Falcon 4i Direct Electron Detector

Unsurpassed image quality, more images per hour

The Thermo Scientific™ Falcon™ 4i Direct Electron Detector features faster detector speeds while maintaining unsurpassed image quality.

Higher detective quantum efficiency (DQE), efficient lossless data compression, and shorter exposure times further optimize productivity for 200 kV and 300 kV cryo-electron microscopy (cryo-EM). The Falcon 4i Detector is available as a standalone option and included with Thermo Scientific Selectris™ and Selectris X Imaging Filters.

The Falcon 4i sensor design is based on the large (14 µm) pixel layout of the Falcon product range. It combines a large signal, generated by large pixels, with unsurpassed low noise levels, due to a Thermo Scientific Ceta™ CMOS camera that is dedicated to electron detection and patented multi-frame Correlated Double Sampling (mfCDS). This produces an unsurpassed signal-to-noise ratio for unambiguous electron event detection (Figure 1), which is the basis of reliable sub-pixel-accurate electron counting.

Without any compromise on SNR, the Falcon 4i Detector has an increased internal frame rate of 320 fps, so typical exposure times are decreased by a factor of 1.3 compared with those in the previous generation Falcon 4 Detector (at 250 fps). With ever shorter exposure times, the “overhead” time of an acquisition becomes a dominant factor in total experiment throughput. Data stream optimization and camera control improvements have enabled a five-fold reduction of camera overhead, to only 0.5 seconds per acquisition.

In addition to improved speed, the Falcon 4i Detector also benefits from an improved DQE at high spatial frequencies. It now fully exploits the sensor’s accurate sub-pixel localization potential by using Electron Event Representation (EER), a patented technology, as its native file format. EER format uses lossless data compression and retains full spatial and temporal resolution, allowing super resolution, and avoids the need for fractioning data.

Key Benefits

High throughput for more images per hour

- Shorter exposure times due to 30% increase in frame rate
- Minimized overhead by optimized data handling and camera control
- Optimized for small coincidence loss

Unsurpassed imaging quality with high DQE

- High DQE over the entire spatial frequency range – ideally suited for small or difficult to detect proteins
- Improved DQE at high spatial frequencies in EER mode

Lossless data compression with EER

- Retains full spatial resolution, allowing true super resolution
- Preserves full temporal resolution and avoids the need for fractioning upfront
- Fully compatible with leading processing pipelines (Relion, CryoSPARC)

Automated data collection

Automated runs and unattended data acquisition through Thermo Scientific application software and support for third-party solutions via scripting interface

The Falcon 4i Detector is fully embedded in Thermo Scientific EPU and Tomography Software. The camera’s output is captured through the Data Management Platform (DMP). The optional EPU Quality Monitor (EQM) performs on-the-fly preprocessing (for motion correction and CTF estimation, including derived parameters) for evaluation of the acquired cryo-EM data during the actual acquisition process.

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

High imaging quality and productivity

The large pixel with low-noise and high-signal, combined with increased readout speed (320 fps) and optimized electron event localization, yields unsurpassed DQE in EER mode (where the electron impact coordinates are stored with one-sixteenth pixel accuracy):

- Large detection probability results in an unsurpassed DQE at low spatial frequency – DQE (0) = 0.92.
- Large detection accuracy provides unsurpassed DQE at higher spatial frequencies – DQE (½ Nq) = 0.72; DQE (1 Nq)) = 0.5.

High DQE at low spatial frequency lets users visualize and align smaller and more flexible proteins. Higher DQE at high spatial frequencies lets users achieve results with fewer images or higher resolutions with the same number of images. The super-resolution performance of the Falcon 4i Detector enhances the effective detection area. This significantly boosts data throughput, leading to faster results as more particles per image can be generated, thus reducing the need for more images.

The increased internal frame rate in combination with improved overhead yields a significant increase in throughput, depending on the use case: 1,200 images per hour have been demonstrated.

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 Detector) 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 ~1 electron per pixel will become a limiting factor for achievable resolution. Therefore, data collection with better temporal resolution (finer fractionation) is required. To this end the Falcon 4i Detector is equipped with EER in which the camera outputs the spatial location of each impacting electron at an accuracy of one-sixteenth (1/16) of a pixel for each raw camera frame (320 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 set up fractionation upfront, which would inevitably lose precious information when camera frames are summed. In short, with the Falcon 4i Detector, all counted camera frames are available with 16k x 16k oversampled event localization in a small file (Figure 2).

