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Falcon 4i Direct Electron Detector – Application note

Contents

Introduction

The Falcon 4i Direct Electron Detector camera is a CMOS-based[1] direct electron detector with a total of 16 million (4096 x 4096) detector pixels, each 14 x 14 micrometer in size. The Falcon 4i Direct Electron Detector can be used in live mode for searching and focusing, or in acquisition mode to record images or stacks (movies) of images. The Falcon 4i can be operated in normal (Integrating or Linear) mode and electron counting (EC) mode. The Electron Event Representation mode (EER)[2] can only be used for the data acquisition, but not for other presets. Both counting and linear mode can only be used for acquiring images in presets other than for Data Acquisition. Data Acquisition can only give images in counting mode. Falcon 4i Direct Electron Detector operation is fully embedded in the Thermo Scientific TEM user interface and in the application software (EPU, Tomography, Maps and Velox).[1] direct electron detector with a total of 16 million (4096 x 4096) detector pixels, each 14 x 14 micrometer in size.

Electron counting (EC) mode

Electron counting is an established method to improve the imaging performance of a camera. The signal transfer performance for a system can be described by Detective Quantum Efficiency (DQE) which is defined as the squared-SNR transfer relationship. A DQE curve describes how well the output of a detector represents the input signal.

$$DQE_{dose}(\vec{f}) = \left(\frac{SNR_{out}(\vec{f})}{SNR_{in}(\vec{f})} \right)^2 \leq 1$$

Without going into much detail in this document, the process of counting optimizes the DQE as follows:

- A single electron hitting the sensor will deposit energy into the silicon.
- On a conventional scintillator-based camera, this will appear as a ‘blob’ of random shape and magnitude in the image (if the dose is low enough for blobs not to overlap). A direct electron detector detects these individual impact events and presents the result as a clean single impact, rather than a randomly shaped blob.
- If a single electron impact is perfectly presented as a single event, the DQE will be close to the theoretical optimum of DQE=1.
- Due to an improved design of the Falcon 4i chip (compared to Falcon 3), a smaller impact of the electron hitting the detector leads to less coincidence loss with a good event localization. As a result, the dose rates used in counting mode can be higher by a factor of 1.44x compared to the Falcon3, while experiencing the same amount of coincidence loss. Together with the improved frame rate (6.25x(320/250)= 8 x higher), the final dose rate per second on Falcon 4i can be increased up to a factor of 12x, compared to Falcon 3, without compromising image quality.

A DQE curve can best be interpreted in the following way: The value at the lowest spatial frequency indicates how well the signal hitting the detector is converted into signal coming out of the detector. This value should be as close to 1 as possible, indicating a high electron hit detection ratio.
The curve at higher spatial frequencies describes how accurately single electron events can be positioned. However, DQE curves are only meaningful for linear detectors. In counting mode, DQE measurements are heavily influenced by the dose rate used (due to the non-linearity introduced by coincidence loss), so that in the case of comparing counting DQE curves for different detectors must be done in the same conditions.

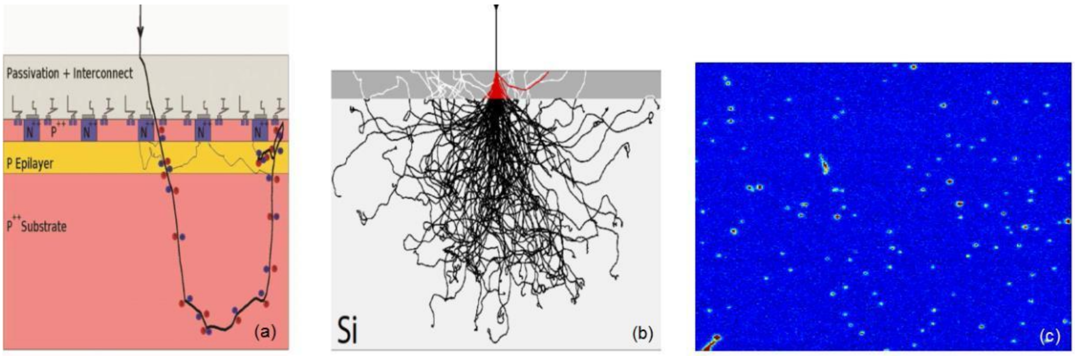


Figure 1. Single electron detection. (a) Random electron path in Thick Si, (b) energy deposited in thinned sensor displayed as red and (c) sensor image with individual electron impacts visible.

Falcon 4i Direct Electron Detector feature overview

The frame rate of the Falcon 4i is 320 frames per second. The image processing up to and including the counting procedure is implemented at the full frames per second. After counting, the full frame images can be summed without penalty. By summing after counting the full fraction rate is reduced to around 35 fractions per second. Further image processing and buffering can be implemented within the hardware constraints and real time requirements needed. If EER mode is used only events will be stored and the full time resolution of 320 fps is preserved.

Integrating mode

The Falcon 4i has two modes to run; Integrating mode (referred to as “Normal” or “Linear”) and counting mode. To convert the readout signal of each pixel to number of electrons, the Integrating mode requires to be calibrated. The determined conversion-factor is used throughout the imaging pipeline to have a link to the actual dose in e^-/nm^2 or dose rate e^-/nm^2.sec. Since each 9th frame is used to correct for noise (Multi Frame Correlated Double Sampling; MFCDS) signal will be lost. This makes the linear mode un-attractive for data collection on dose sensitive applications. Therefore, the mode is only used for imaging at LM mag range in EPU.

For certain applications (EPU) the Integrating mode is not available for data collection.

Counting mode

A single electron hitting the sensor will deposit energy which appears as a “blob” of random shape. To avoid coincidence loss, a dose rate is aimed for that results approximately in one electron hitting a group of 6x6 pixels per frame. Which adds up to a dose rate of approximately 0.025 (epf) [=1/(6^2)]. The counting algorithm will determine for each “blob” the center position as “electron hit location” per frame. This can be done with sub pixel accuracy, therefore generating super-resolution data. In standard counting the events are represented using a b-spline on a 4kx4k grid.

