thermo scientific

DATASHEET

Glacios Cryo-TEM

A complete solution for macromolecule structure determination using Single Particle Analysis

Cryo-EM Single Particle Analysis (SPA) can determine the three-dimensional structure of proteins, protein complexes and other biological macromolecules at near-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 challenge of SPA lies in its complex multi-component workflow, which includes a cryo-electron microscope (cryo-EM) with specific detectors and software. The Thermo Scientific™ Glacios Cryo-TEM offers a comprehensive solution with hardware, software and support to allow any structural biology lab to successfully adopt 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 electron 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 conformational classification and particle averaging, these 2D projection images are recombined into a 3D reconstruction to determine the particle structure at near-atomic resolution (Figure 1).

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 performance and experimental setup.

Small footprint. Compact hardware architecture minimizes installation requirements for easy adoption.

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

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

Components of SPA workflow

SPA consists of several critical components, from sample preparation to data collection.

First, the sample must be purified and frozen, as was previously stated. Subsequently, the quality of the vitrification and particle distribution is verified in a screening step. Autoloader-equipped cryo-TEMs are recommended (such as the Glacios Cryo-TEM or the Thermo Scientific™ Talos™ Arctica cryo-TEM). If the sample

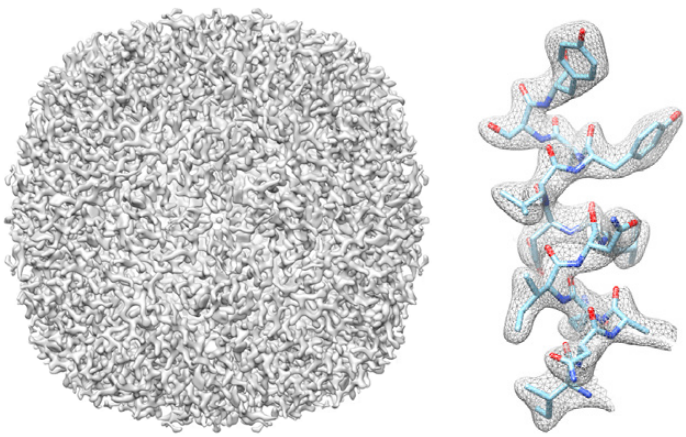

Figure 1. Apoferritin data collected on a Glacios Cryo-TEM with a Falcon 3EC Detector using EPU Software. 282 micrographs were collected in a 12-hour period, and 60,746 particles were used for the reconstruction to achieve 2.5 Å resolution. The Fourier Shell Correlation (FSC) shows this resolution on the left, while the atomic structure docking on the right demonstrates the structures that are visible at this resolution.

ThermoFisher SCIENTIFIC

is of sufficient quality, data acquisition is performed on the same instrument, so it is essential to choose the right cryo-TEM for this step. The new Glacios Cryo-TEM with integrated SPA data acquisition software (Thermo Scientific™ EPU™ Software) and an embedded detector (Thermo Scientific™ Falcon™ 3EC 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

When configured with the Falcon 3EC Detector and embedded data collection EPU Software, the Glacios Cryo-TEM becomes a standalone SPA data acquisition microscope. It not only performs the critical sample screening 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 come 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 Arctica 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 EPU automated sample screening 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.

Optimized for cryo-imaging

The Falcon 3EC Detector is the first electron counting detector to combine full embedding in Thermo Scientific application software with ultimate detector quantum efficiency (DQE) performance (how effectively a camera can produce images with a high signal-to-noise). There is no need to switch cameras in an SPA experiment since low-magnification mode is also supported, leading to easier experimental setup and increased throughput.

The superior large pixel design of the Falcon 3EC Detector is tailored to low-dose life sciences applications and makes it excel in high signal and low noise (highest signal-to-noise ratio). This DQE value has a particular effect on the detector’s ability to view small, low-contrast objects, which are important for SPA. The Falcon 3EC Detector offers the highest DQE and largest effective area among detectors (Figure 3).

Extremely fast, yet high DQE

The Falcon 3EC Detector offers both “Fast” and “Electron counting” modes to cover the needs of a SPA experiment; there is no need to switch cameras during the EPU Software run. In “Fast mode” (i.e. linear/integration mode), you can set up exposure times as short as 0.1 second, yet the penalty on the DQE is minimal. This makes the mode ideal for screening, auto-alignments and EPU Software setup. “Fast mode” is also the choice for phase plate activation and data collection for fast initial model building.

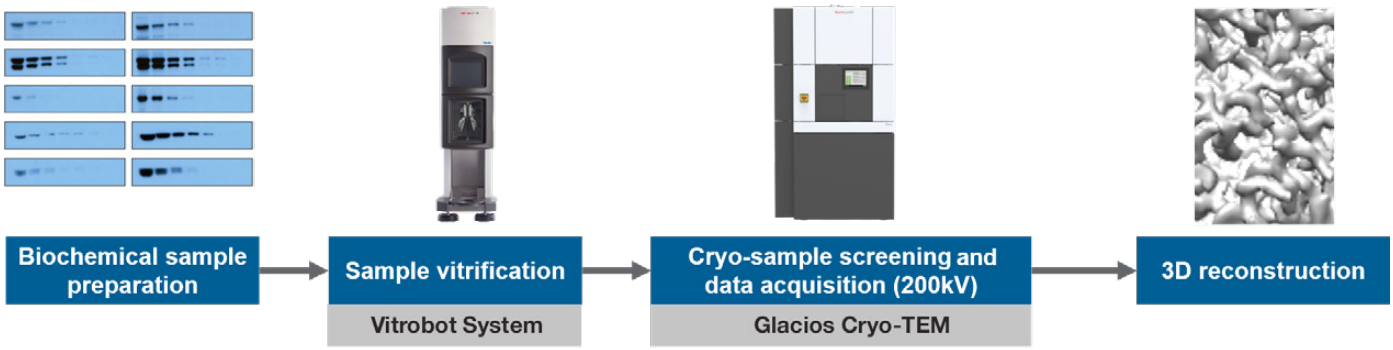

Figure 2. Schematic representation of the SPA workflow. After the biochemical steps of protein purification and verification, the samples are ready for cryo-investigation. Cryo-grids are prepared using the Thermo Scientific™ Vitrobot™ System. Subsequently, they are transferred into the Glacios Cryo-TEM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

