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DATASHEET

# Glacios Cryo-TEM with Falcon 4

## A complete solution for macromolecular structure determination using single particle analysis

Cryo-EM single particle analysis (SPA) can resolve the three-dimensional structure of proteins, protein complexes and other biological macromolecules at atomic resolution. This is possible thanks to vitrification, where specimens are rapidly frozen, preserving their biologically relevant native states. SPA has transformed the field of structural biology, leading to new insight into numerous biological processes. The Thermo Scientific™ Glacios™ Cryo-TEM offers a comprehensive SPA solution with hardware, software and support to allow any structural biology lab to successfully get started with SPA.

### The SPA technique

In order to perform SPA, macromolecules of interest must first be purified using traditional molecular biology methods. If the sample is intact and in its native state, it is then rapidly frozen into a thin layer of vitreous ice for structural preservation. Data acquisition can subsequently begin once protein density, distribution and ice quality have been optimized.

During SPA data acquisition, low-dose imaging is used to minimize sample damage, resulting in images with low contrast. To improve overall signal, and to ensure the sample is imaged from all angles, thousands of images at different orientations must be collected. After classification and particle averaging, these 2D projection images are recombined into a 3D reconstruction to determine the particle structure at atomic resolution (Figure 1).

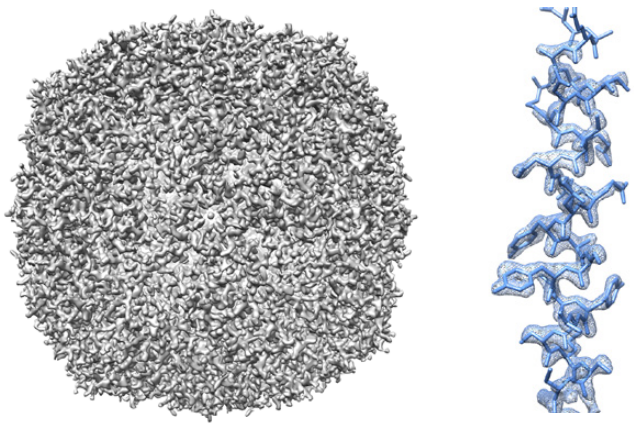


Figure 1. Apoferritin data collected on a Glacios Cryo-TEM with a Falcon 4 and AFIS Detector using EPU Software. 186 micrographs were collected with the throughput of 258 images/ hour due to AFIS implementation, and 19919 particles were used for the reconstruction to achieve 2.2 Å resolution.

### Key benefits

**Complete solution.** Integrated and embedded components ensure seamless usage, with a single user interface and a single contact point for support and training.

**Enhanced ease of use:**
- Innovative high-level automation combined with user guidance ensures optimal experiment setup and performance
- EPU Quality Monitor Software enables on-the-fly preprocessing of SPA data
- EPU automatic grid square clustering assists in the selection of the best grid squares
- EPU Multigrid optimizes microscope by enabling unattended usage

**Small footprint.** Compact hardware architecture minimizes installation space requirements for standard labs.

**Workflow connectivity.** Seamless contamination-free sample transfer between Autoloader-equipped instruments (Krios, Talos Arctica, and Glacios Cryo-TEMs).

**Optimized for improved performance and productivity.** Best imaging performance with the latest generation electron counting detector Falcon 4. The powerful combination of aberration-free image shift (AFIS) and the Falcon 4 Detector significantly enhances SPA throughput.

**End-to-end support.** The Accelerate portfolio service package provides the knowledge and confidence needed to keep moving forward with SPA regardless of previous experience.

### Components of SPA workflow

SPA workflow consists of several critical components, from sample preparation to data collection (Figure 2). First, the sample must be purified and frozen, as was previously stated. Subsequently, a high-end 200kV Glacios Cryo-TEM is used for data collection to optimize the sample and ultimately to provide the high-resolution map. The Glacios 200kV Cryo-TEM features an industry-leading autoloader, or cryogenic sample manipulation robot, ideal for sample optimization and high-resolution data collection.

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Cryo-EM experiments can produce several terabytes of data, which require considerable computational resources. During data analysis, powerful algorithms use averaging to boost the signal of individual images and determine needed parameters for 3D reconstruction.

The Glacios Cryo-TEM with integrated SPA data acquisition software (Thermo Scientific EPU 2 Software) and an embedded detector (Thermo Scientific Falcon™ 4 Detector) delivers a complete and compact cryo-EM solution at 200 kV. These cutting-edge components ensure optimal and effective performance.