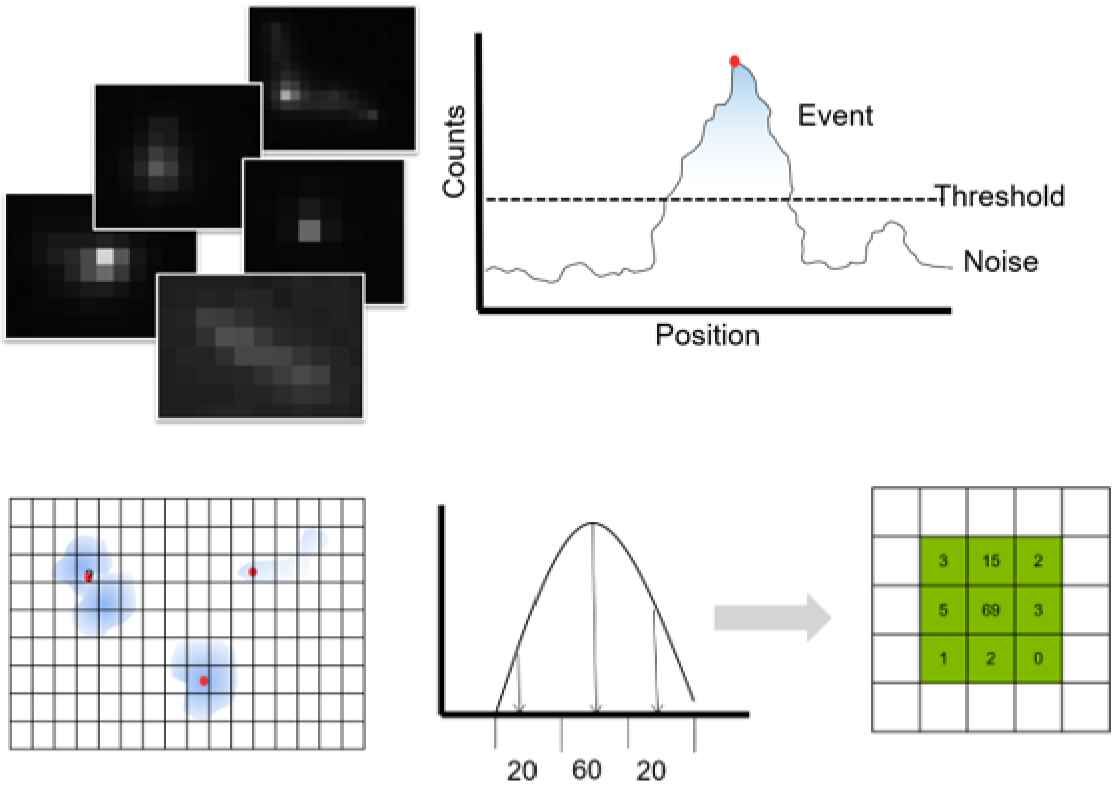


Figure 2.Counting determines the position of an electron hit on the sensor with sub-pixel accuracy.

Electron event representation (EER)

Data storage is becoming a more relevant topic in Low Dose imaging of radiation sensitive material for 3-D reconstruction beyond 2 Å resolution. Electron event representation offers a way to store data at shorter times and full spatial resolution (above a certain magnification and dose rate that is!). After counting, the electron impact position is determined with sub-pixel accuracy. Storing the position of the electron and the frame time greatly reduces the amount of data to record without losing information. Acquiring data in the EER format can be activated in the EPU software by selecting EER from the fractionation options. A separate application note is available that deals with processing of Falcon 4i EER data.

MFCDS

Multi frame correlated double sampling (MFCDS) is used to remove the reset noise generated normally when reading out frames. Because the reset noise for all frames in one MFCDS stack is the same, subtraction fully eliminates the reset noise. This reduction is helping the counting algorithm to better locate and detect the electrons hitting the sensor.

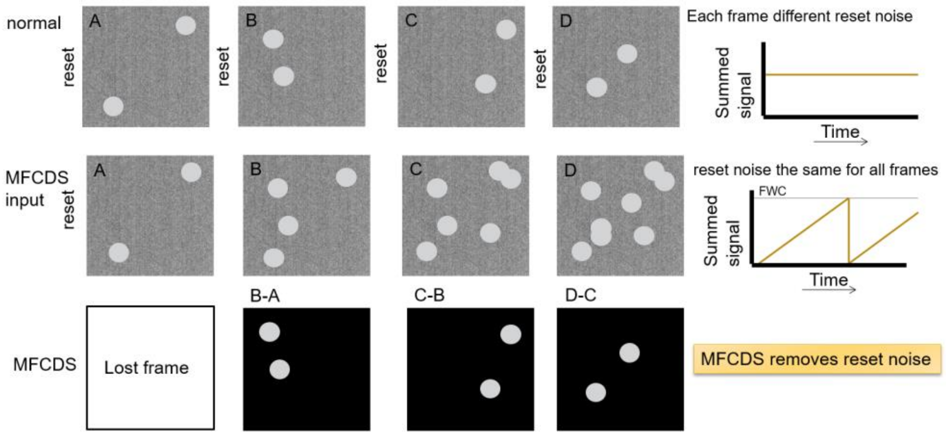


Figure 3 Multi frame correlated double sampling (MFCDS) explained. By only resetting the sensor once for frame readout, results in less readout noise.

Acquisition modes

In EC mode, the electron dose within a fraction is summed over the frames included in the fraction.

Warning for saturation:

Since all images are written with 2-byte data depth, at rare use case scenarios that data range could not be enough to record the actual signal. The image pipeline saturates at 2^15=32,768 (signed integer limit). Currently, the average ‘blob’ size is 307 counts total per primary event. Collect 100 (e^-/pixel) in a final image: 100*307=30,700 counts would just fit. Collect 200 (e^-/pixel), 200*307=61,400 counts, which would result in a over-saturated image.

However, the use case is intended for a total dose per image of approximately 40 (e^-/Å^2) at a pixel size of close to 1 (Å/pixel), this fits fine. On the other hand; if the pixel size would be 2 (Å/pixel) the allowed sample dose would be (2^2=)4*40=160 (e^-/pixel) which would lead to a saturated final image. The fractions will not saturate, only the final image. Therefore, in case a movie is recorded no effect would be seen in the further processing and alignment of the individual fractions. Since the movie processing software works with 32 bit internal architecture and can therefore handle the movies not resulting in over saturated fraction averaged results.

Response curve and coincidence loss

When the number of electron events per exposed frame, becomes too high, the response of the counting algorithm will be underestimated, meaning too many electrons arrive to the chip in the time of the acquisition of a single frame. Therefore, some pixels might get multiple hits. The resulting count in such a pixel will lead to a single counting event and thus to loss of electrons during the counting process. Figure 4 shows the amount of co-incidence loss or response of the chip over a wide range of electron dose rates.

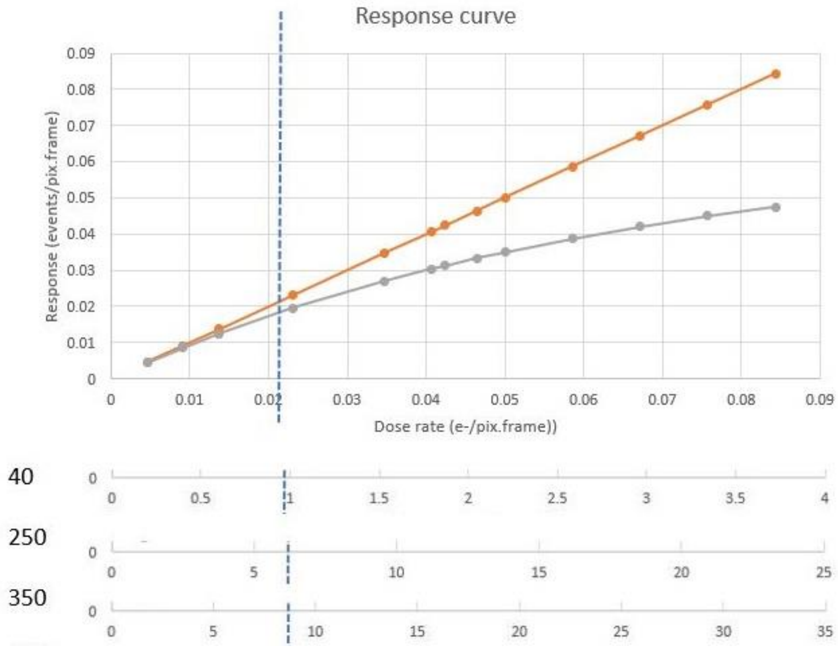


Figure 4 Response curve as measured for Falcon 4i: Orange curve indicates the applied dose rate on the camera. Grey curve shows the dose rate as seen from the counting algorithm. The vertical blue line indicates the dose rate of 0.025 (e^-/pix/frame). The various x-axes indicated are for dose rates in (e^-/pix/sec) for different frame speeds.

