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DATASHEET

Falcon 4 Direct Electron Detector

Enhance productivity with high throughput and the best image quality

The Falcon 4 is the next step in direct electron detection: a large pixel layout, with high signal-to-noise ratio, has been combined with speed and efficient data compression for optimized productivity without compromising image quality.

The Thermo Scientific™ Falcon™ 4 has an improved detective quantum efficiency (DQE) over the entire spatial frequency range, while exposure times are typically 10 times shorter than with its predecessor. Built-in data compression and integration with the data acquisition software ensure a productivity boost and efficient daily operation.

The optimized Falcon 4 sensor design is based on the proven large (14 µm) pixel layout of the Falcon product range. This further reduces the unsurpassed low noise levels, while also improving electron localization for lower coincidence loss. Combined with the increased internal framerate (250 fps), this allows collection of the best quality images at high throughput.

The Falcon 4 is the first direct electron detector to utilize electron event stream-based data handling. Images are stored in a new format: the Electron Event Representation format (EER – patent pending) features powerful lossless compression, while preserving full temporal (250 fps) and spatial resolution (events are localized to one sixteenth of a pixel).

The Falcon 4 is fully integrated in Thermo Fisher's EPU & Tomography software. To further enhance productivity and ease of use, the camera's output can be automatically captured through EPU Data Management (powered by Athena) allowing easy organization of large datasets and streamlined interfacing with your image processing system.

The optional EPU Quality Monitor performs on-the-fly pre-processing (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.

Key benefits

Best imaging quality
- Highest DQE over the entire spatial frequency range – ideally suited for small or difficult to detect proteins
- Optional EPU Quality Monitor for on-the-fly quality assessment

High throughput for faster results
- 10 times shorter exposure times than its predecessor
- Optimized for small coincidence loss
- Minimized offload times by optimizing data handling

Efficient data compression
- Lossless data compression using Electron Event Representation (EER) technology (patent pending)
- EER compression retains full spatial and temporal resolution, allowing super resolution and avoids the need for fractioning

Ease of use
- Fully embedded in Thermo Scientific EPU and Tomography software for smooth operation
- Built-in EPU Data Management (powered by Athena) offers easy project administration and automated organizing of images and metadata for remote and collaborative access – while maintaining full backward compatibility with existing workflow setups

With its unique combination of highest image quality, high throughput, efficient lossless data compression and a streamlined solution for data management and quality monitoring, the Falcon 4 enables the productivity boost that is required by today's demanding scientific and industrial communities.

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Best image quality

The Falcon 4 is the next step in direct electron detection: a large low-noise pixel design is combined with increased readout speed (250 fps). Furthermore, the sensor design is optimized for electron event localization, further reducing coincidence noise.

The result is a further reduction of noise level, such that incoming electrons at a dose rate of 1.5 e/p/s are detected with:

- A large detection *probability* resulting in an unsurpassed DQE at low spatial frequency – DQE(0) = 0.90
- A large detection *accuracy* providing unsurpassed DQE at higher spatial frequencies – DQE(½ Nq) = 0.70; DQE (1 Nq) = 0.28

Overall, the DQE over the entire frequency range was improved, making the Falcon 4 ideal for fast high-resolution data collection on a wide range of particle sizes. In particular the unsurpassed DQE at low frequencies makes it ideally suited for small or difficult to detect proteins.

High throughput for enhanced productivity

The 6 times higher internal frame rate in combination with improved electron event localization yields exposure times that are reduced up to 10-fold as compared to the previous generation detector. In addition, for each exposure, significant time savings have been realized on overhead (dark image recording, offloading the data from the camera). Overall, this significantly boosts data throughput (use case dependent: up to 600 images/hr can be achieved), leading to faster results and subsequently the need for less microscopy time.

Additionally, improved radiation hardness allows the Falcon 4 to benefit from a much improved, non-interfering, reactive dose protection mechanism for easier and faster set-up of experiments.

