K3 IS User Manual

Models 1026 and 1027

Gatan, Inc.
5794 W. Las Positas Blvd.
Pleasanton, CA 94588
t +1.925.463.0200
service-gatan@ametek.com
www.gatan.com

Document # 1027.40004 Rev 002

Publisher
Gatan, Inc.

Technical editors
Gatan *In-Situ* Team

Document version
002

www.gatan.com

Table of contents

1 Regulatory compliance and safety instructions

IMPORTANT

Before installing and operating this product, and to avoid the risk of injury and potential hazards, read and review the regulatory pamphlet and follow all safety instructions.

2 Introduction

The K3® IS direct detection camera is a successor to the innovative K2® IS direct detection camera. The camera contains a direct detection transmission complementary metal-oxide-semiconductor (CMOS) detector, which gives the highest resolution images compared to any other electron-imaging sensor available today. The camera constantly collects images from the sensor at 1502 frames per second. These frames are then transferred at full-speed through a high-bandwidth link into custom, dedicated hardware designed for real-time, high-throughput image processing. Because of the detector speed and custom processing hardware, the detector can identify and record individual electron events (counting) as they reach the sensor. By counting every single electron event, the camera can eliminate the background noise typically seen in devices that simply read out the charge deposited by an electron striking a piece of silicon. By removing this source of noise, the camera delivers higher image quality and sensitivity than previously available in any electron-imaging device.

1.1 Configurations

The K3 IS direct detection camera system includes the dedicated hardware to enable the electron-counting operation.

The K3 IS direct detection camera system includes the camera, controller, microscope interface box (MIB), PC (K3 IS server), and a multi-Terabyte, high-speed RAID array. The K3 IS system was designed from the ground up to remove bottlenecks in the data acquisition process. One example of this optimization is to move the hardware responsible for electron counting from a standalone processor unit, as found in the K2, to custom, high-bandwidth hardware processing as integrated into the K3 IS PC. These and other improvements help remove bottlenecks associated with collecting large volumes of data.

The K3 IS is available with two different sensor formats, as detailed in Table 1.

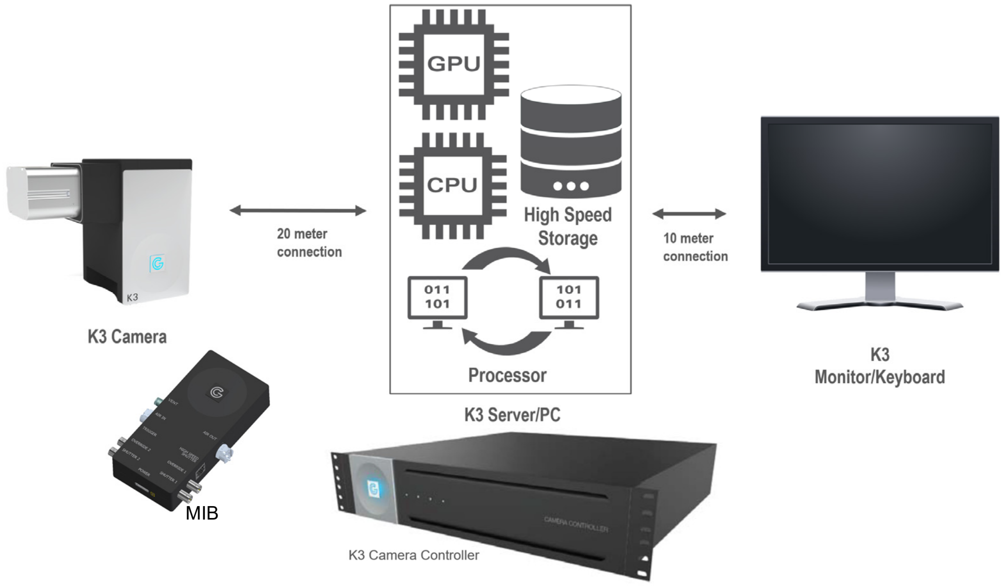


Figure 1: ***Overview of the K3 IS system components.***

Table 1: ***K3 IS models and sensor format.***

Model number	Product name	Sensor format (px)
1026	K3 IS Base	3456 x 3456
1027	K3 IS	5760 x 4092

The configuration of Model 1027 can be purchased directly, or this configuration can be achieved by upgrading either the K3 (model 1025) or the K3 IS Base (model 1026) cameras as detailed in Table 2.