When acquiring a data set at a dose rate of 8.0 (e^-/pix.sec) approximately 17 % of the electrons are missed in the counting. However, in EPU the ribbon will correct for the loss and show the true value for dose rate in (e^-/$Å^2$.sec). From this the total dose is calculated multiplied by the exposure time for the full acquisition.

Analysis of the MTF and DQE data for Falcon 4i EC show that the DQE at ½ Nyquist remains good even until a dose rate of 13 eps. Recent 3D reconstructions of Apo-ferritin recorded at 10 eps resolve details up to 0.2 nm. When extrapolating the DQE data, a rate higher than 10 eps could still be useful. However, it could already require significantly more particles to average, to produce good signal close to 0.2 nm resolution. Further investigation is ongoing.

Is there an optimal dose rate for EPU data collection?

It depends on the situation. Initial modeling of the EPU data collection process indicated an optimal dose rate in the range from 4 to 5 eps for most efficient data collection speed (considering the image quality and the number of images recorded per hour). This is true for an optimal sample not having preferential orientations and close to perfect particle distribution. For other cases where larger number of images are required, due to preferential orientations, or sparse particle distribution, a higher dose-rate of even 15 eps (because of the increased speed of data acquisition) will be beneficial.

Prerequisites

At the beginning of your Falcon 4i Direct Electron Detector session, please make sure that

- the Falcon 4i DEC camera is installed & connected
- the camera power interface (CPI) is on.

- the image processing unit (CMTS) is installed and connected
- Athena (the storage server) is installed, connected, and running

Back

CMTS

Front

Athena

- the remote connection to Athena is active (see Remote connection to Athena)
- the Falcon 4i Direct Electron Detector target has the correct temperature (TEM UI indicator)

- gain reference images have been acquired and are up to date for the acquisition mode used (see Falcon Reference Image Manager)
- a magnification calibration for the physical pixel size of the Falcon 4i Direct Electron Detector has been saved (a service action, which is done upon installation)
- the TEM User Interface and TIA / Velox are installed and running
- the column valves are open, and the electron source and high voltage are on.
- the fluorescent screen is retracted.

Falcon Reference Image Manager

The Reference Image Manager is used to acquire gain reference images. In linear mode and EC mode, one gain reference image is required. Purpose is to make the camera response uniform across the chip for each pixel. And to correct for artefacts introduced by the counting algorithm (especially pronounced around camera defects and the chip border).
For sufficient quality of the gain reference images, it is advised to use the exposure times pre-defined in the Reference Image Manager:

- EC mode: Exposure time 10s, # images to average: 45.
- Linear (integrating) mode: Exposure time 10 sec, # images to average: 10.

Careful consideration about the dose rate for Gain reference acquisition for counting mode is less critical then was before. We now can obtain a gain reference at a dose rate 7.8 (e^-/pix.sec) (total of 5000 e^-/px). With 5000 e^- there is sufficient noise statistics in the gain.
For linear mode the dose rate required should be with a range from 20 – 200 (e^-/pix.sec).

Longer exposure times decrease the beam shot noise and therefore increase the quality of the gain reference.

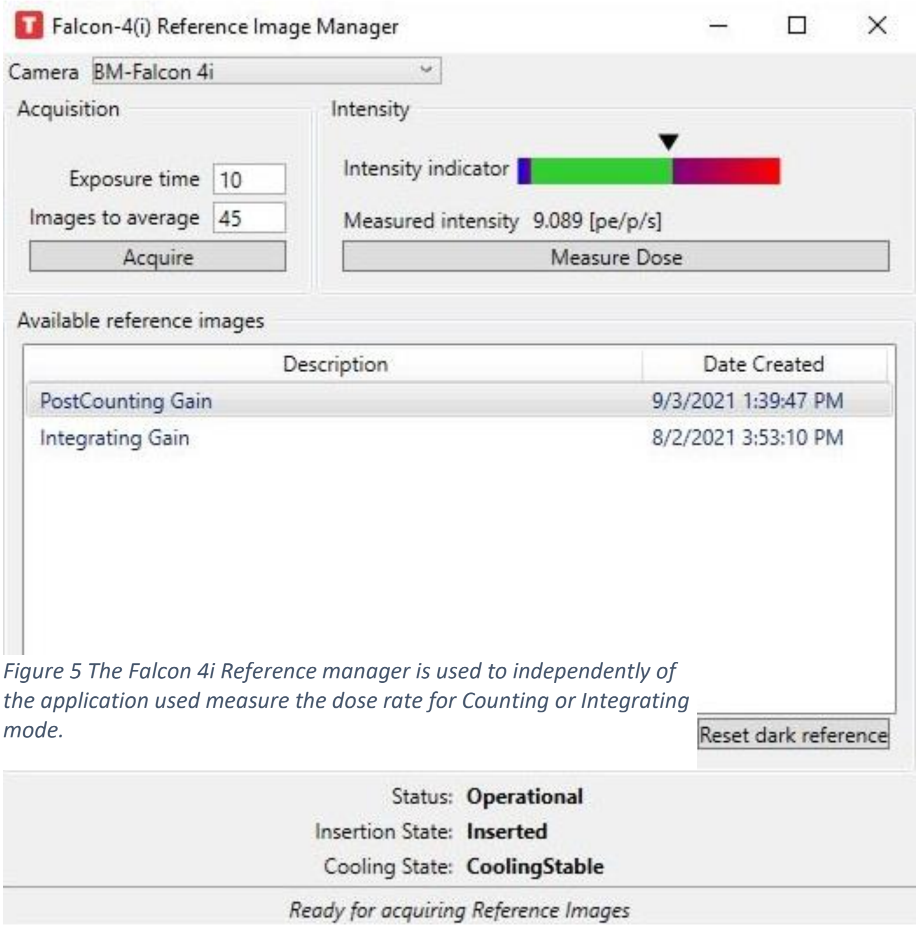


Figure 5 The Falcon 4i Reference manager is used to independently of the application used measure the dose rate for Counting or Integrating mode.

Gain reference acquisition

To acquire a suitable gain reference image, move the stage to a hole in the sample (or unload the sample from the stage). Set the microscope to the acquisition parameters you are going to use for your experiment

- Check that the beam illuminates the entire camera chip and that it is centered.
- Retract the flu screen and make sure that the camera is inserted.
- Take a test image and make sure that no beam fringes are visible. Create an FFT of the test image for un-wanted lines or an X, Y-cross in them. Basically, only a central peak should be visible in the autocorrelation.
- Measure the dose rate with the "Measure Dose" button in the Reference Image Manager. The dose rate should be within the green range of the intensity indicator in the Reference Image Manager. It is advised to record the gain for EC and EER at the 7.8 (e^-/pix.sec) dose rate.

The location of the EER gain reference file can be found in folder:

```
C:\Titan\Data\EF-Falcon4\Reference Images\300 kV\20210911_105204_EER_GainReference.gain
```

For further processing we refer to the processing Application note.