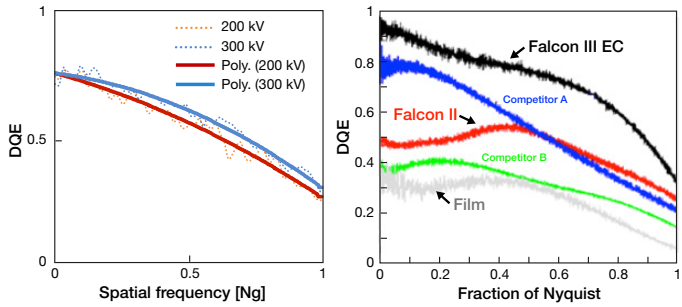

Figure 3. (left) DQE measurement of Falcon 3EC Detector at 200 kV (red) and 300 kV (blue) in "EC mode" at a dose rate of 1 e/px/s. (right) DQE comparison of various detectors by MRC Cambridge (Greg McMullan and Richard Henderson 2017). Competitor A is in counting mode, competitor B is in integrating mode and the Falcon 3EC Detector is in EC mode at a dose rate of 0.7 e/px/s.

Ultimate DQE performance

After the EPU Software setup and a quick initial modeling (to check sample quality), the same camera can be used for extended high-resolution data collection by switching to "Electron counting mode." This enables imaging at extremely low doses with enhanced DQE. The patented noise reduction improves the signal-to-noise ratio even further, which results in unsurpassed direct electron detection. The Falcon 3EC Detector is the ideal camera for high-resolution SPA of small or large structures alike, due to the combination of high DQE and large effective area. Furthermore, fewer images are needed to create a reconstruction due to the superior quality of the detector.

EPU 2.0 Software

EPU Software is an embedded software solution on the Thermo Scientific cryo-TEMs for SPA acquisition. Its new intuitive user interface features more automation and screening capabilities, allowing for straightforward planning and execution of SPA experiments.

Advanced SPA workflow with EPU Software

Once the vitrified samples are loaded into the Glacios Cryo-TEM the EPU Software automatically acquires an atlas of each grid. The ice quality is then examined, clustered and color coded (Figure 4). You can then use this information to quickly decide which grid would be suitable for data collection. The EPU Software also assists with data collection by offering adjustable templates. These mark the areas where data collection will take place and are displayed over the grid atlas. Once the data collection conditions are set up, the experiment will run unattended and images from each selected grid square will be acquired.

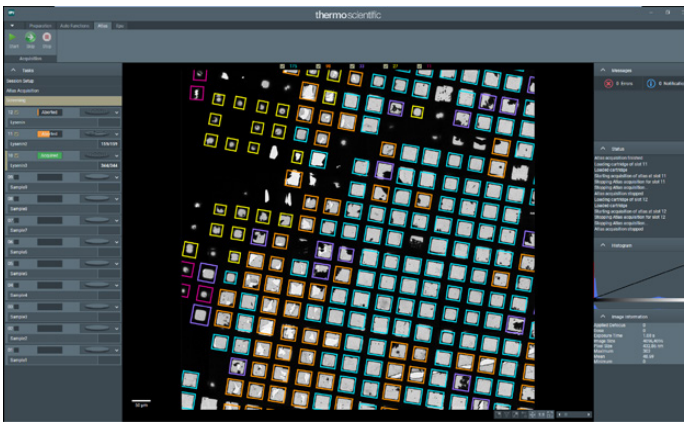

Figure 4. Screening of the cryo-grids is done automatically in EPU Software. Ice quality on the grids is grouped and color coded for easy assessment.

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 you.

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 you the expertise and insight necessary to jump-start your scientific productivity.

End-to-end support

From the moment your system is installed, Thermo Fisher Scientific will help fast-track your 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 Fisher Scientific applications experts help you achieve technical proficiency and will empower you to efficiently conduct research.

We stay in touch, so you stay in control

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

Glacios Cryo-TEM is your 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 introducing this powerful technique into your research.

thermo scientific

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
- Ceta 16M CMOS camera
- Windows® 7 Operating System
- Low Dose software suite for minimized electron dose during cryo-TEM operation
- System enclosure

Optional Configurations

- Volta Phase Plate solution
- Falcon 3EC Direct Electron Detector
- EPU Software for SPA screening and data acquisition
- STEM and TEM tomography software
- HAADF STEM detector
- On-axis BF/DF detectors
- Vitrobot System for vitrification
- Accelerate integrated service and application support packages

Technical highlights of Falcon 3EC Detector

- 4,096 x 4,096 sensor size
- 14 x 14 μm^2 pixel size
- On-axis, bottom-mounted, retractable
- 40 fps to storage rate
- 2 detection modes:
  - Fast mode (non-electron counting)
  - EC mode (electron counting)
- Imaging performance 4k x 4k DQE @ 0.5Ny:

Fast mode (10–120 e/p/s)	0.3 (200 kV)
EC Mode (1 e/p/s)	0.55 (200 kV)

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 1.67 meter)
- 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)

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

ThermoFisher SCIENTIFIC