Table 2: ***Upgrading to K3 IS (Model 1027) from other K3 models.***

Starting model number	Upgrade part number	Resulting configuration
K3, model 1025	1025.IS	K3 IS, model 1027
K3 IS Base, model 1026	1026.IS	K3 IS, model 1027

1.2 Counted mode

The K3 IS sensor was carefully designed such that the point-spread function (PSF) is slightly larger than the 5 µm physical pixel size. As a result, each incoming electron deposits signal in a small cluster of pixels. The high-speed K3 IS electronics can recognize, locate, and count each electron event (at 1502 frames per second at every pixel).

The K3 IS can count electrons with sub-pixel precision (super-resolution mode) due to the advanced electron counting algorithm, and the carefully designed pixels, the accuracy of electron localization on each pixel is improved to one-quarter of a pixel. This results in a quadrupling of the effective number of pixels (pushing beyond the Nyquist information limit to even higher resolution), as well as a further improvement of the detective quantum efficiency (DQE) and modulation transfer function (MTF). Practically, this means that the field of view can be increased for the same end resolution allowing the researcher to capture much more data per image.

In Counted mode, each electron event nominally results in one count in the image. The raw images are dark subtracted, gain-normalized, and processed in hardware to detect individual electrons. The resulting summed electron counts are displayed as an image. In this mode, keep the beam at low levels (4 – 40 e^-/pixel/s) to avoid coincidence loss (Section 1.19). This mode can be used with binning levels of 0.5x, 1x, 2x, and 4x. Binning 0.5x corresponds to the super-resolution mode found in the K2 camera.

In the K3 IS Counted mode, noise can be greatly reduced, particularly because with Gatan's transmission sensor, no scattered electrons are re-entering the detector from below.

1.3 Linear mode

In Linear mode, the charge is collected, integrated during the exposure, and read out to provide the image. No electron counting is performed in this mode. The sensor electron charge proportional to the total energy deposited by the incoming primary electron is accumulated. This mode benefits from direct detection's improved DQE arising from the transmission detector and inherent higher conversion efficiency. In Linear mode, there is no requirement to keep charges separated, and this allows for dose rates much higher than in Counted mode. Of course, if very high doses are used, the user must remain aware of the total dose capability of the camera and avoid saturation (See Section 1.21 Saturation for more information) as well as unnecessary beam/electron exposure to the camera.

1.4 Raw frames and image frames

The K3 IS reads the sensor at a rate of 1502 frames per second. This sensor readout rate enables real-time electron counting and minimizes coincidence loss in counting mode at dose rates up to 40 e/pix/s. These raw sensor frames are accumulated to create the image frames written to disk, where the number of accumulated frames depends upon the camera mode of operation. More information on the camera modes that impact raw frame accumulation can be found in Section 1.16.2.

Table 3: ***K3 IS imaging modes and raw frame accumulation.***

Imaging mode	Raw frame accumulation
Standard, non-CDS	20
High-speed, non-CDS	5
Standard, CDS	10
High-speed, CDS	3

In the full ROI mode, the sensor is read at 1502 frames per second, and the readout rate is scaled for the other ROI modes. Scaling the sensor readout rate in the different imaging modes leads to mode-dependent frame rates that are not integer frame per second values. The available framerates are inherent to the sensor readout and cannot be altered. The maximum framerates for each imaging mode can be found in the tables in Section 1.15.6.

3 Getting started

1.5 Power-up sequence

To start from the power off:

1. Close DigitalMicrograph® (DM) software if it is open.
2. Turn on power to the K3 IS camera controller using the blue, G touch button.
3. Wait at least 3 min for the K3 IS camera to fully power on.
4. Turn on the small red power switch on the K3 IS PC.
5. Log into to the K3 IS PC:
   - Username = valuedgatancustomer
   - Password = $admin
6. Launch DM software.
7. Wait for the camera monitor light to turn green and for the camera temperature to reach -20 °C.
8. Insert camera and start live view.