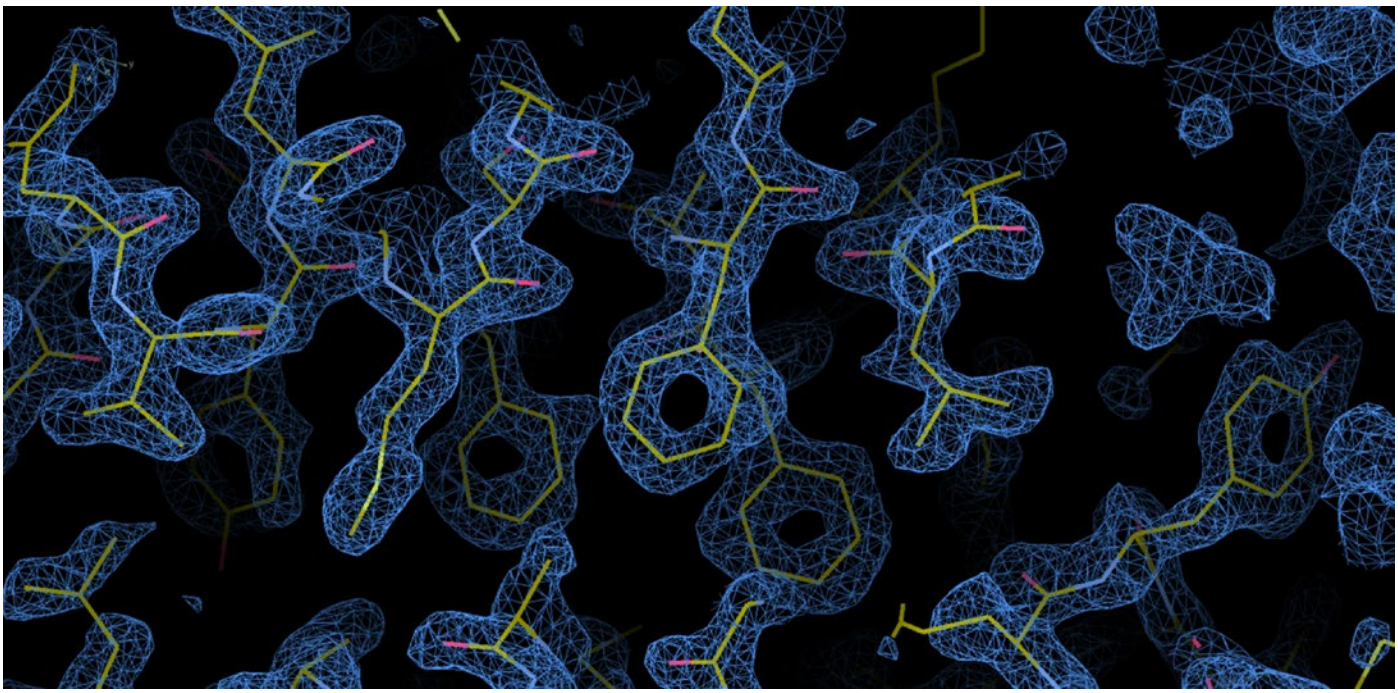
Efficient data compression

An important challenge for today's cryo-EM community is the efficient handling of increasingly large datasets. To overcome the data overload, traditional direct electron detectors have been summing raw camera frames into dose fractions – either with intra-fraction drift correction (Falcon 3EC) or without. This efficiently reduces the data size, but also severely compromises the information content of the resulting movie.