## Glacios Cryo-TEM

### Reproducible, optimal tool performance

The Glacios Cryo-TEM enables ultimate performance with the brightest 200 kV X-FEG optics and state-of-the-art column design along with a constant-power objective lens. Thermal and mechanical stability ensure ideal optical performance, while self-assessment functionality guarantees the optimum starting point for SPA or tomography. Additionally, self-assessment automatically evaluates the optical status of the microscope, indicating if any steps require adjustment.

### SPA data collection

If configured with the Falcon 4 Detector and embedded data collection EPU 2 Software, the Glacios Cryo-TEM becomes a standalone SPA data acquisition microscope. It not only performs the critical sample optimization steps but also collects the final data used to solve the high-resolution structure.

### Small footprint to simplify installation

The new hardware architecture of the Glacios Cryo-TEM has been specifically designed with a smaller footprint and easier access path without sacrificing performance. In many cases, this avoids the additional investment and unwanted downtime that comes with modification of existing lab infrastructure (or even the need for a purposely built lab) to accommodate the instrument.

### Designed-in connectivity

The Glacios Cryo-TEM offers a robust and contamination-free designed-in connectivity with the Thermo Scientific Talos™ Arctica™ Cryo-TEM, Thermo Scientific Tundra™ Cryo-TEM and the Thermo Scientific Krios™ Cryo-TEM, allowing the exchange of AutoGrid cassettes and capsules between all (“plan 3”) Autoloader-equipped instruments. This connectivity, combined with the automated sample screening offered by EPU 2 Software, enables grids to move between systems seamlessly. The same cryo-grid and the acquired grid atlas can be loaded from the Glacios Cryo-TEM directly to the Krios Cryo-TEM if higher resolution data collection is needed on the sample at 300 kV. With this connectivity, the Glacios Cryo-TEM ensures that you will get the most out of any sample. Besides optimal mechanical connectivity, the newly introduced EPU Data Management (powered by Athena) Software ensures the best connectivity for reproducible operation and storage of acquired data.

## Optimized for cryo-imaging

The Falcon 4 Detector is the latest generation direct electron detector with counting capabilities from Thermo Fisher Scientific. It is fully embedded into the Thermo Scientific application software and features optimal detective quantum efficiency (DQE) performance (how effectively a camera can produce images with high signal-to-noise) combined with a significant productivity improvement. The large pixel design of the Falcon 4 Detector is tailored to low-dose life sciences applications and excels in high signal and low noise (highest signal-to-noise ratio). For further productivity enhancement, the Glacios Cryo-TEM can be equipped with the new Thermo Scientific Selectris™ Energy Filter which contains a Falcon 4 Detector behind the filter.

### Highest productivity with the highest DQE

The Falcon 4 Detector has the highest DQE over the entire frequency range. The high DQE values make the camera optimal for SPA low-contrast objects. In particular the unsurpassed DQE at low frequencies make it ideally suited for sub-100 kDa or difficult-to-detect proteins. As a result, fewer images are needed for a 3D reconstruction.

With an improved internal frame rate of 250 fps and improved pixel design the exposure time on the Falcon 4 Detector is typically 10 times shorter than on its predecessor, taking less time to acquire the same number of images. Together with the ultimate DQE, microscope productivity is significantly boosted from this advance.

The Falcon 4 Detector is the first direct electron detector to utilize electron event stream-based data handling, a technology called Electron Event Representation (EER). EER efficiently compresses the data, while preserving full temporal and spatial resolution (super resolution to 16k), which results in the highest image quality.

Falcon 4 is fully integrated with Thermo Fisher Scientific application software, such as EPU 2, which make daily operations easy and efficient.

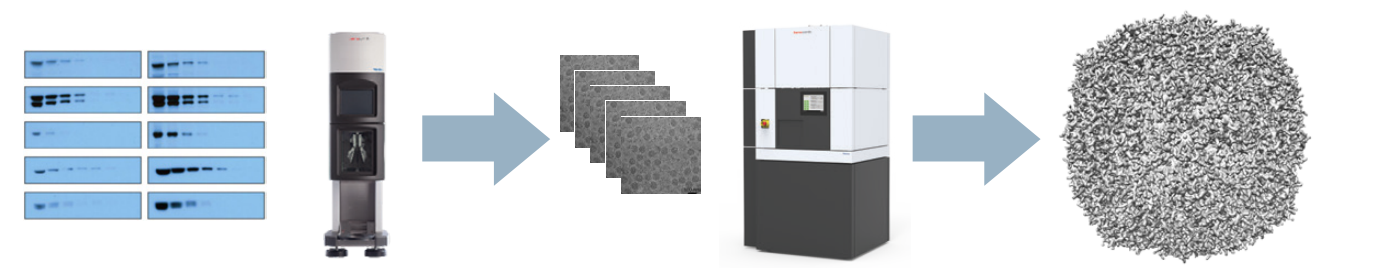



Figure 2. Schematic representation of the SPA workflow. After the biochemical steps of protein purification and verification, the samples are ready for cryo investigation. Cryo-grids are prepared using the Thermo Scientific Vitrobot™ System. Subsequently, they are transferred into the Glacios Cryo-TEM for sample optimization and high-resolution data acquisition followed by 3D reconstruction of the protein.