IMPORTANT
When ending a DM session, do not quit and immediately restart DM software. DM software requires a few seconds to save data when closed

1.6 Power down sequence

Under normal circumstances, you can leave the K3 IS camera running. If necessary, you can power down the K3 IS camera completely.

To completely power down the K3 IS camera:

1. Warm up the camera by setting the temperature to 20 °C.
2. Close the DM software.
3. Turn off the PC using the Shut Down option.
4. Turn off the K3 IS camera controller using the blue, G touch button.

1.7 Insertion and retraction

There are three ways to insert the K3 IS:

1 Right-click on the K3 IS camera icon in the **Microscope Diagram** and select **Insert**.

2 Select **Insert Camera** from the **Camera** menu using the list of pull-down menu items in DM.

3 If not already inserted, DM will automatically insert the camera when **View** or **Capture** are clicked from the K3 camera palettes.

There are three ways to retract the camera:

1 Right-click on the K3 IS camera icon in the **Microscope Diagram** and select **Retract Camera**.

2 Use the **Camera** dropdown menu.

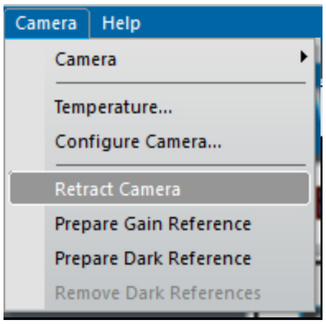


Figure 2: DM drop down menu to retract the K3 IS.

1 The camera will automatically retract if inserted and unused for longer than the auto retraction delay that has been set. See 1.9 Auto retraction for more information.

The insertion and retraction process for K3 takes about 20 s.

1.8 Insertion indicators

When the camera is inserted, the camera logo on the camera lights up. Also, the camera icon within the **Microscope Diagram** in DM changes from the retracted state to the inserted state, as shown in **Error! Reference source not found.**.

Figure 3: Camera icon in retracted state (left) and inserted state (right).

1.9 Auto retraction

The K3 IS includes an auto retraction feature that keeps the camera safe by retracting it if it is not in use for longer than the time specified in the **Auto Retraction** field from the dialog box accessed from the menu **Camera** → **Configure Camera** → **Insertion**. The default auto retraction time to 600 s.

IMPORTANT
Auto Retraction delay should be longer than the longest intended image; otherwise, the camera may unexpectedly retract if you are taking several long, single exposures (typically 30 s or longer).

1.10 Camera status

A **Quick Scan** feature is included to assess the camera's health. This scan is available from the setup icon in the **Camera Monitor** palette, as shown in **Error! Reference source not found.**.

