate instrument data.

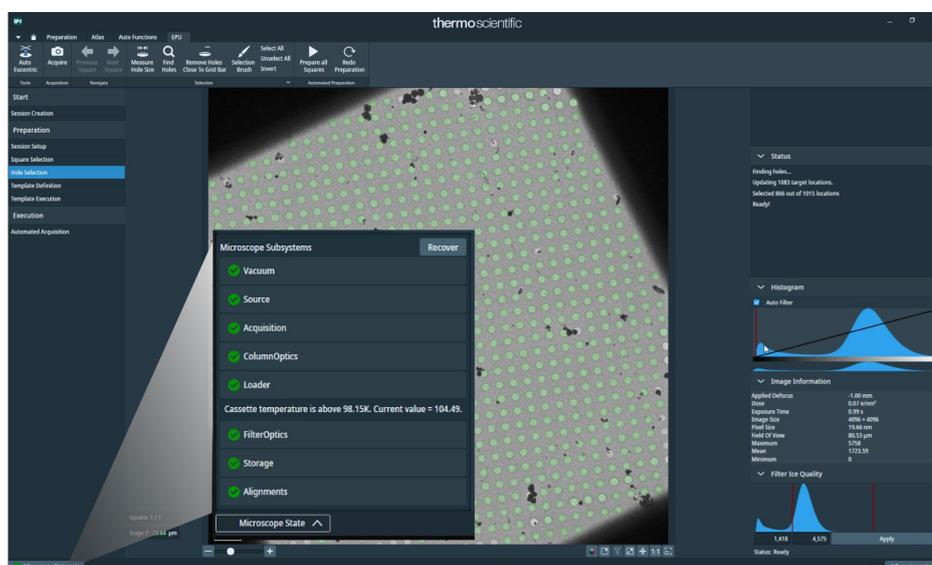


Figure 4. A screenshot of the Thermo Scientific EPU 3 Software user interface showing the traffic lights elements with all elements in green, meaning that the microscope is ready for imaging.

## Technical highlights

- High-brightness X-FEG electron gun
- Flexible accelerating voltage: 80 to 200 kV
- Cryo-Autoloader for automated and contamination-free loading of cassettes containing up to 12 AutoGrids
- Temperature management software, including liquid nitrogen autofill and scheduling of post-cryo-cycle cool down
- Automatic condenser, objective, and SA apertures
- Computerized 4-axis specimen stage with  $\pm 70^\circ$  alpha tilt
- Cryo-stage with single-axis holder
- Symmetric constant power objective lens to minimize image aberrations and lens hysteresis while switching between LM-SA-Mh imaging and diffraction
- Wide 11 mm pole piece gap
- Rotation-free imaging upon magnification changes
- Primary control unit including two 24" monitors to be placed within 10 m of the column
- Flu Camera: All manual and automatic alignments can be executed with the search and view camera
- Low-dose software suite for minimized electron dose during cryo-TEM operation
- Thermo Scientific Smart EPU Software for automated SPA sample screening and high-resolution data acquisition
- Full system enclosure to protect against environmental interference
- Aberration-free image shift (AFIS) between grid holes for shorter relaxation times
- Fringe-free imaging (FFI) for enhanced throughput with multiple image acquisitions per grid hole

## Optional configurations

- Falcon 4i Direct Electron Detector
- Selectris Energy Filter
- EPU Quality Monitor Software

- Embedded CryoSPARC Live
- EPU Multigrid Software
- Maps Software
- STEM\* and TEM tomography software
- Thermo Scientific Tomography 5 Software
- Tomo Live Software
- Thermo Scientific Inspect 3D Software
- HAADF STEM detector\*
- On-axis BF/DF detectors\*
- MicroED Package
- Thermo Scientific™ Vitrobot™ System
- Thermo Scientific™ Ceta™ D or Ceta 16M Camera
- Thermo Scientific Phase Plate Solution
- Accelerate Integrated Service and Application Support packages
- Customer witness acceptance test

\*STEM imaging is not compatible with FFI

## Installation requirements

- Environmental temperature: 18–23°C
- Temperature stability: within 1°C p-p/24 h (compatible with air conditioning class ASHRAE 2001)
- Door height: 2.32 m (can optionally be reduced to 2.00 m)
- Door width: 0.99 m
- Ceiling height: 2.8 m
- Weight distribution maximum: 700 kg/m<sup>2</sup> (without Selectris Filter), 750 kg/m<sup>2</sup> (with Selectris Filter)
- Frequency: 50 or 60 Hz ( $\pm 1\%$ )
- Compressed air supply with pressure range of 6 to 8.5 bar
- Nitrogen (N<sub>2</sub>) supply with pressure range from 1 to 3 bar
- Liquid nitrogen (LN<sub>2</sub>) for continuous LN<sub>2</sub> filling
- LAN connection for Thermo Scientific RAPID Service
- Room humidity 60% max

### Falcon 4i Direct Electron Detector performance

Sensor size	4,096 x 4,096 pixels / 5.7 x 5.7 cm <sup>2</sup>
Pixel size	14 x 14 $\mu\text{m}^2$
Internal frame rate/frame transfer to computer	320/320 fps (EER mode)
File formats	EER (native) MRC, TIFF, LZW TIFF
Detection modes	Electron counting Survey (fast linear mode)
Imaging performance (counting)	DQE (0) = 0.91 DQE (0.5 Nq) = 0.62 DQE (1.0 Nq) = 0.33

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