## EPU 2 Software

EPU 2 Software is an embedded software solution on the Thermo Scientific cryo-TEMs for SPA acquisition. Its simplified user interface, advanced automation and screening capabilities allow for straightforward planning and execution of SPA experiments.

### Advanced SPA workflow with EPU 2 Software

Once the vitrified samples are loaded into the Glacios Cryo-TEM, the EPU 2 Software automatically acquires an atlas of each grid. Ice quality is then evaluated and individual grid squares are automatically grouped into categories of similar quality (Figure 3).

This information can then be used to quickly decide which grid would be suitable for data collection. The EPU 2 Software also assists with data collection by offering adjustable templates. Once the data collection conditions are set up, the experiment will run unattended and images from each selected grid square will be acquired.

The automated data acquisition itself is accelerated through the use of image/beam shifts rather than a number of mechanical stage movements. This is possible due to aberration-free image shift (AFIS), a new optical mode, which performs large beam shifts without off-axis coma and astigmatism.

### EPU Multigrid Software

With the need to optimize the productivity of microscope time the optional EPU Multigrid Software comes into play. It enables queuing multiple automatic EPU sessions for the different grids in the Autoloader, allowing full tool utilization by making it possible to automatically set-up and execute multiple unattended sessions. It also facilitates efficient scheduling of work for multi-user facilities and permits fast screening by queuing short runs to be executed overnight or over a weekend.

### EPU Quality Monitor and Data Management Software

With lots of data coming from the microscope, proper data management is important. With EPU Data Management (powered by Athena) Software the camera's output can be automatically sorted, allowing easy organization of large datasets and streamlined interfacing with your image processing system. This data can be easily viewed and shared.

The optional EPU Quality Monitor Software performs on-the-fly preprocessing (for motion correction and CTF estimation, including derived parameters) for evaluation of the acquired SPA data during the actual acquisition process. This allows users to judge the quality of the acquired data and optimize the data acquisition while it is happening.

The combination of EPU 2, EPU Quality Monitor, and Data Management software helps to generate high-quality results quickly, with confidence, and with the same ease of use for which EPU is already known.

## Accelerate integrated service and applications support

Beyond state-of-the-art hardware, knowing how to operate the instrument and how to interpret the resulting data is also crucial for a successful SPA project. That is why Thermo Fisher Scientific developed the Accelerate portfolio. From the first engagement until agreed-upon results are reached, technical expertise and resources are arranged to help the user.

The Accelerate service portfolio is a new approach to scientific success, right from the start. It includes frequent touchpoints with application experts and a unique combination of support elements, providing the user the expertise and insight necessary to jump-start their scientific productivity.

### End-to-end support

From the moment the system is installed, Thermo Fisher Scientific will help fast-track research with cryo-TEM workflow validation, using a real biological sample, on-site training and consultations, remote support and access to our Scientific Workflows App. Thermo Scientific applications experts help users achieve technical proficiency, so they can efficiently conduct research.

### We stay in touch, so users stay in control

With quarterly reviews of learning progress and consistent remote monitoring of the system's health and status, users have the insight needed to keep the cryo-TEM running optimally and their scientific goals on track. A dedicated Customer Success Manager will coordinate the entire program, tailored for each user's individual needs.