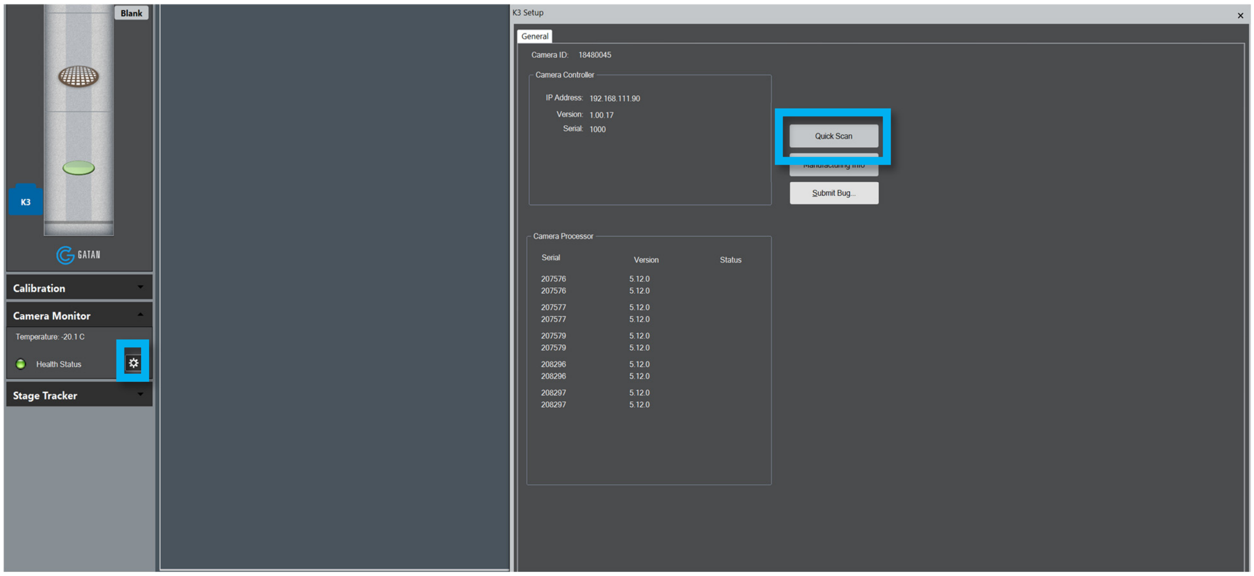


Figure 4: ***Quick Scan location within Camera Monitor setup.***

After pressing the **Quick Scan** button, a screen appears with numerous details showing camera status. When a quick scan is performed by an end-user, a successful result is for all lights to be green.

If one or more lights are not green, then an error condition exists within the camera system that should be resolved before data acquisition. Some error conditions can be corrected by the end-user, while others require Gatan Service.

1.10.1 User correctable error conditions

If one or more of the Sync Status, SATA Link Status, or Memory Status lights are not green:

1 Press the **Reset Data Link** button.

2 Wait for 1 min.

3 Close **Quick Scan**.

4 Reopen **Quick Scan** to verify all status lights are green.

If the lights are still not green:

1 Press the **Reset Data Link** button.

2 Wait for 5 min.

3 Close **Quick Scan**.

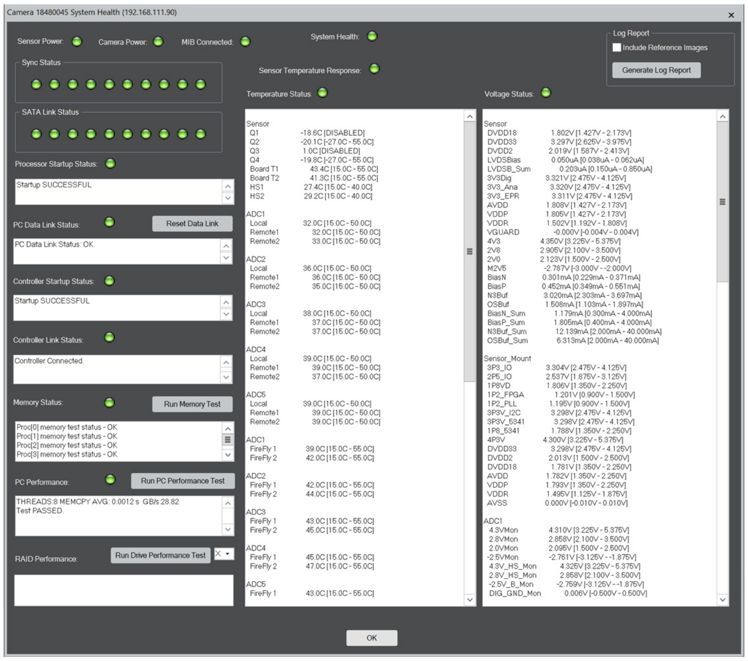


Figure 5: ***Typical results from Quick Scan showing healthy camera operation (all green lights).***

4 Reopen **Quick Scan** to verify all status lights are green.

If the lights are still not green:

1 Shut down the camera and PC using the power off sequence described in Section 1.6.

2 Restart the camera and PC using the power-on sequence described in Section 1.5.

If the problem persists after a full restart of the system, contact your local Gatan service office as listed at www.gatan.com/contact.

1.10.2 Error conditions requiring Gatan service

If the **Temperature Status** or **Voltage Status** lights are orange, you can continue to use the camera but should report this to Gatan service by contacting your local Gatan service office as listed at www.gatan.com/contact. To help the service team diagnose the issue, press the **Generate Log Report** button in the top right corner of the **Quick Scan** window, and send the resulting *.zip file when you contact service.

