APPLICATION NOTE

# Imaging beam-sensitive and low-contrast soft materials

The Thermo Scientific™ Falcon™ 4 Direct Electron Detector offers the best imaging quality and highest throughput for fast resolving of the finest structures of extreme beam-sensitive nanomaterials, such as soft materials.

Characterization of nanostructures in soft materials, such as macromolecular samples, is vital for understanding and manipulating their functions. However, it has been historically challenging to image soft materials by electron microscopy (EM) techniques due to beam damage and their low visibility due to weak scattering of light elements.

The Falcon 4 Detector, available on Thermo Scientific Talos™, Glacios™, Krios™ and Spectra™ (S)TEMs (scanning/transmission electron microscopes), is ideal for fast, high-resolution data collection on extremely beam-sensitive, soft materials. The Falcon 4 Detector is a CMOS-based direct electron detector with a total of 16 million (4096x4096) detector pixels and large, low-noise pixel design (14x14 µm for each pixel). It provides an unparalleled detective quantum efficiency (DQE) over the entire spatial frequency range with a full temporal resolution (250 frames per second). The sensor design is optimized for detecting and counting electron localization with sub-pixel spatial resolution (one-sixteenth of a pixel), as shown in Figure 1.

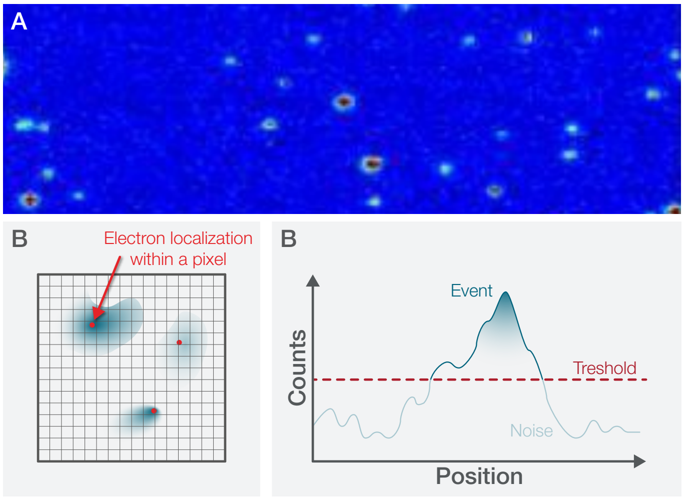


Figure 1. Single electron detection: (a) sensor image with individual electron impacts visible (energy deposited in sensor displayed as red dots); (b–c) schematics to show that electron counting determines the position of an electron hit on the sensor with sub-pixel accuracy (one-sixteenth of a pixel).

This reduces the unsurpassed low noise levels, while also improving electron localization for lower coincidence loss. When combined with cryo-EM, imaging of the soft materials with the Falcon 4 Detector can benefit from increased image contrast and also protect specimens from electron beam damage. Figure 2 provides direct visual representation of a soft structure in a native, frozen-hydrated state by the Talos Arctica Cryo-TEM with the Falcon 4 Detector. Next, the key benefits of using the Falcon 4 Detector on imaging soft materials are described separately.

