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DATASHEET

Krios G4 Cryo-TEM

Atomic resolution with enhanced productivity and compact design

The new Thermo Scientific™ Krios™ G4 Cryo-Transmission Electron Microscope (Cryo-TEM) is the most compact TEM in its class. A thorough redesign of the mechanical base frame and system enclosure has reduced microscope height below 3 m, allowing the instrument to be installed in labs with a ceiling height below 3.04 m (10 ft) – avoiding costly room renovations.

The Krios G4 Cryo-TEM has improved ergonomics for easier sample exchange. Data acquisition set up is easier and faster thanks to enhanced automation, systematic user guidance, and Advanced Performance Monitoring (APM), a built-in self-diagnostic function that ensures microscope alignments are optimized for high-resolution data acquisition.

Simultaneously, the system's productivity and high-resolution performance have been enhanced through a combination of new optical modes, improved optical specifications, and on-the-fly preprocessing.

Equipping the Krios G4 Cryo-TEM with the Thermo Scientific Falcon™ 4 Direct Electron Detector, which is also available in

Key benefits

Easier to fit into new or existing labs. Redesigned internal base frame and system enclosure reduce height below 3 m (10 ft) while enhancing system performance and avoiding costly room renovations

E-CFEG for truly atomic resolution and high throughput. Low energy spread ≤0.3 eV enables higher contrast at high resolutions (≤2.0 Å); Improved contrast at high spatial frequencies to reach higher resolutions in less time

Best-in-class image quality. 0.12 nm information limit and linear distortion of <0.5%. Optional Falcon 4 Detector has the highest DQE in its class

Enhanced productivity for more structures:

- Improved drift performance allows you to start your experiments faster
- EPU automatic grid square clustering assists in the selection of the best possible grid squares
- The combination of fringe-free imaging (FFI), aberration-free image shift (AFIS), and the optional Falcon 4 Direct Electron Detector enhances single-particle-analysis (SPA) throughput
- EPU Quality Monitor optimizes SPA data through on-the-fly pre-processing
- EPU Multigrid enables automated SPA data acquisition on multiple grids
- Advanced Performance Monitoring (APM) ensures the best optical starting point is used for automated SPA runs

Workflow connectivity. Designed for the easy exchange of cryo-EM-sample cassettes, facilitating a smooth transfer of specimens between Autoloader-equipped instruments

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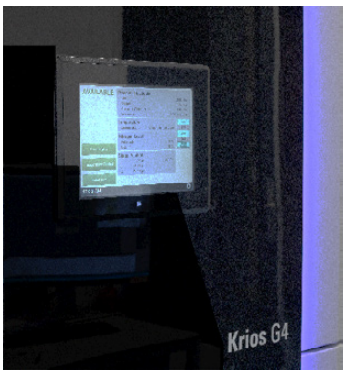
combination with the innovative Thermo Scientific Selectris™ Imaging Filter, further increases productivity and performance. Adding Thermo Fisher's cold field emission gun (E-CFEG) even enables atomic-resolution imaging.

The latest generation of the award-winning Krios platform is now even further optimized for automated high-productivity applications such as single-particle analysis (SPA), cryo-electron tomography (cryo-ET) and micro-electron diffraction (MicroED), allowing every user to get the best possible performance out of every experiment.

Fits more easily into new and existing labs

Installing a high-end cryo-electron microscope can be challenging and often requires significant room renovations due to instrument size. Thanks to its completely redesigned mechanical base frame and system enclosure, the Krios G4 Cryo-TEM has a system height of <3 m, avoiding these costly and challenging renovations.



As part of the redesign, the system ergonomics have also been improved. More specifically, the sample loading area has been lowered, allowing safe and convenient access—there is no need to climb a ladder to load cryo-EM samples.

An interactive touch screen is available close to the sample loading area in order to indicate and control key microscope parameters that facilitate the sample loading process. This is especially beneficial when the instrument is being operated remotely.

Maximized productivity

The Krios G4 Cryo-TEM features a built-in self-diagnostic function, Advanced Performance Monitoring (APM), which evaluates if the microscope alignments are optimized for acquiring high-resolution data. Automated alignment routines allow the instrument to be tuned to its ideal starting point for SPA or cryo-ET. Combined with the instrument's thermal and mechanical stability, APM can ensure that the ideal alignment is available for all users.

A typical SPA experiment starts by screening vitrified samples in order to find the best possible grid areas. Built-in Thermo Scientific EPU Software checks specimen quality and facilitates SPA data acquisition. With full control over the Autoloader, all 12 grids in an Autoloader cassette can be automatically batch screened. After the creation of a grid atlas, grid squares are automatically clustered based on their ice quality (i.e., presence, thickness); this guides grid-square selection. In combination with the improved stage stability of the single axis holder (resulting in reduced drift after sample insertion) these features make the assessment of sample quality, as well as data acquisition set up, more efficient.

In preparation for high-resolution data acquisition, EPU Software can automatically perform daily tuning of essential alignments such as focus, eucentricity correction, stigmation, and coma correction.