WARNING
If any of the temperatures or voltages are critical, the camera will shut down to prevent further damage.

1.11 Setting temperature

The temperature of the K3 IS must be cooled to its operating temperature before acquiring data and returned to ambient temperature when not in use.

1 The **User Mode** must be set to **Power User** to change the camera temperature. See 1.25.1 User modes for more information.

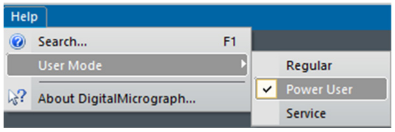


Figure 6: Set User Mode to Power User to change camera temperature.

2 In the **Camera** menu, select **Temperature…** and enter the setpoint value. The typical operating temperature is -20 °C.

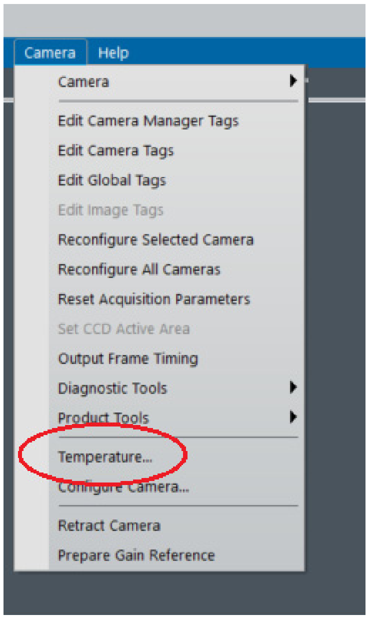


Figure 7: Temperature option in Camera menu.

WARNING
If the camera chamber needs to be vented, set the camera temperature to +20 °C, then wait for the warm-up to complete before venting.

The camera temperature is displayed in the **Camera Monitor** palette on the left side of the DM window.

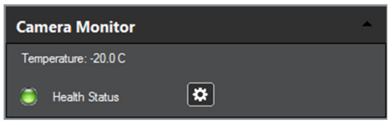


Figure 8: K3 IS sensor temperature in Camera Monitor.

WARNING
Never operate the camera above +22 °C, or with a vacuum above 1.5 E^{-5} Torr.

Check the flow of cooling water periodically. If the flow rate of the cooling water deviates significantly from the value initially set (~15 L/h), make sure the lines are not obstructed and adjust the pressure regulator to bring the flow back to the original level. If the water flow stops while the Peltier cooler is on, damage to the camera may result.

WARNING
Ensure there are no airflow restrictions around the PC, K3 IS power supply, and monitor.

1.12 Shutter configuration

The **User Mode** must be set to **Power User** to access basic shutter configuration settings. See 1.25.1 User modes for more information. Once in **Power User** mode, basic shutter configuration is accessed through the **Camera** → **Configure Camera…** dropdown item.