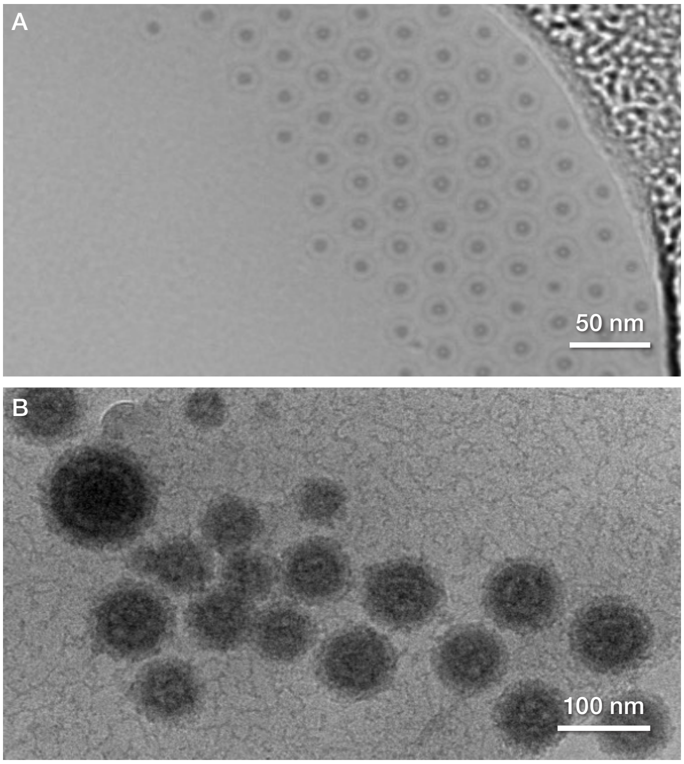


Figure 2. Low-dose, 4K TEM images acquired with the Falcon 4 Detector: (a) block copolymer micelles in water, sphere-sphere packing assembled from blends of PAA-PI-PS and PAA-PS (150/30 nm small spheres), (b) block copolymer giant spherical aggregations in water, with internal phase separation assembled from PAA-PI-PS (100–200 nm spheres); *sample courtesy of Prof. Thomas Epps, III, University of Delaware.*

## High DQE for the best image quality on nearly "invisible" soft materials

Soft materials are typically weak phase objects due to their low atomic number and related weak electron scattering cross-section. However, TEM images count mainly upon the intensity (amplitude, etc.) of the scattering electron wave. Therefore, imaging of soft materials with a scintillator-based camera is problematic, due to its low detection limitation to distinguish the weak contrast from background noise. This makes the soft materials, such as two-phase polymer, nearly "invisible" under the Thermo Scientific Ceta™ camera (Fig. 3a and 3c).

The Falcon 4 Detector is the next step in direct electron detection: a large, low-noise pixel design combined with an optimized sensor for electron event localization and coincidence noise reduction. The signal transfer performance for a detector can be described by detective quantum efficiency (DQE). A single electron hitting the sensor will deposit energy into the Si. On a conventional scintillator-based camera, this will appear as a blob-like random shape in the image. The Falcon 4 Detector can detect these individual impact events and present the result as a clean, single impact with sub-pixel spatial accuracy (Figure 1).

The Falcon 4 Detector has improved large detection probability and accuracy, resulting in unsurpassed DQE at the both low and high spatial frequencies. In particular, the unsurpassed DQE at low frequencies makes it ideally suited for detecting soft materials with weak contrast. As a result, the atomic structure of the two-phase polymer can be detected in Fig. 3b and 3d.

The Falcon 4 Detector can be operated in normal (integrating or linear) mode and electron counting (EC) mode. In linear mode, the direct detector integrates the total charge generated when a single electron hits a pixel and keeps read noise or the variation in the signal. In this mode, the Falcon 4 Detector can barely detect the light contrast of soft materials in low-dose conditions.

EC mode is an established method to boost the imaging performance of the Falcon 4 Detector, especially at low electron doses. The main benefit of EC is that it dramatically lifts the DQE across all spatial frequencies and completely removes signal read noise and variability associated with electron scattering.

The result is a massive reduction of noise level, such that the atomic structure of the soft materials can be clearly identified by the Falcon 4, even with a low dose of 30 e/Å2 (Fig. 3b and 3d). Note that no staining/modification was applied on the soft materials for enhancing image contrast.

Overall, the Falcon 4 Detector offers the best image quality for imaging soft materials without the sample modification required by conventional cameras, such as negative staining using uranyl acetate, to increase the contrast of the soft materials against the substrate.