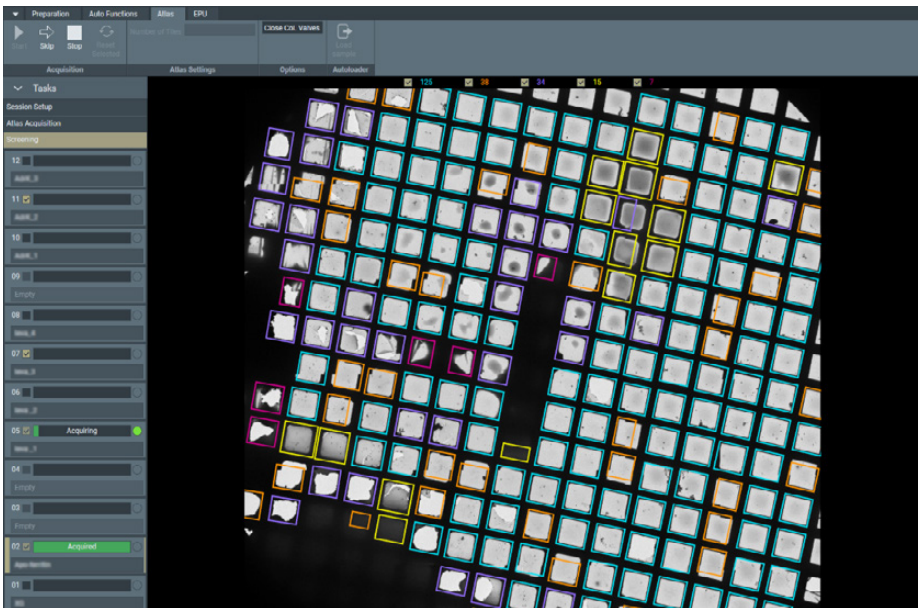



Figure 3. EPU 2 software can automatically acquire Atlases for the specimens in multiple slot positions and indicates the suitability of grids for high-quality data acquisition. Ice quality on the grids is grouped and color-coded for easy assessment.

## Glacios Cryo-TEM is the complete workflow solution for adopting cryo-EM SPA

The ability to study native structures offers unique insights that cannot be achieved with any other technique. This makes SPA an essential part of every structural biology lab.

With a small footprint, superior performance, innovative automation and end-to-end support, the Glacios Cryo-TEM offers a complete package for adopting this powerful technique.

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## Technical highlights of Glacios Cryo-TEM

- High-brightness X-FEG electron gun
- Flexible accelerating voltage: 80–200 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-axes specimen stage with ±70° alpha tilt
- Cryo-stage with single axis holder
- Symmetric constant power objective lens for minimizing image aberrations and lens hysteresis during mode switching between LM-SA-Mh imaging and diffraction
- Wide pole piece gap of 11 mm
- Rotation-free imaging upon magnification changes
- Primary control unit including two 24" monitors to be placed within 10 meters of the column
- Digital FluCam: All manual and automatic alignments can be executed with the search and view camera
- Thermo Scientific Ceta™ 16M CMOS camera
- Windows® 10 Operating System
- Low-dose software suite for minimized electron dose during cryo-TEM operation
- Thermo Scientific EPU 2 Software for automated SPA sample optimization and data acquisition
- System enclosure
- AFIS: Aberration-free image shift between grid holes for shorter relaxation times.

## Optional Configurations

- Thermo Scientific Phase Plate solution
- Thermo Scientific Falcon 3EC or Falcon 4 Direct Electron Detector
- EPU Quality Monitor Software for on-the-fly preprocessing
- EPU Multigrid Software
- STEM and TEM tomography software
- HAADF STEM detector
- On-axis BF/DF detectors
- Thermo Scientific Vitrobot System for vitrification
- Thermo Scientific Selectris Energy Filter
- Thermo Scientific Ceta or Ceta D 16Mpix CMOS camera
- Accelerate Integrated Service / Applications Support packages to accelerate customer innovation and enhance productivity

## Floorplan – installation requirements

- Environmental temperature: 18°C – 23°C
- Temperature stability: 0.8°C p-p per 24 hr (Compatible with air conditioning class ASHRAE 2001)
- Door height: 2.30 meter can optionally be reduced to 1.96 m
- Door width: 0.90 meter
- Ceiling height: 2.80 meter
- Weight distribution maximum: 700 kg/m$^2$
- Double earth connection
- Frequency: 50 or 60 Hz (±3%)
- Compressed air supply with pressure range of 5–7 bar
- Nitrogen $N_2$ supply with pressure range of 1–10 bar
- Liquid nitrogen ($LN_2$) for continuous $LN_2$ filling
- Sulfur Hexafluoride ($SF_6$) gas in proper ventilated room
- LAN connection for Thermo Scientific RAPID™ Service (Remote Access Program for Interactive Diagnosis)

| Falcon 4 Direct Electron Detector performance | |
|---|---|
| Sensor size | 4,096 x 4,096 pixels / 5.7 x 5.7 cm$^2$ |
| Pixel size | 14 x 14 µm$^2$ |
| Internal frame rate/frame transfer to computer | 250/240 fps |
| File formats | EER (includes 16k x 16k super-resolution data, all frames accessible – fractionation not required)<br>MRC |
| Detection modes | Electron counting<br>Survey (fast linear mode) |
| Imaging performance (counting) | DQE (0) = 0.85<br>DQE (0.5 Nq) = 0.60<br>DQE (1.0 Nq) = 0.20 |
| Automation support | Integrated in Thermo Scientific EPU 2 and Tomography Software<br>Compatible with 3rd party software via TEMscripting |

Find out more at **thermofisher.com/EM-Sales**

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