The EER file format feeds seamlessly into the Relion and CryoSPARC data processing pipelines, ensuring efficient processing and an overall enhancement of single particle analysis (SPA) and tomography workflow productivity. Alternatively, EER images can always be converted into common file formats (MRC, TIFF, LZW TIFF) when required.

Automated data collection

The Falcon 4i Detector is fully integrated in our single particle acquisition (EPU) and Tomography Software, enabling smooth daily instrument operation and data acquisition. The camera's output is recorded through the DMP. This platform also 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. Users can easily view all the project data and metadata at the microscope, as well as remotely through a secure connection, and can comment on 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, etc.) are visualized and allow on-the-fly analysis of incoming image quality. Based on its results, users can optimize acquisition parameters and filter data sets according to the quality indicators.

In combination with the Falcon 4i Detector, these solutions help to generate high-quality results quickly (Figure 3), confidently, and with the same ease of use for which EPU software is known.

Additionally, the proven radiation hardness allows the Falcon 4i Detector to benefit from a non-interfering, reactive dose protection mechanism for easier and faster setup of experiments.

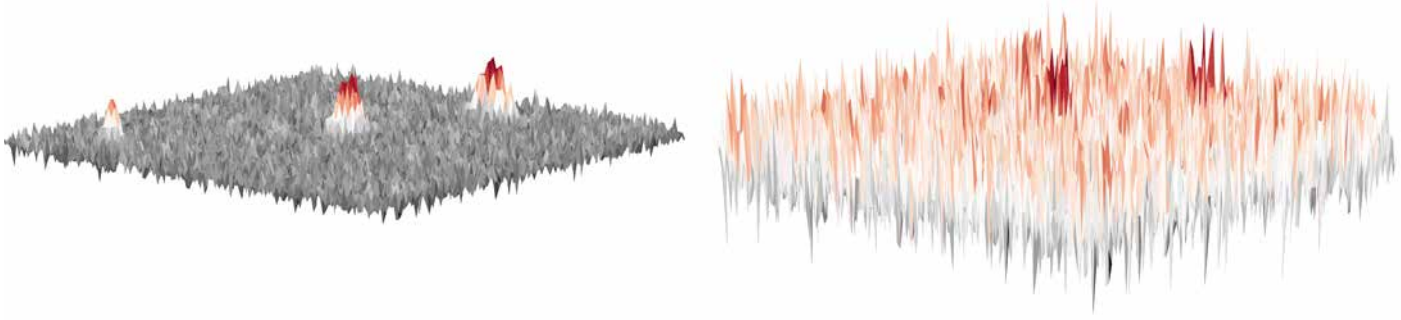
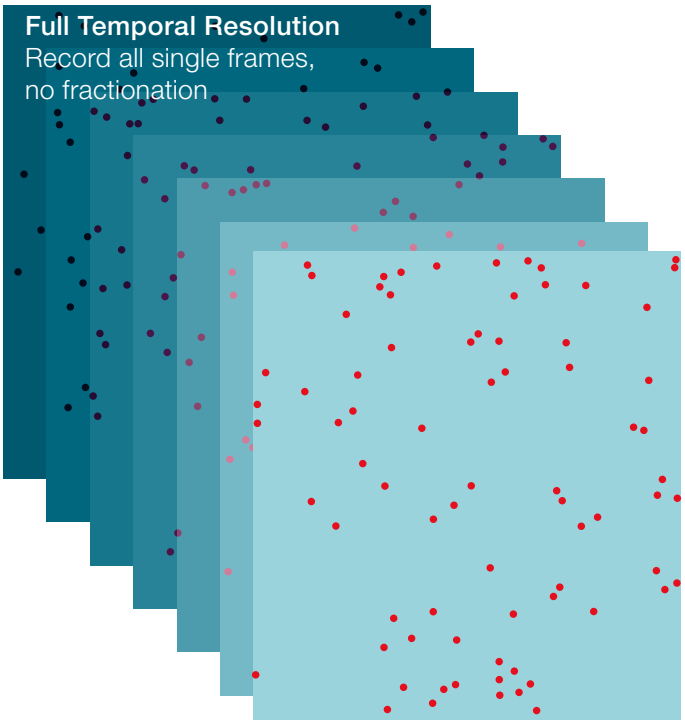


Figure 1. Electron event detection with background noise. Left: three single electron events are detected. Due to the large pixels on the Falcon 4i Detector with high signal and low background noise, the electron events are unambiguously distinguishable from the background. Right: the same electron events in a detector with smaller pixels generate less signal in a noisier background.