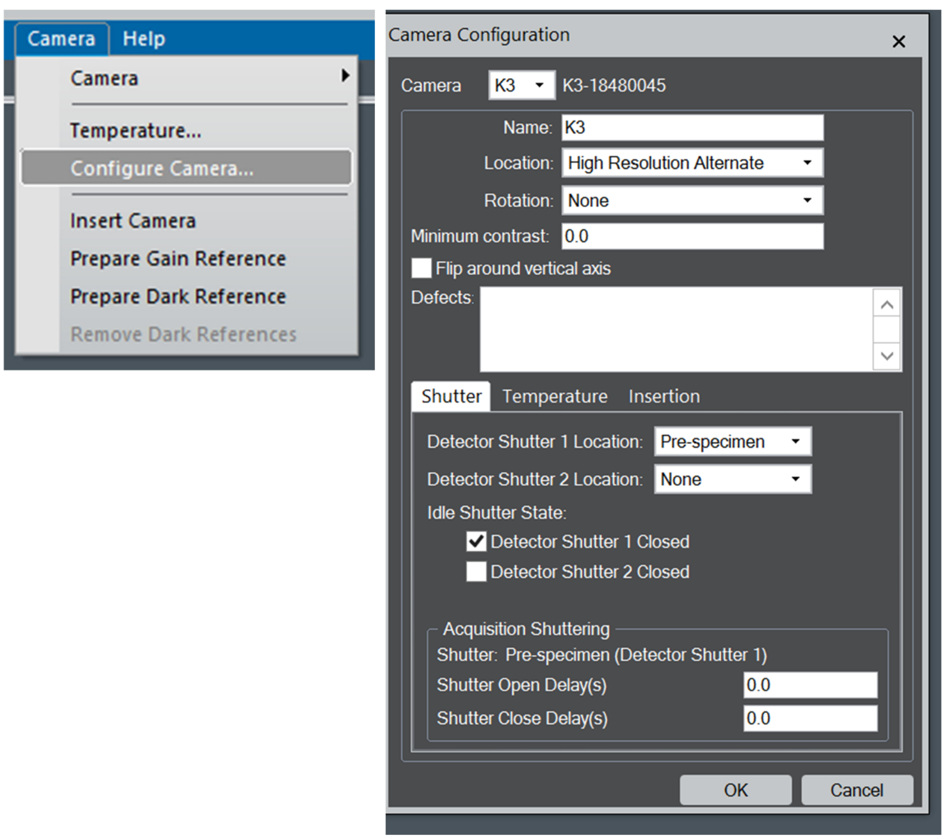


Figure 9: Basic shutter configuration options.

A shutter above the sample should be set to automatically close when both the transmission electron microscope (TEM) viewing screen is raised, and the camera is inserted but not viewing/capturing. This default state protects your sample from unnecessary beam exposure/damage while not actively viewing the sample or capturing data.

1.13 Step-by-step data acquisition

1.13.1 Single images

To acquire a single image:

1. Align TEM as you would for any data collection session.
2. Start in a location of the sample where beam exposure, damage, or contamination is acceptable, not at the best location on the sample.
3. Start DM software if it is not running.
4. Within DM software, check that the **Camera Monitor** is green.
5. On the TEM, check that the electron beam is on and the viewing screen is down.
6. On the TEM, check that beam intensity on the viewing screen is normal for TEM imaging (beam visible, but not a very small spot).
7. Select TEM imaging or **In Situ Imaging** technique within DM software.
8. From the **Imaging** or **In Situ** technique in DM software, check that binning is set to 1 and Counted mode is selected. Set the readout area as desired (Full is recommended as the starting point).
9. Start live view and wait for camera insertion if needed, as indicated in the microscope graphic.
10. Check that the live view exposure time is reasonable (0.1 s is recommended as the starting point).
11. Lift the viewing screen by double-clicking on it in DM software.
12. Observe the dose rate given in the dose rate monitor in DM software, check that it is a number and not red (Section 1.20).
13. Confirm that the image looks like what you expect to see. Often the magnification needs to be set lower than on most other cameras. (Section 1.22).
14. If capturing high-resolution images, look at the live FFT in the DM software to adjust focus, objective stigmation, and magnification (Section 1.22). If a binning other than 1x is desired, set binning to the desired setting.
15. Adjust the brightness in the TEM software so the dose rate on the camera (shown in dose rate monitor) is in the recommended range, accounting for binning (Section 1.20 and 1.19).
16. In the DM software, check that the dose rate on the sample is not too high for your sample. Calculate the dose rate on the sample by dividing the dose given in the dose rate monitor by the view exposure time.
17. Set the capture exposure time to the desired value (if unsure, try 1s) and set # of frames to the largest value (it is recommended that the number of frames should be no less than ceil[capture_exp_time / live_view_exp_time]).

18 If desired, check the save image checkbox to automatically save each image to disk as it is captured. If so, check that the save path is set to the desired location (Section 1.16.2).

19 If a GPU is installed and the correct license present, a **Correct Drift** checkbox is visible. If this is selected, drift i automatically measured and corrected during capture, resulting in a drift corrected image. This will slow down image capture somewhat. For more details on drift correction, see Section 1.18.1.

20 Move to the desired location on the sample and wait for any sample drift to stop. (Section 1.18.1).

21 Click the **Capture** button. The resulting image is displayed in a different workspace and not the live view workspace.