Remark: the lifetime of a gain reference is about 1 month.

Dark reference

Acquisition of dark reference images is done automatically (every 5 minutes) for the Falcon 4i Direct Electron Detector (see Reference images).

The Reset counting dark reference button re-sets the currently used dark reference. A new dark reference is automatically acquired with the next image acquisition.

Falcon Dose Protector

The Falcon 4i Direct Electron Detector is protected from damage by irradiation for a high electron dose by a dose protector. The dose protector is a reactive dose protection, meaning several frames are readout during acquisition to check if the applied dose is safe. In case the dose is too high, the beam will be blanked and if needed the camera will be retracted. For Falcon 4i the diffraction mode is disabled, and the camera is retracted when switching to diffraction.

Manual image acquisition - Velox

Project definition.

To define a project a user needs to specify where to store data and what folder name etc. The ‘Preferences’ window allows the user to define the Auto save path, sub-directories, File name construction (better avoid spaces in Subdirectories).

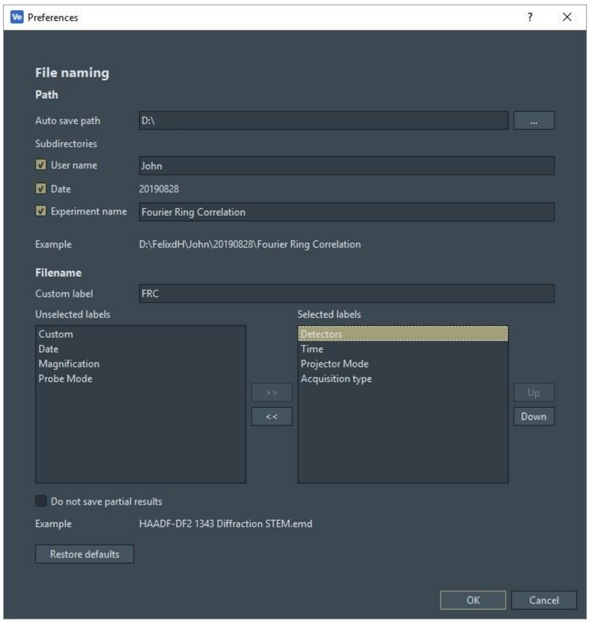


Figure 6 Preferences window to describe where to create the project.

Falcon 4i live mode

The Falcon 4i camera can work in live mode. In Velox from version 3.3 onward. The live mode can be operated under linear and counted mode conditions. Since the linear mode is operating with higher dose rates, consequently at faster frame rates a good image of the sample can be obtained. The frame rates depend on the dimension of the readout area; 4kx4k areas a maximum frame rate of 2-3 fps can be realized, at 1kx1k frame rates get better for live imaging close to 20 fps. At 10-20 fps (100-50 msec exposure times) the user will perceive close to live performance. Live searching and focusing is then a possibility. Velox will limit the maximum possible exposure rate according to the readout area size.

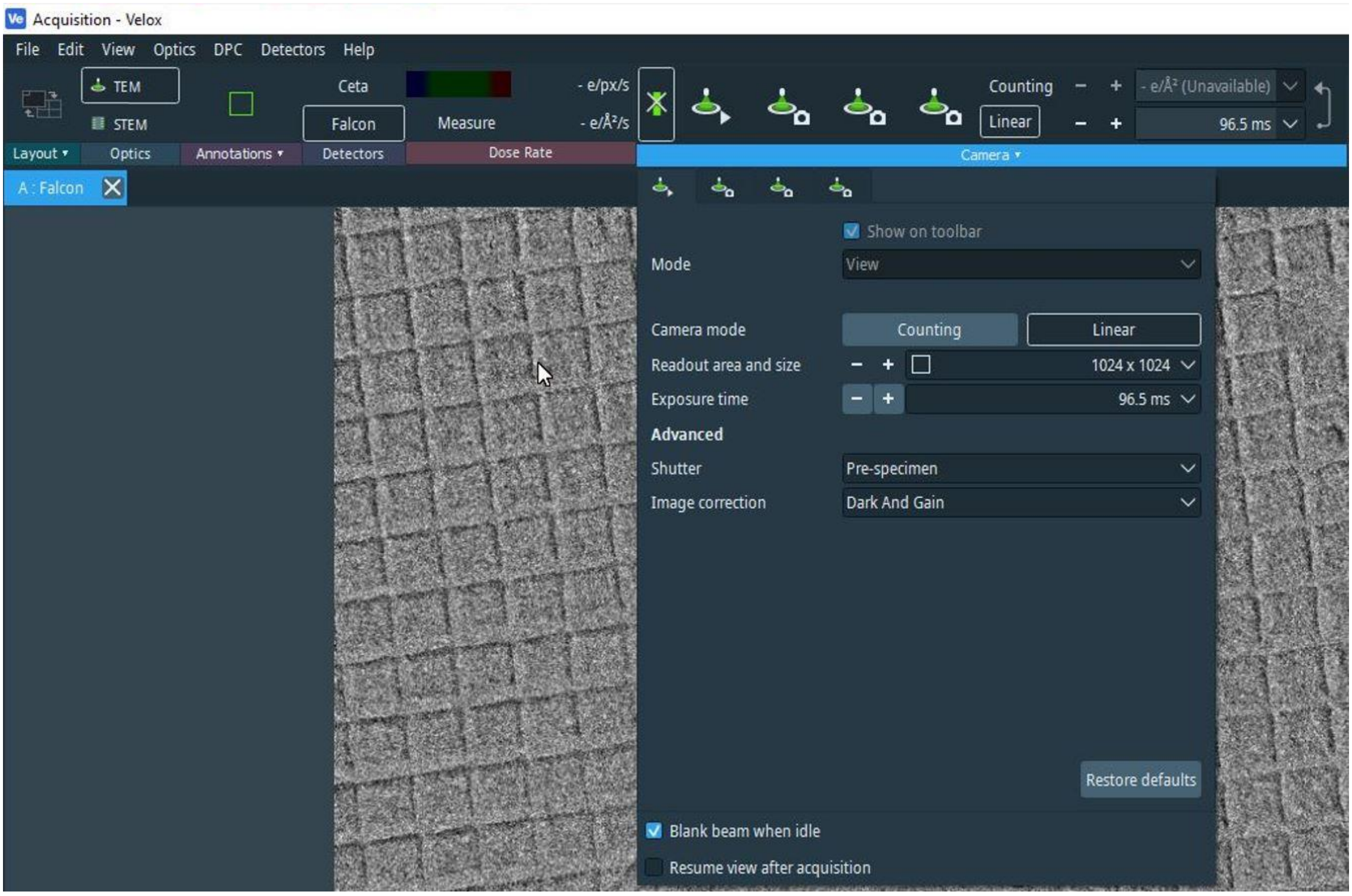


Figure 7 Setting up live mode for Falcon 4

NOTE: the radiation damage on the specimen will be higher at linear mode and reached sooner than for counted mode dose rates. Good practice is to keep an eye on the dose rate used and estimate what the total dose will be when operating in live mode on the same area for more than a minute.

“Double-click-center-feature”

When in live mode, by double-clicking using the left mouse button on a feature in the image, the stage will be triggered to move the object in the center of the image (when the magnification calibrations are valid). This takes away lot of time from the user trying to center some area using the joystick. More centering options are existing in Velox, please see Velox manual.

