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.
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.

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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.
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.

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.
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® 10 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 μm² 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:
<table>
<thead>
<tr>
<th>Mode</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fast mode</td>
<td>0.3 (200 kV)</td>
</tr>
<tr>
<td>EC Mode</td>
<td>0.55 (200 kV)</td>
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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/m²
- Double earth connection
- Frequency: 50 or 60 Hz (±3%)
- Compressed air supply with pressure range of 5–7 bar
- Nitrogen N₂ supply with pressure range of 1–10 bar
- Liquid nitrogen (LN₂) for continuous LN₂ filling
- Sulfur Hexafluoride (SF₆) gas in proper ventilated room
- LAN connection for Thermo Scientific™ RAPID™ Service (Remote Access Program for Interactive Diagnosis)