22 After capturing several images and deleting any undesired ones, the entire workspace with all its images and their relative positions on the screen can be saved by right-clicking on the **Workspace** tab and selecting **Save workspace as…,** rather than saving individual images one-by-one.

1.13.2 Image stacks

To acquire an image stack

See Steps 1-15 above

1 Set the capture exposure time to the desired total exposure time for the entire stack. Set the **# of Frames** to the desired value. Press tab or enter to allow the software to validate whether this number is possible. The resulting stack will have the number of frames set from this field.

2 To capture many image sub-frames, not just a single image, check the save stack checkbox, and verify that the save path is set to the desired location (Section 1.16.2).

3 If a GPU is installed, and the correct license present, a correct drift checkbox is visible. If this is selected, drift is automatically measured and corrected during capture. This will slow down image capture somewhat. For more details on drift correction, see Section 1.18.1.

4 Move to the desired location on the sample and wait for any sample drift to stop. (Section 1.18.1).

5 Click the **Capture** button. The resulting summed image (not a stack) will be displayed in a different workspace (not the live view workspace). This summed image will only be saved automatically to disk if the save image checkbox is also checked. The individual stack frames have all been saved as individual *.dm4 files in the location specified as the save path. To view the full-stack, right-click somewhere in the **Workspace** and select **Open Series…** In the dialog box that opens, select the first image in the series. DM software automatically detects how many frames are in the series by the file names.

6 Drift correction can be applied after data collection if saved as a stack. This is accessed using the process image technique. (Section 1.18.1).

1.13.3 Continuous capture

To stream data to disk (e.g., continuous data capture)

See Steps 1-15 above, but select the **In Situ Imaging** technique, not the **TEM Imaging** technique

1 Set the dataset name and location through the K3 IS Setup. If this step is skipped, then the DM software will warn that the folder location for in situ data has not been configured.

2. Set the view exposure time based on the framerate desired in the streamed dataset. To specify framerate, rather than an exposure time, press the IS Setup button, and enter a framerate.
3. Set the lookback duration by pressing the **IS Setup** button. 5 s is a recommended starting point. For more detail, see Section 1.16.1.
4. Start the live view if it is not already running. The **Lookback Buffer** now begins to fill until it reaches the specified duration.
5. Move to the desired location on the sample.
6. When the behavior of interest is seen, click the record button to start capturing.
7. Data can be played back using the IS player. To open a dataset, right-click on the **Workspace**, and select **Open InSitu Dataset…**, and select the base folder of the in-situ video dataset, which will have a folder named Hour_00 inside. The most recently collected dataset is pre-selected by default when the dialog opens.

4 K3 IS operation

1.14 Overview

This section describes the modes and configuration options for operating the K3 IS camera. Several screenshots of DM software are used as references to present and explain the camera features. For more information on setting up DM additional software features, refer to Section 5 DigitalMicrograph software.

1.15 Imaging modes

When the K3 IS camera is selected, two camera palettes are available to configure the operating modes and acquire data. The two palettes are labeled **K3 In-Situ Acquisition** and **K3 Camera**.

As discussed in Sections 1.2 Counted mode and 1.3 Linear mode, the K3 IS can operate in **Linear** and **Counted** modes. These modes can be selected from both K3 camera palettes, as shown in **Error! Reference source not found.**10. The options on these palettes are similar between **Linear** and **Counted** modes except for binning.