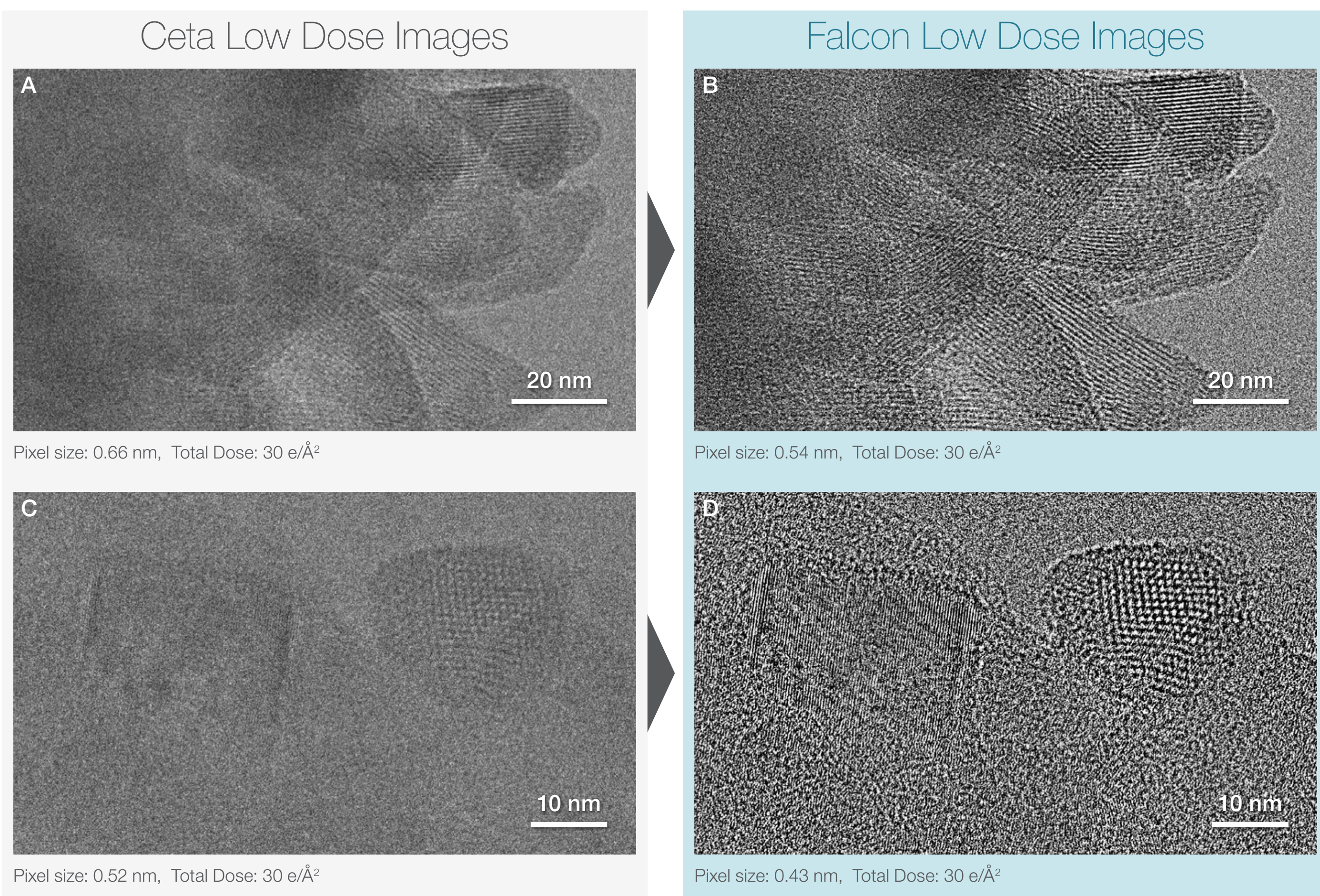


**Figure 3. Comparison of low-dose images taken by the Ceta Camera and the Falcon 4 Detector: (a,c) two-phase polymer containing C, N, H, O, Ca, K, Li, Cl elements, acquired with the Ceta Camera on a Talos TEM at 200 kV; (b,d) two-phase polymer containing C, N, H, O, Ca, K, Li, Cl elements, acquired with the Falcon 4 Detector on a Talos TEM at 200 kV. The lattice structure of the polymer is more visible with the Falcon 4 Detector.**
***Sample courtesy: Prof. Zhiyang Yu, Department of Chemistry, Fuzhou University.***

## High throughput for productivity and protection on beam-sensitive soft materials

Soft materials are generally very beam-sensitive, requiring short exposure times for imaging even under cryogenic conditions. For example, the PCL-PTHF block copolymer is one of the most beam dose-sensitive soft materials. The experimental data with the Falcon 4 Detector proves that a PCL and PTHF layered structure cannot survive a beam dose as low as 4 e/Å$^2$.