Acquisition definition.

Acquiring images using Velox requires the user to set the acquisition parameters. First, the user must measure the dose rate and take care the rate is within the required regime. Further, specify the following parameters: 'Show on tool bar' (if used: toggle it on), Mode, Readout area and size, Dose, Exposure time, align frames, Store fractions, Shutter, Image correction and if Blanker should be active when idle. Manual image acquisition settings of Falcon 4i Direct Electron Detector are defined through the 'Camera' settings window in Velox per acquisition button defined.

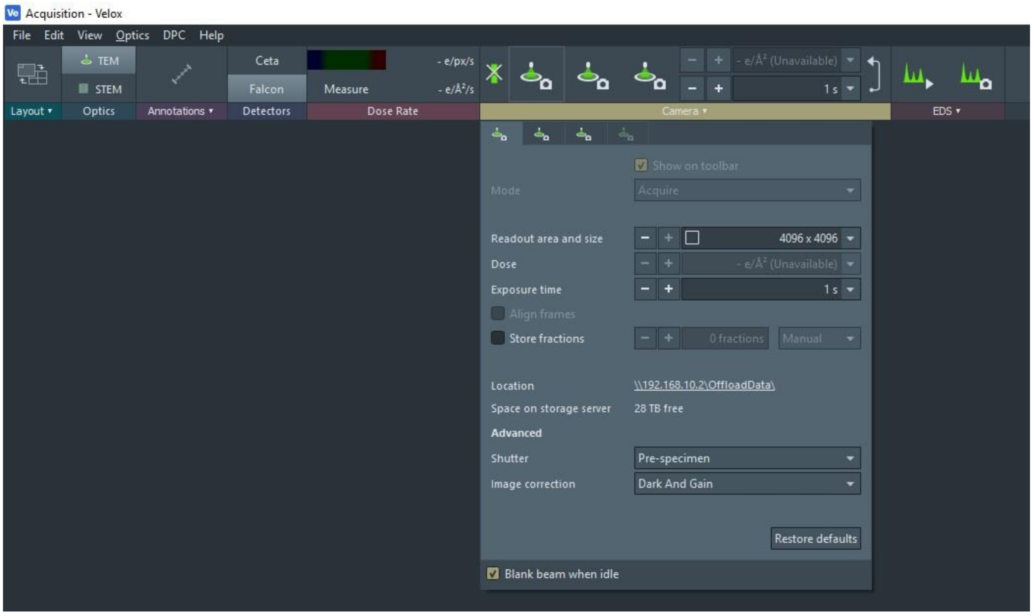


Figure 8 Setup for Velox acquisitions.

The second action needed is to set the required dose for the type of acquisition. The exposure time to set depends on the dose rate and the total dose that is needed for the acquisition. A typical total dose of 40 e-/Å2 is quite common. For a dose rate of 8.1(e-/pix.sec) at a pixel size of 0.5 (Å/px) an exposure time of 1.23 seconds would be the result. To acquire the image the user needs to click (left mouse button) on the previously defined

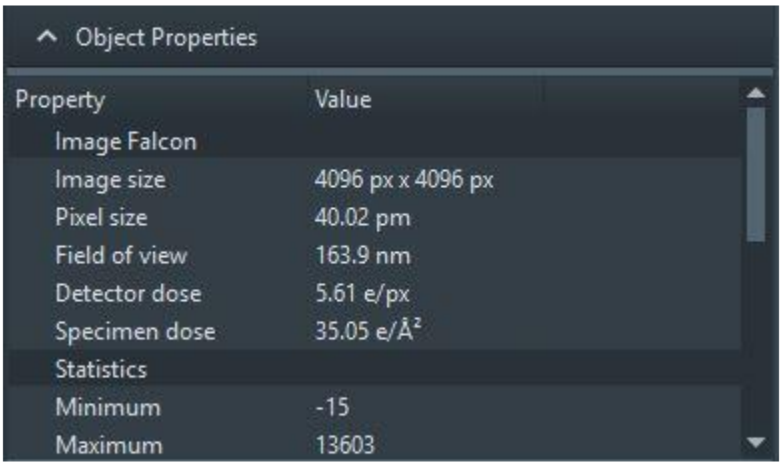


Figure 9 Object properties window shows the values of the relevant image parameters.

acquisition button . Subsequently, the recording will begin, and the recorded image appears in Velox. In case movies are acquired the resulting file is stored on the Athena server.

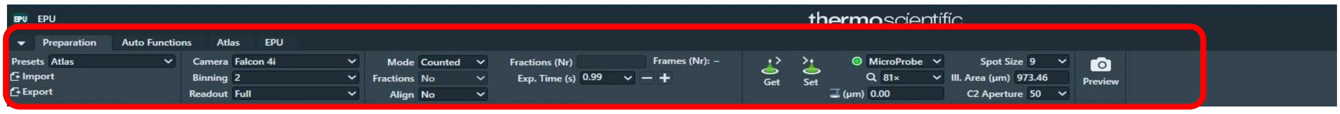
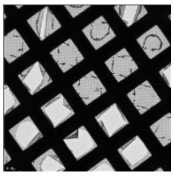
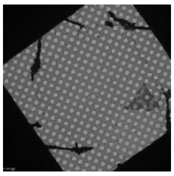
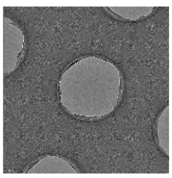
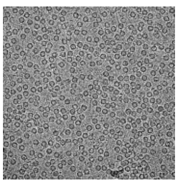


Figure 10 Shown is the Atlas preset ribbon.

EPU – setup

Table 1.1

Preset	Magnification	C2 aperture	Ill. area	Mode	Defocus	Electron dose
Atlas 	Lowest possible magnification that shows required details; no cut-off from cryo-box	Depends on magnification; typically, 150 µm	Filling the CCD square or energy filter entrance aperture marking on the fluorescent screen. If CCD button is pressed a green square is shown.	Micropro be	None	Should be displayed as 0.00 e/nm²; <1 nA on the flu screen
Grid square 	Adjust to image an entire grid square area on the camera.	Same as data acquisition: 50 - 70 µm	Illuminate entire grid square	Micropro be	-200 µm	As low as possible; <0.03 nA on the flu screen
Hole/Eucentric height 	Adjust to have one foil hole and the edges of neighboring holes in the field of view	Same as data acquisition: 50 - 70 µm	10-15 µm, depending on magnification	Preferably Micropro be	-25 to -75 µm	Ideally not over 0.3 e/nm²; as low as possible
Data acquisition; Autofocus; Drift measurement; Conditioning; Thon-Rings 	Adjust to pixel size 0.5 – 1.5 Å/px	50 – 70 µm, depending on desired beam diameter and dose rate	0.5 - 3 µm, depending on magnification (check for beam fringes), hole size, and desired dose rate. On Glacios/Talos 1.65 µm at 50 µm C2 (parallel ill. In nanoprobe).	Nanoprob e	-0.5 to -3 µm; not available for Autofocus	30 e/Å² over the entire exposure time (or higher, if required)

- Bring the sample to eucentric height.
- Activate the EPU Preparation tab.
- Define Camera Settings for all modes of EPU, including low magnification (LM) modes
- Define Optics settings for all modes of EPU, (see table 1.1)

Select the Data Acquisition preset from the Optics preset dropdown menu. Define the Direct Detector Exposure Settings:

  - Mode: Counted
  - Fractions: Manual, Auto, Maximum, EER

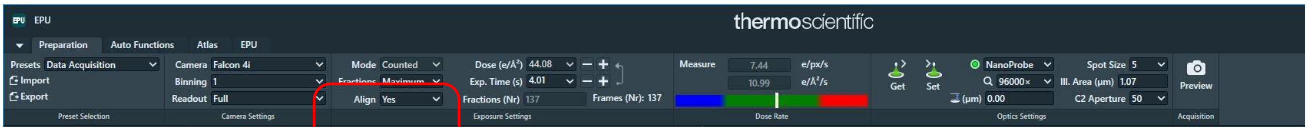


Figure 11 Data acquisition preset, indicated (red box) the Fractions and Align frames option.