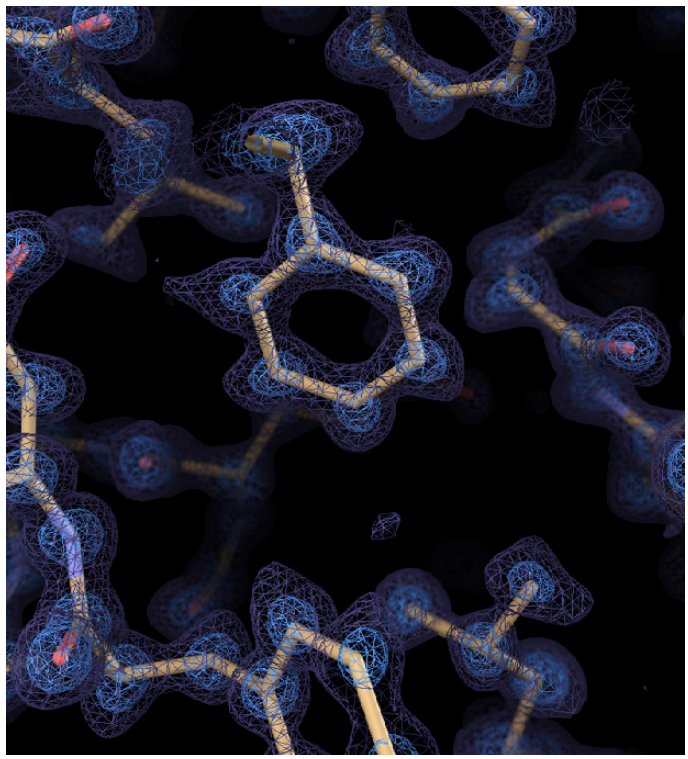
The automated data acquisition itself is accelerated using image/beam shifts rather than several mechanical stage movements. This is possible due to aberration-free image shift (AFIS), a new optical mode which performs large beam shifts without off-axis coma and astigmatism, as well as the collection of more images per foil hole through fringe-free imaging (FFI).

The EPU Multigrid option automates SPA data acquisition of multiple grids in an Autoloader cassette, enabling long, unattended SPA acquisition runs.

Additionally, EPU Quality Monitor is an optional on-the-fly preprocessing tool (consisting of motion correction and CTF estimation, including derived parameters) that evaluates the acquired SPA data during the actual acquisition process. This allows you to judge the quality of acquired data remotely and consequently optimize the data acquisition while it is happening. This way, the highest quality data can be acquired in the most efficient way possible.

Atomic resolution for 3D reconstruction

The Krios G4 Cryo-TEM has a proven track record of high-resolution imaging for a wide variety of particles: the vast majority (>95%) of published structures at or below 4 Å have been determined using Thermo Scientific Cryo-TEMs[1]. To allow for the reconstruction of increasingly smaller molecules at increasingly



3D reconstruction of Apoferritin at 1.2 Å atomic resolution. Data taken on the Krios G4 Cryo-TEM with E-CFEG and the Selectris/Falcon 4 Detectors in counting mode. *Image courtesy of P. Emsley, MRC LMB Cambridge.*

[1] Analysis of EMDB data August, 2020.

higher resolutions, the information limit has been improved to 0.12 nm and linear distortion to below 0.5%, ensuring the best possible boundary conditions for high-resolution imaging.

To meet the growing need for high-resolution, high-quality imaging, the Krios G4 Cryo-TEM features a cold field emission gun with a low energy spread (E-CFEG, ≤0.3 eV), which contributes to enhanced resolution and better imaging contrast. Especially for resolutions <2.0 Å (a domain often linked to small protein research) the cold FEG can be of significant value. For example, at a target resolution of 1.5 Å, the contrast is enhanced 2× compared to the high-brightness X-FEG. Additionally, the E-CFEG enhances data throughput, and thus, productivity.

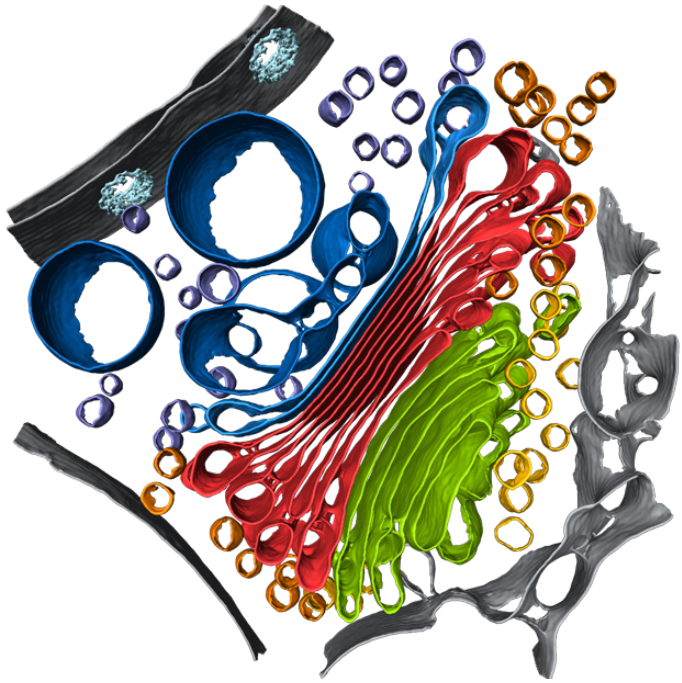
The Krios G4 Cryo-TEM can also be equipped with the optional Selectris Imaging Filter with included Falcon 4 detector to enhance sample resolution even further.