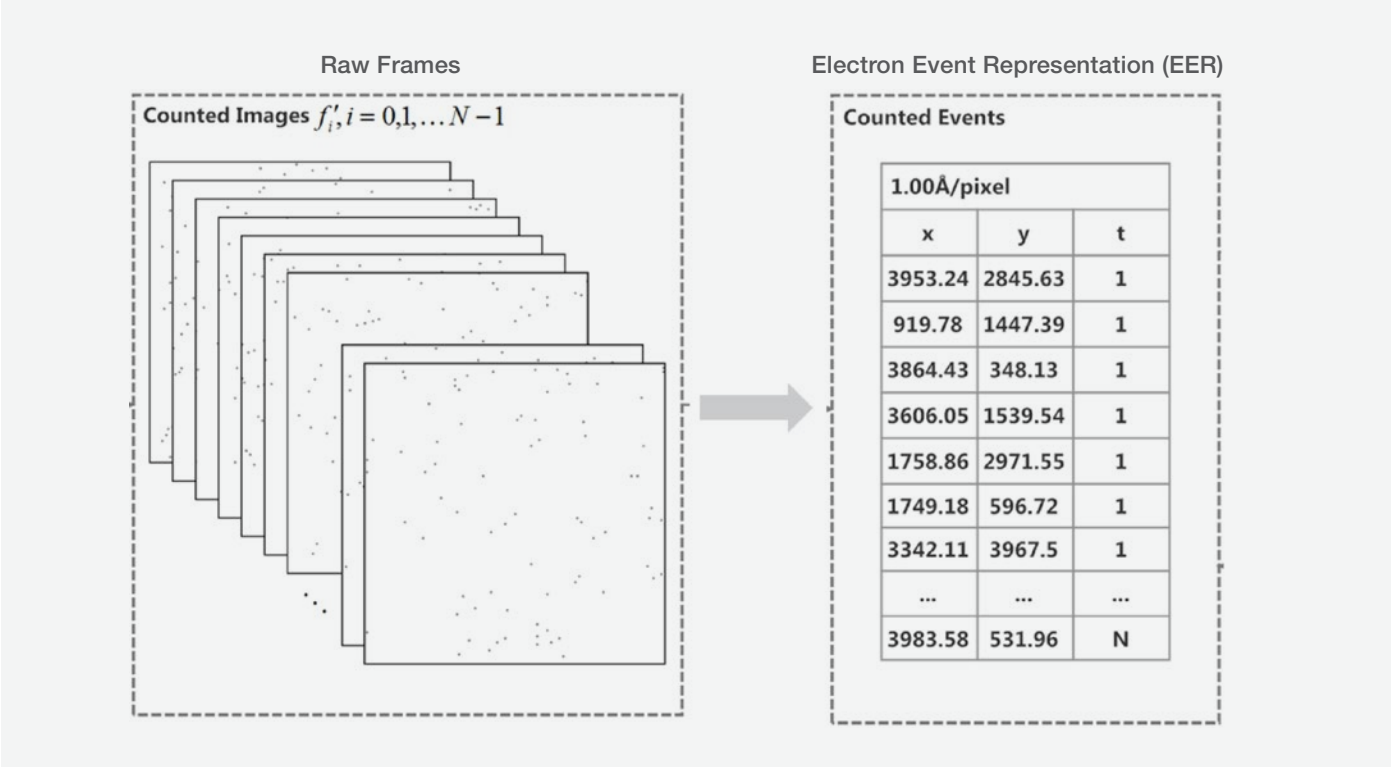
Data quality and processing algorithms are expected to improve to a point where today's dose fractions of typically 1 electron per pixel will become a limiting factor in the achievable resolution. Therefore, data collection with better temporal resolution (finer fractionation) is required. To this end the Falcon 4 is equipped with Electron Event Representation (EER), a patent-pending technology where the camera outputs the location of each impacting electron at an accuracy of one sixteenth ($1/16^{th}$) of a pixel for each raw camera frame (250 fps).

This electron event stream from the camera is then further reduced in size by lossless data compression (using run length encoding). This eliminates the need to setup fractionation upfront whereby inevitably precious information is lost by summing camera frames. In short, with Falcon 4 all counted camera frames are available with 16k x 16k oversampled event localization in a small file.

The EER file format feeds seamlessly into the Relion 3 and cryoSPARC pipelines ensuring an efficient processing and an overall enhancement of the SPA and tomography workflow productivity. Alternatively, EER images can always be converted into common file formats like MRC and TIFF when required.



Falcon 4 high throughput illustrated:
2.0Å Apoferritin reconstruction – from 80 images/6000 particles acquired in 10 minutes (SPA with EPU data acquisition on Krios G4).



Falcon 4 Electron Event Representation (EER): counted events of all raw frames are available for processing with full temporal resolution (250 frames per second) and spatial resolution (events are localized to one sixteenth of a pixel). This super resolution capability allows maximally benefiting from the Falcon 4's superior DQE at high spatial frequencies.