- For Manual, Auto and Maximum the Exposure time determines how many “Frames (Nr)” will be recorded. This number of frames cannot be changed by user input, but only by increasing or decreasing the exposure time. Use the “Fractions (Nr)” field to subdivide the total amount of frames into fractions that will be saved as a data stack during data acquisition. Note that the number of frames per fraction is always divided equally over all fractions. We advise to use “Manual” and give a number that allows all frames to be distributed of the Fractions. If that is not possible the leftover frames will automatically be given to the last fraction. The last fraction is such cases can be 2 to 3 times larger in number of frames then the others, which should not pose a big problem for processing.
- However, to avoid large last fractions, make sure that the number of fractions to be written out is a multiple of 9 The same time adjust the exposure time such that the number of frames is also a multiple of 9. Adjust to the combinations in the Table below.

	18	27	36	45	54	63	72	81	90	99	108
81		27						9			
90	45	30		18	15			10	9		
99		33						11		9	
108	54	36	27		18			12			9
117		39						13			
126	63	42			21	18		14			
135		45		27				15			
144	72	48	36		24		18	16			12
153		51						17			
162	81	54			27			18			
171		57						19			
180	90	60	45	36	30			20	18		15
189		63				27		21			
198	99	66			33			22		18	
207		69						23			
216	108	72	54		36		27	24			18
225		75		45				25			
234	117	78			39			26			
243		81						27			
252	126	84	63		42	36		28			21
261		87						29			
270	135	90		54	45			30	27		
279		93						31			

Figure 12 Combination of possible number of frames to distribute in to fractions for a given total of Frames recorded in EPU.

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- For a 4.73second exposure we would get 4.73x 34.25 (=EPU-frames per second) = 162 frames to distribute over fractions. Say we want a fraction time of 0.25 seconds; meaning we have 4 fractions per second therefore a total of 4*4.73sec=18.9 fractions to acquire. Which is not a multiple of 9. The nearest multiple of 9is 18 (allowed by the table in Figure 12). Meaning 162 frames can be divided in 18 fractions. In this way we avoid that camera hardware will add the extra frames to the last fraction.
- To ensure motion correction is accurate, subsequent processing requires a dose rate of 0.5 to 1 (e^-/px/fraction), for a pixel-size around 1 (A/px). However, at this pixel size it is highly recommended to use the EER format, which gives full time resolution.

Note: The software will automatically change the exposure time to the next nearest time that represents a full number of frames. But not necessarily a multiple of 9.

- Export the EPU settings (Export button).
- Activate the Atlas tab. Set up a new session.
- Acquire an Atlas.
- Activate the EPU tab. Set up a New Session.

EPU_2.13.0

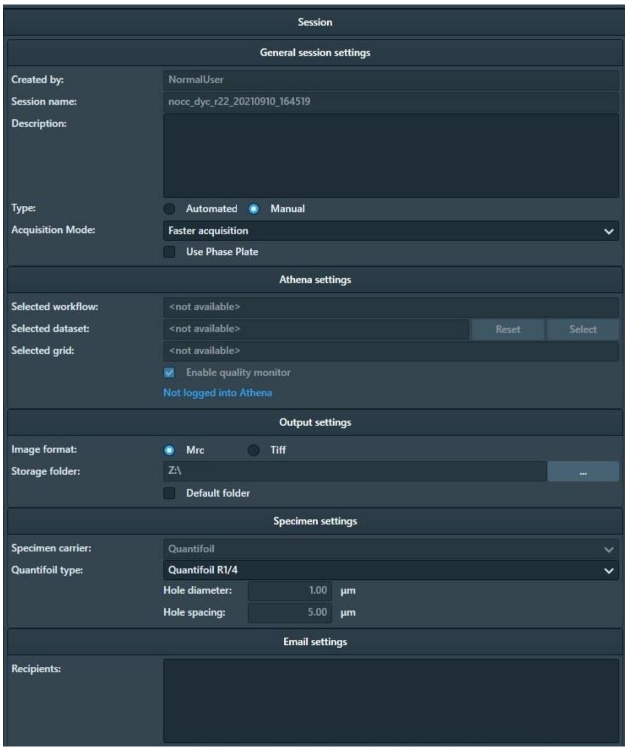

Figure 13 For each EPU run the user needs to setup a new session.

- Define a session name, a username, and an Output directory for the session
- Select Type: Manual selection or Automated (for large number of grid squares)
- Select acquisition mode: Accurate (Every foil hole accurately centered) or Fast (clustered acquisitions without hole-recentering, either by stage move or image shift =AFIS, up to 12 µm)
- In case Athena is present, also specify the 'Workflow', 'dataset', 'grid' type and if quality monitoring is needed. But log in to Athena first and create a project.
- Select Image format: MRC or TIFF (only applies to saved images not movie format)
- Select Specimen carrier and hole spacing, e.g. Quantifoil R2/2
- Press Apply.
- Follow the EPU setup according to the EPU Software user's guide.

EPU – electron counting/linear mode

- Select Mode "Counted" and "EER" in the camera Fractions Settings of the Data Acquisition presets
- The dose rate must be in the range of ~1 to 10 (e^-/pix.sec) for "Counted" and below 200 (e^-/pix.sec) for "Linear". Check on FluCam if screen current is in the range of 0.16 nAmp when in TEM mode. To measure the dose rate, navigate over a hole in the sample, use the Dose Rate section and press the "Measure" button (this will set the illumination settings as defined in the ribbon in the "Optics Settings"). The dose rate value will appear when the Data acquisition presets are selected, and the Falcon mode is set to Counted/EER. Be aware that the dose reading for the dose rate in (e^-/$Å^2$.sec) is corrected for co-incidence loss (TEM UI version 7.10 and later). This does not apply to the dose rate given in (e^-/pix/sec).
- Warning; to adjust to the right dose rate follow procedure given in the "tips and tricks" section.

Figure 14 Only Data acquisition preset has its own does rate measurement button. It is required to first measure dose rate for counted mode.

- When a total image dose has been specified, e.g. 40 $e^-/Å^2$, pressing the Calculate button will automatically set the required exposure time. The actual exposure time is automatically rounded to the nearest frame time or fraction time. The resulting dose and dose-rate is corrected for co-incidence loss.
- Pressing "Calculate" will also automatically set the number of fractions (in case the option Fractions: "Auto" has been selected). In electron counting mode, the fractionation scheme is defined so that each stored fraction contains approximately 1 (e^-/pix) dose for the specified Dose and Exposure Time.
- Copy the Data Acquisition presets to Autofocus and Drift measurement using the Get/Set functionality. The exposure time can be greatly decreased for these Presets, typically to 1 s or less.