Workflow connectivity

Screening for optimized samples is an essential component of a successful cryo-EM workflow, verifying sample quality in terms of both biochemistry and vitrification. The Krios G4 Cryo-TEM fits seamlessly into the SPA and cryo-ET workflows with convenient and contamination-free sample transfer.

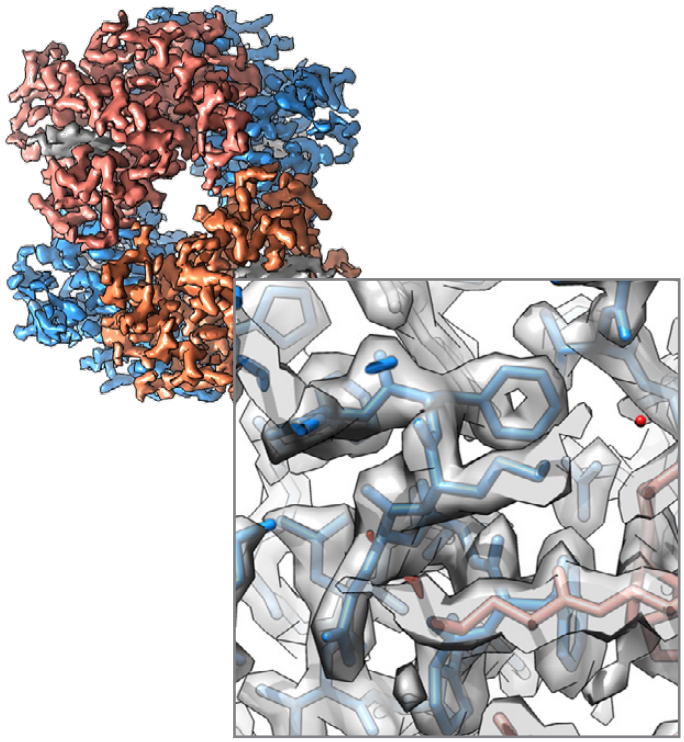
For SPA, the built-in connectivity of the Autoloader capsule and cassette systems ensures a robust and contamination-free transfer of multiple samples between the Thermo Scientific Glacios™ Cryo-TEM, the Thermo Scientific Talos Arctica™ Cryo-TEM, and the Krios G4 Cryo-TEM without needing to manipulate individual grids.

Similarly, for cryo-ET, samples can be transferred from sample fabrication (in the Thermo Scientific Aquilos™ Cryo-FIB) to tomography data collection in the Krios G4 Cryo-TEM.



3D visualization of a Golgi apparatus from the green alga *Chlamydomonas reinhardtii.* The unicellular alga was flash-frozen without any artificial stains or fixatives. Prior to imaging with cryo-ET, 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 by Thermo Scientific Amira™ Software. *Data courtesy of Dr. Benjamin Engel, Department of Molecular Structural Biology, Max Planck Institute for Biochemistry, Martinsried, Germany.*

Membrane protein from SARS-Cov-2 (3a ion channel, 62 kDa) resolved to 2.1 Å with Selectris X Imaging Filter with Falcon 4 camera.
Kern et al. 2021, doi: https://doi.org/10.1101/2020.06.17.156554.



Human hemoglobin (64 kDa) resolved to 2.0 Å using 10 eV filtering with Selectris X Imaging Filter.

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Technical Highlights	
Source	X-FEG (extreme high-brightness field emission gun) or low-energy-spread cold FEG (E-CFEG)
Accelerating voltage	80–300 kV
Cryo-autoloader	Automated and contamination-free loading of cassettes (up to 12 grids)
Temperature management software	Includes liquid nitrogen autofill and cool down scheduling
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)
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	Self-assessment of optical microscope status, combined with automated alignments, ensures ideal experimental conditions
Room size requirements (L × W × H)	17’ × 22’ × 10’
AFIS (aberration-free image shift)	Enhancing throughput with shorter relaxation times when moving coma-free between grid holes
FFI (fringe-free imaging)	Enhanced throughput with multiple image acquisitions per grid hole
Thermo Scientific EPU 2 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, or extend up to 300 meters from the column (optional)
Detectors (optional)	• Falcon 4 Direct Electron Detector • Thermo Scientific Ceta™ D Camera • Thermo Scientific Ceta 16M Camera • HAADF STEM detectors • On-axis BF/DF detectors
Energy filter (optional)	• Selectris Imaging Filter • Selectris X Imaging Filter • Gatan BioContinuum Energy Filter
Other options	• Cs Image Corrector • Thermo Scientific Phase Plate Solution

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