The Falcon 4 Detector can be used with much lower dose rates as compared to scintillator-based cameras. This allows you to use much lower electron doses while still getting better image quality. The combination of low dose rate and high image quality makes the Falcon 4 Detector ideally suited for high-quality tomography acquisition.

When compared to the previous generation of direct electron detection cameras, the Falcon 4 Detector has gotten a significant speed boost. The typical exposure times for the Falcon 4 Detector are reduced by up to 10 times, owing to its six times higher internal frame rate in combination with improved electron event localization. This significantly boosts data throughput (use case dependent: up to 250 images per hour can be achieved), leading to faster tomography acquisition and, subsequently, the need for less microscopy time. More importantly, together with improved radiation hardness, this also allows the Falcon 4 Detector to benefit from a much improved, non-interfering, reactive dose protection mechanism for easier and faster set up of experiments.

Additionally, the high sensitivity of the Falcon 4 Detector allows for the detection of incoming electrons at a dose rate as low as 1 e/p/s, enabling minimum electron dose imaging with fewer artifacts. This capability minimizes beam damage and therefore increases the yield in the imaging experiments on beam-sensitive soft materials.

## Efficient data compression and ease of use for tomography acquisition/reconstitution on soft materials

3D structural analysis of the soft materials (Figure 4) is critical to understand the relationship between their structure and function. Resolving 3D structures of soft materials requires reconstruction of large datasets from tomography acquisition. Data storage is becoming a more relevant topic in low-dose imaging of radiation-sensitive soft materials for 3D reconstruction beyond 2 Å resolution. The Falcon 4 Detector 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). EER offers a way to store data in an optimal way; after counting, the electron impact position is determined with sub-pixel accuracy; storage of the position of the electron and the frame time greatly reduces the amount of data to record without losing information. This powerful lossless compression preserves full spatial resolution (events are localized to one-sixteenth of a pixel) for tomography reconstruction of 3D structures.