“Align” option described.

For obtaining better images the “Align” option was added to the design of the camera. Once “Align” is activated (select “Yes” in the edit box for the option in EPU) the camera CAB/CMTS will apply a proven procedure to achieve alignment of the frames over the acquisition time.

Some factors limit the performance of the alignment algorithm. The exposure time and specimen/image movement are involved in proper working of the alignment. The “Align” option is not activated by software when the exposure time is shorter than 2 seconds, because of a too limited number of frames.

Two types of specimen(image) movement can be defined. First type is specimen drift. The second type is movement induced by the electron beam hitting the specimen area. When the electron beam hits the thin ice layer, the impact of the beam induces a movement of the layer. It is also observed that the image is affected by charge built up on the illuminated area for the first frames as described in the literature[3]. [3].

The first type, specimen drift, can be handled perfectly (Figure 4) by the implemented algorithm for the “Align” option. That is when the drift is less than 50 (nm/sec). Most EPU runs prove to have drift rates well below 50 (nm/sec). Since the first type of specimen movement is well described by a vector across to whole image for each pixel to be the same at a certain moment, the second type of specimen movement not necessarily exerts the same constant vector for each pixel in the image. Therefore, the “Align” algorithm will not solve movement of the second type. Motioncor2 will be required to reduce the second type of specimen/image movement over the fractions recorded (in using patch tracking).

Because specimen drift is corrected at frame level, we advise to use “Align” always, since within a fraction you then benefit also from drift correction. Motioncor2 cannot correct drift, occurring within the fraction time.

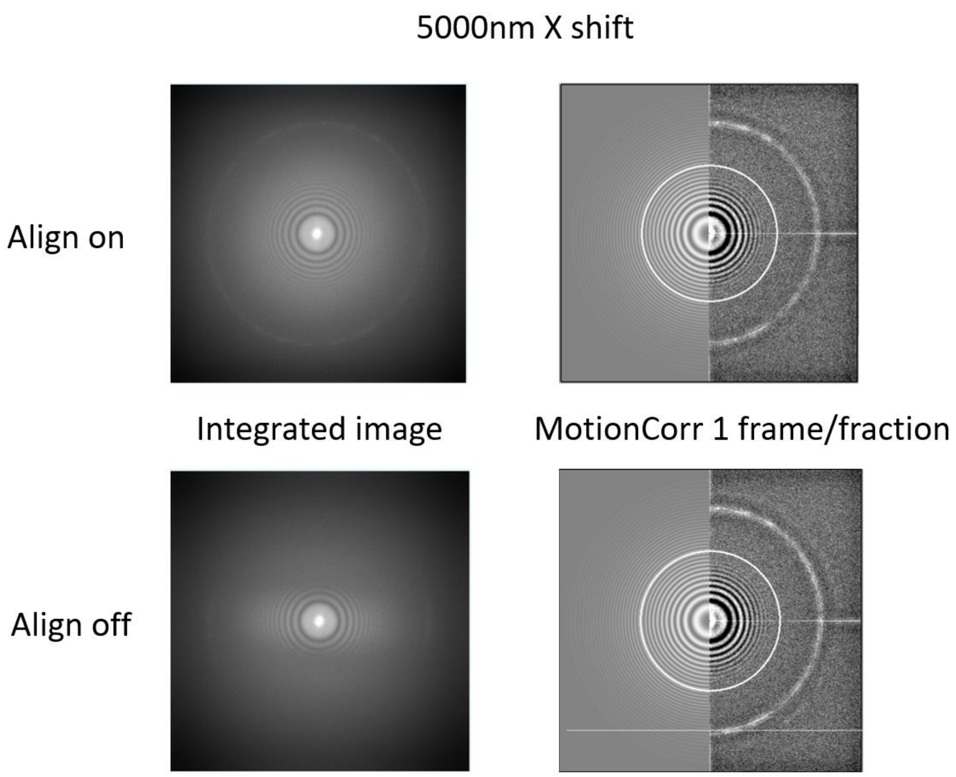

Figure 15 Effect of alignment on recently moved position to introduce some drift.

Dose Rate Measurement

There are several ways to measure the dose rate on the camera.

- Gain Reference Manager: "Measure Dose" button in Falcon Reference Image Manager; this will give the dose in (e/pix.sec).
- EPU data acquisition preset: "Measure Dose" button in the Data Acquisition preset will become available when selecting "counted" mode in the Data Acquisition preset in EPU.
- Velox detectors tab in ribbon: Choose Falcon camera and "Measure Dose" button will become available.

Data Storage Server: Athena

All images acquired through the "Acquire" button in the CCD/TV camera control panel or via Velox and during an EPU run are stored on Athena and can be accessed from the TEM PC via Remote Desktop connection.