Full Spatial Resolution All localized events	
Coordinates	
x	**y**
3953.24	2845.63
919.78	1447.39
3864.43	3864.43
3606.05	1539.54
1758.86	2971.55
…	…
3983.58	531.96

Figure 2: Electron Event Representation: Counted events of all raw frames are available for processing with full temporal resolution (320 fps) and spatial resolution (events are localized to one-sixteenth of a pixel). This super resolution capability maximizes the benefit of the Falcon 4i Detector's superior DQE at high spatial frequencies.

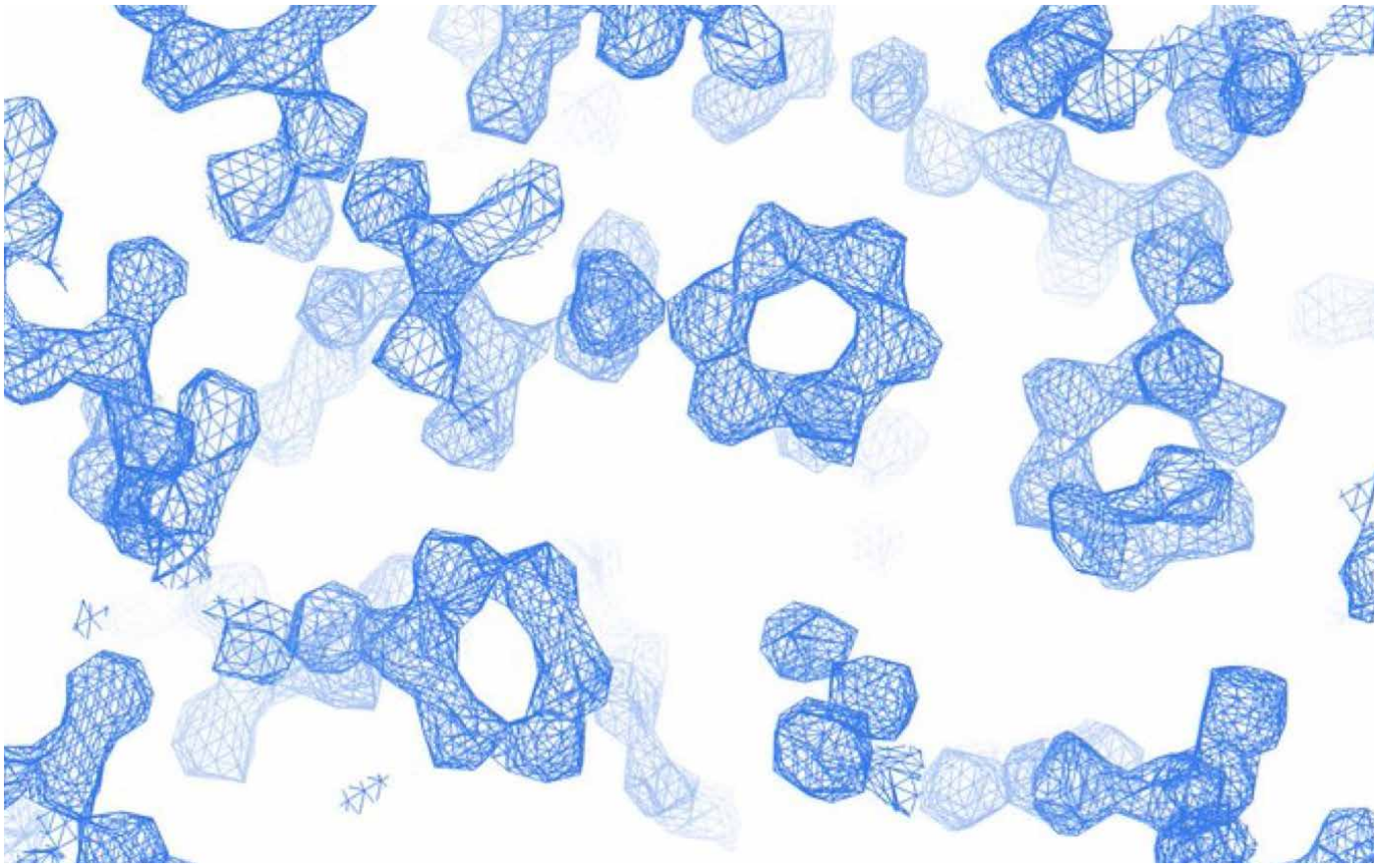
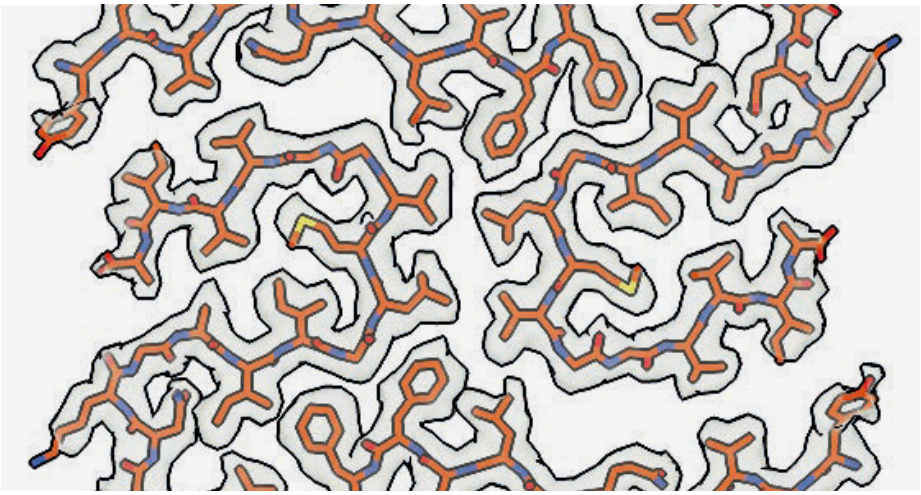