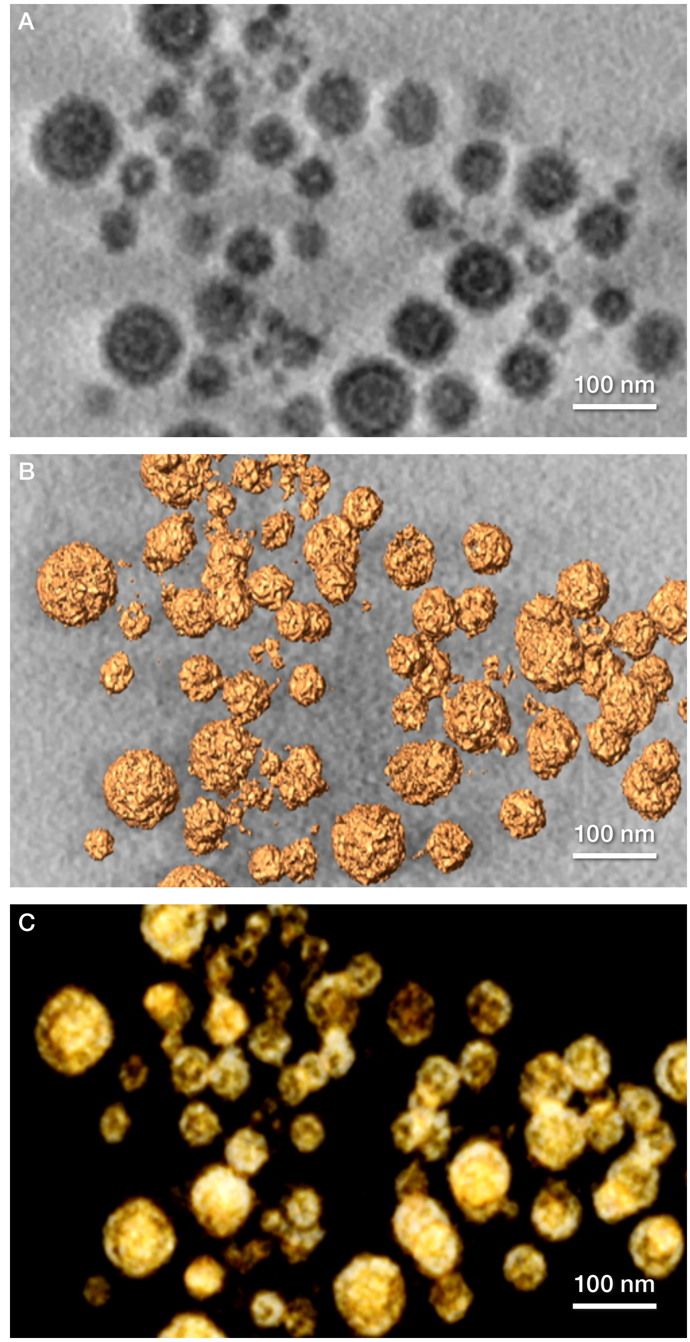


**Figure 4. Reconstructed tomography on beam-sensitive soft materials acquired with the Falcon 4 Detector on a Talos TEM at 200 kV: (a) cross-section view, (b–c) reconstructed volume rendering views of block copolymer giant spherical aggregations in water, with internal phase separation assembled from PAA- PI-PS (100–200 nm spheres).**
***Sample courtesy of Prof. Thomas Epps, III, University of Delaware.***

Additionally, the Falcon 4 Detector is fully integrated into both Thermo Scientific EPU Software (for single particle acquisition) and Tomography Software, allowing for smooth daily instrument operation and data acquisition.

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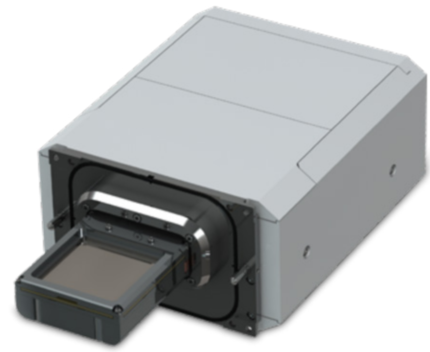

Figure 5. Falcon 4 Detector with a large (14 µm) pixel layout.

### System requirements

The Falcon 4 Detector is available on the Krios, Glacios, Talos, and Spectra (S)TEM platforms (running under Windows 10) at 200 kV and 300 kV.

The Talos (S)TEM is a 200 kV scanning/transmission electron microscope that delivers fast, precise, quantitative characterization of nanomaterials in multiple dimensions. With innovative features designed to increase throughput, precision, and ease of use, the Talos (S)TEM is ideal for advanced research and analysis across academic, government, and industrial research environments.

| Key specifications | | |
|---|---|---|
| Camera architecture | Direct electron detection | |
| Sensor size | 4,096 × 4,096 pixels ~ 5.7 x 5.7 cm | |
| Pixel size | 14 x 14 $\mu m^2$ | |
| 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<br>• Survey mode (fast linear mode) | |
| Imaging performance 4k x 4k DQE in EC mode | **300 kV**<br>• DQE 0 Nq 0.9<br>• DQE 0.5 Nq >0.7<br>• DQE 1 Nq >0.35 | **200 kV**<br>• DQE 0 Nq 0.9<br>• DQE 0.5 Nq >0.6<br>• DQE 1 Nq >0.23 |

Table 1. Key specifications of the Falcon 4 Direct Electron Detector

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