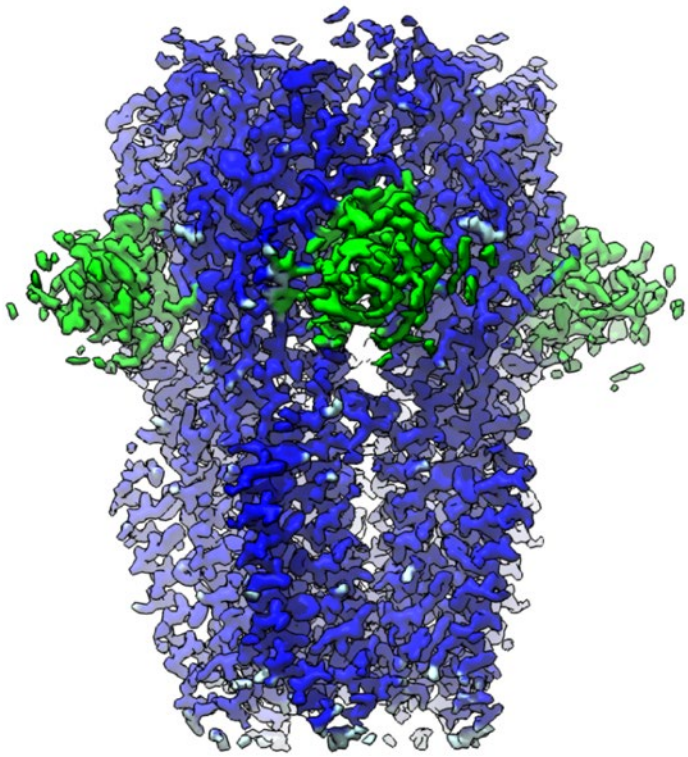
Ease of use

The Falcon 4 is fully integrated in Thermo Fisher Scientific's single particle acquisition (EPU) & Tomography software allowing for smooth daily instrument operation and data acquisition. For single particle analysis, the Falcon camera now includes a data management and optional on-the-fly pre-processing solution.

EPU Data Management (powered by Athena) comes pre-installed on the Falcon 4 hardware and facilitates the organization, viewing and sharing of single particle cryo-EM data. EPU streams data and metadata directly to a project which is set up prior to the start of an experiment. You can easily view all the project data and metadata at the microscope as well as remotely through a secure connection, and can comment and share it with collaborators.

Single particle analysis data acquisition can be further augmented with the optional EPU Quality Monitor which processes incoming EPU data on-the-fly. Drift correction and CTF estimation (including derived parameters such as defocus, phase shift, astigmatism, …) are visualized and allow on-the-fly analysis of the quality of the incoming images. Based on its results, users can optimize acquisition parameters and filter data sets according to the quality indicators.

In combination with the Falcon 4, these solutions help to generate high quality results quickly and with confidence and with the same ease of use for which EPU software is known.



3D SPA reconstruction of the Homopentameric GABAA receptor at 2.1Å resolution. Images are acquired with the Falcon 4 and EER and processed with Relion 3. *Image courtesy of: Radu Aricescu, MRC-LMB Cambridge, UK and Abhay Kotecha, Thermo Fisher Scientific, Eindhoven, Netherlands.*

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System requirements

Compatibility: the Falcon 4 detector is available on Krios, Glacios, and Talos platforms (running under Windows 10) at 200 kV and 300 kV.

Key specifications	
Camera architecture	Direct electron detection
Sensor size	4,096 × 4,096 pixels ~ 5.7 x 5.7 cm^2
Pixel size	14 x 14 μm^2
TEM Operating voltage	200 kV, 300 kV
Mounting position	On-axis, bottom mounted, retractable
Frame rate	250 fps internal frame rate
File Format	EER super resolution up to 16k x 16k. All counted frames (~240 fps) accessible/written to disk, fractionation not required MRC
Detection modes	Electron Counting mode Survey mode (fast linear mode)
Imaging performance 4k x 4k DQE* in EC mode	DQE (0) = 0.90 DQE (½ Nq) = 0.70 DQE (1 Nq) = 0.28 *measured at 300kV, 1.5 e/p/s

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

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