Figure 3. The Falcon 4i Detector offers high resolution and high throughput. Here, a 1.2 Å resolution reconstruction of apoferritin was created from 3,600 images of 297k particles acquired in 6 hours (EPU data was acquired on the Thermo Scientific Krios G4 Cryo-TEM). Recorded at a dose rate of 5.4 e/p/s.

System requirements

Compatibility: The Falcon 4i Direct Electron Detector is available on Thermo Scientific Krios™, Glacios™, and Talos™ Cryo-TEM platforms (under Windows 10) at 200 kV and 300 kV. A Falcon 4i Upgrade Kit is available for the Falcon 4 Detector. The Falcon 4i Upgrade Kit upgrade is also applicable for the Thermo Scientific Selectris and Selectris X Imaging Filters.

Falcon 4i Direct Electron Detector performance		
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 voltages	200 kV, 300 kV	
Internal frame rate	320 fps	
Frame rate to storage	320 fps (EER mode)	
Camera overhead time	0.5 s per acquisition	
File formats	EER (native), MRC, TIFF, LZW TIFF	
Lifetime (<10% DQE degradation)	5 years in normal use (1.5 Ge/px)	
Modes	Electron counting mode Survey mode (fast linear mode)	
Imaging performance in EER mode (4k x 4k)	300 kV	200 kV
DQE (0) DQE (½ Nq) DQE (1 Nq)	0.92 0.72 0.50	0.91 0.62 0.33



Cryo-EM structures of Amyloid-β 42 filaments isolated from human brain. Data was collected at a flux of 8 e/p/s; magnification of 0.74 A/pix; 40 e/A2 total dose and a throughout of 570 movies/hr resulting into a 2.5 Å reconstruction. (Data collected in collaboration with MRC – Laboratory of Molecular Biology, Cambridge) doi: https://doi.org/10.1101/2021.10.19.464936

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