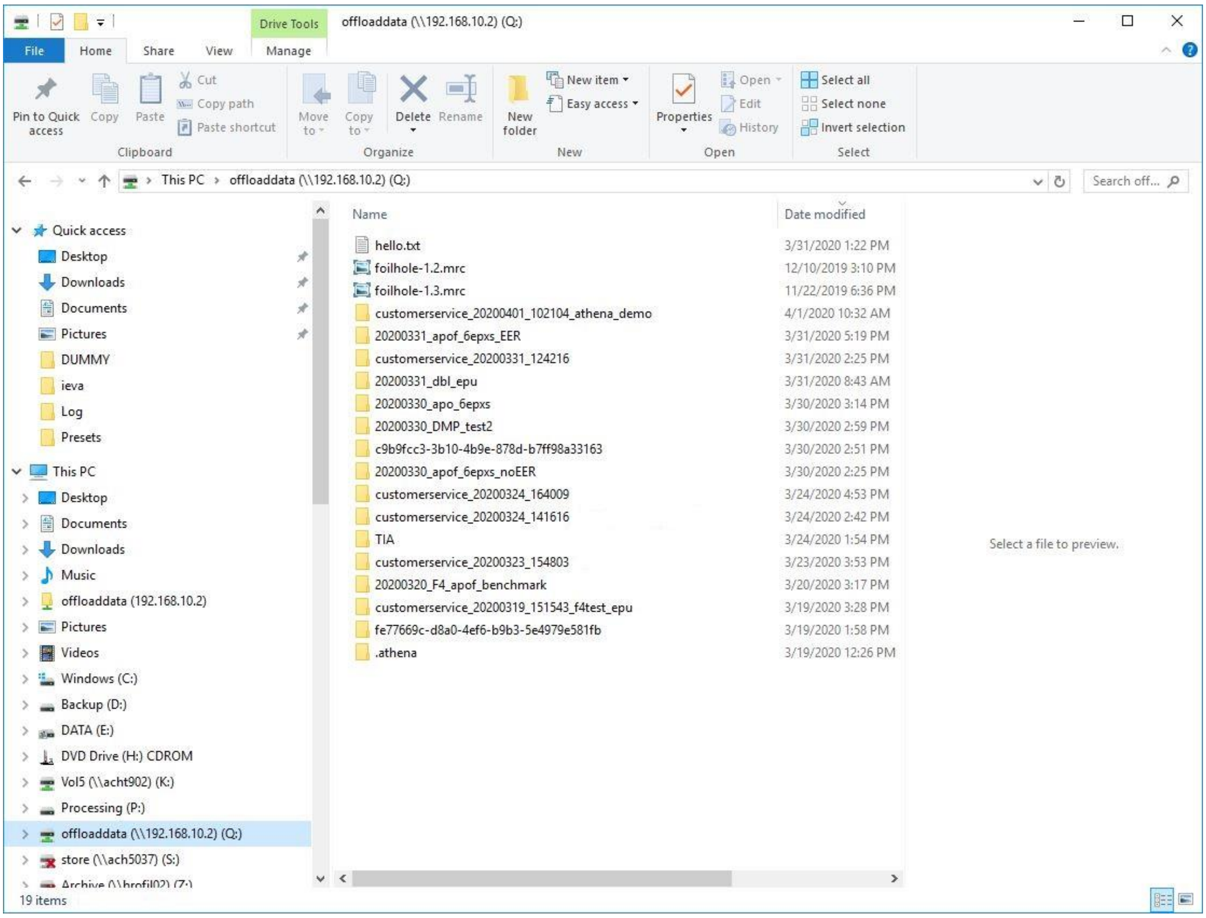

Figure 16Setup of the offload server.

Athena is connected to:

- Image processing unit (CMTS) by 10 Gbit link to transfer data
- User network by 10 Gbit link
- TEM PC by 1 Gbit link (see remote connection storage server)

The Athena storage server contains the hard disk drives for data storage (~40 TB). Image offloading to Athena is done in parallel to image acquisition.

Remote connection to Athena.

Remote Desktop connection setup is done by a Service engineer during the Falcon 4i Direct Electron Detector software installation. It is configured as follows:

IP Server Address 192.168.10.2 (Manual: 192.168.10.1)

IP address of the corresponding TEM PC network slot: 192.168.10.2

Should the remote desktop connection be lost, reconnect through the Remote Desktop Connection on the TEM PC.

Figure 17 Remote Desktop credentials.

Verify that the connection is established by identifying the StorageServerService in the Services tab of the Windows task Manager.

When the remote desktop connection is established, it is enough to mount the Storage Server as a network drive on the TEM PC via Windows Explorer. Map a network drive to ‘Q:\OffloadData’ on the storage server. Please contact the local service engineer for details.

Comments and tips

Intensity Indicator in Falcon Reference Image Manager

Dose measurement in EC mode is only reliable within a dose rate below approximately 13 (e-/pix.sec). Higher dose rates will cause saturation. To make sure to be in the correct dose rate range, roughly adjust dose in “normal” mode to about 13 (e-/pix.sec). At a screen current of approximately 0.1 nA (TEM mode), the switch to EC mode and measure and fine adjust again. If decreasing/increasing intensity in EC mode does not change in measured dose, the detector is most likely oversaturated. Further reduction will enhance the counting accuracy as depicted in the response curve.

The intensity indicator shows which region of dose rate to use for unperturbed and efficient imaging.

Falcon 4i EC example acquisition settings, pixel output, resulting image (stack) size

Pixel output in EC mode: Dose fractions and final image consist of summed frames with subpixel accuracy. Recorded as 16-bit signed for dose fractions and final image. In case of EC frame recording stored as 8-bit signed.

Automatic adjustment of acquisition time

Due to the discrete number of frames the exposure time is checked with the hardware and adapted to the nearest time that encompasses full frames, therefore a warning is given when the exposure time is adjusted.

Table 1.2

Falcon mode	Image dimensions	Readout area	Binning factor	Exposure time [sec]	Frames per fraction	# of fractions	Image data size	Bit depth
EC	4k x 4k	Full	1	1	1	37	0.6 GB	1 Byte
EC	4k x 4k	Full	1	2	2	37	1.2 GB	2 Byte
EC	4k x 4k	Full	1	4	12	12	0.38 GB	2 Byte
EC	4k x 4k	Full	1	10	10	37	1.2 GB	2 Byte
Falcon EER mode	4k x 4k	Full	Total Dose ($e^-/Å^2$)	Dose rate (e/pix.sec)	Exp time (sec)	pixel size	Image data size	byte/e-
EER	4k x 4k	Full	30	9.3	2.5	0.088	528 MB	1.5
EER	4k x 4k	Full	30	10	1.25	0.05	300 MB	1.5

All final 4k x 4k images, full frame, binning 1 are 32MB in size (16 Bit = 2 Byte bit depth).

Tips and tricks for setting up counting.

Some help for setting up the ‘counting’ mode is required since we have the problem of “wrong side of the response curve”. In changing, for instance, from Ceta camera to Falcon 4i camera ‘counting’ mode, a very different dose rate is required.

The problem arises from the fact that in the condition for either, the green arrow (at ‘say’ spot size 10) or, the red arrow (at ‘say’ spot size 2), a similar dose rate will be found from the counting algorithm. Due to co-incidence, electron hits are missed that should be counted and therefore a lower rate is given. This leads to artifacts in the images. Also take care with this when setting up for creation of a Gain reference!

The easiest procedure is.

1. Check the screen current for the mag you want to setup the Falcon 4i camera.
    a. Lower the FluCam screen. (un-blank beam if required)
    b. Screen current should be adjusted by changing spot size to a value close to 0.050 (nAmp).
2. Start the Falcon reference manager in the “Microscope Software Launcher”.
3. Select the counting mode for measuring the dose rate.
4. Lift the Flu-Cam screen and insert the Falcon camera.
5. Measure the dose rate. (It should be between 2 and 13 eps)
6. Adjust spot size to the dose rate you need.
7. Check screen current again.

Another way is to always start from spot size 11 to set the right dose rate for counting and a beam illuminating the full camera (FluCam).

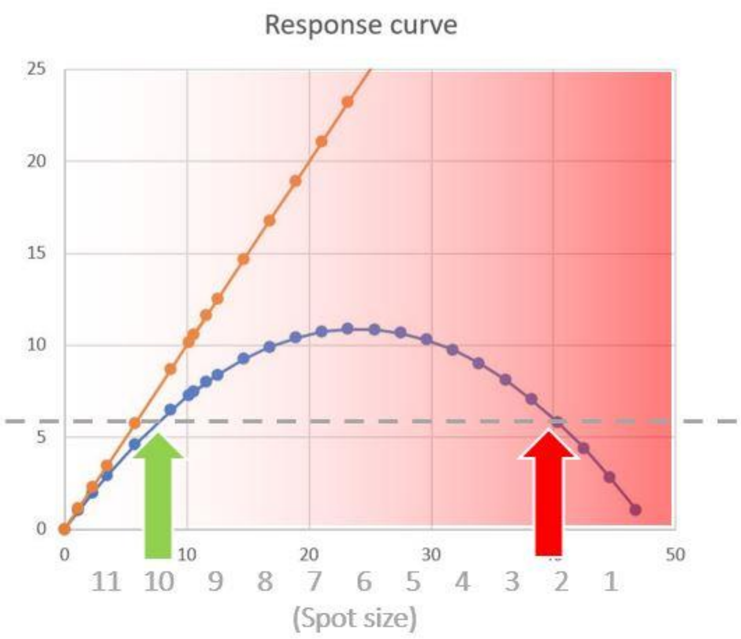

Figure 18 "Wrong side of the response" curve problem.

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