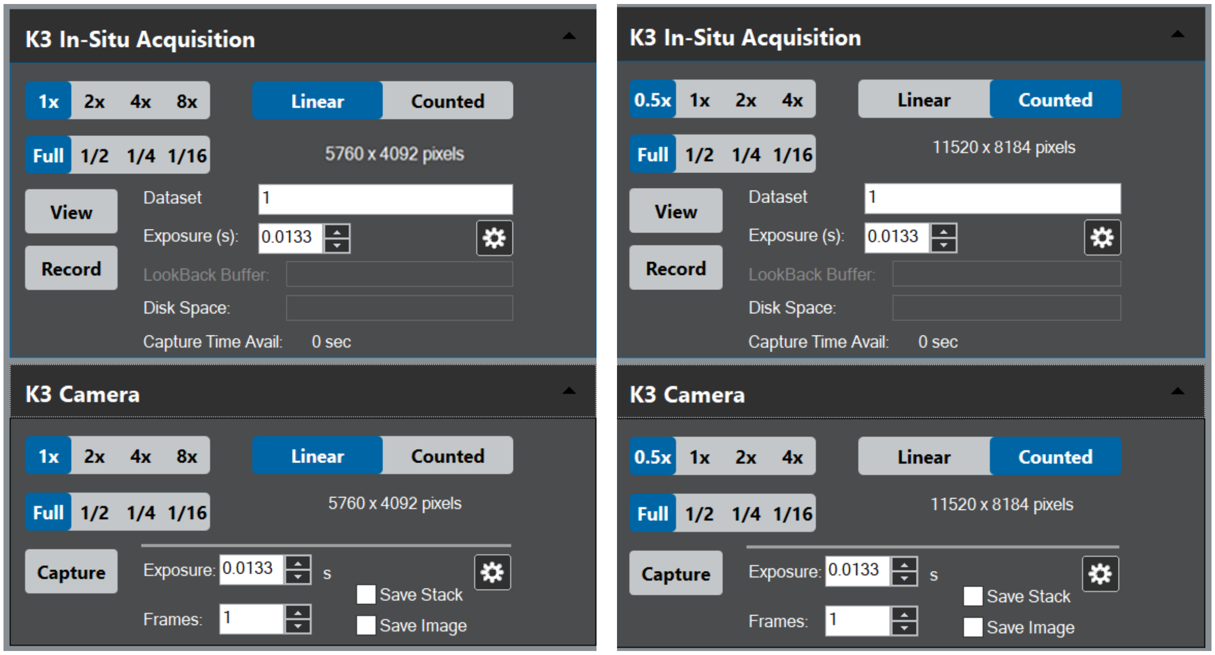


Figure 10: ***K3 In-Situ Acquisition and K3 Camera palettes for Linear (left) and Counted (right) modes.***

1.15.1 K3 In-Situ Acquisition palette

The **K3 In-Situ Acquisition** palette is used to set conditions for live view and *in-situ* data recording. The functionality of the controls is labeled in **Error! Reference source not found.**11 and described in the sections as indicated:

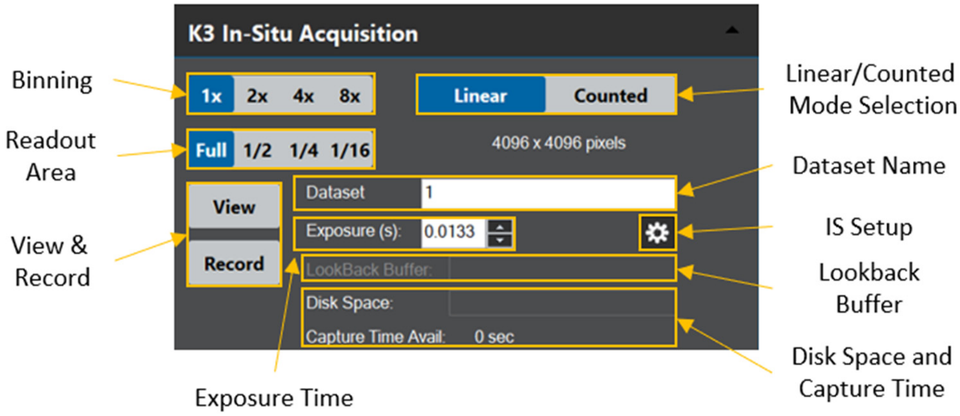


Figure 11: ***K3 In-Situ Acquisition palette.***

- **Linear** / **Counted** mode selection (Sections 1.2 & 1.3)
- Binning (Section 1.15.3)
- Readout area (Section 1.15.4)
- **Dataset** name (Section 1.15.5)
- **Exposure(s)** time (Section 1.15.6)
- View and Record (Section 1.15.9)
- **Lookback Buffer** (Section 0.15.10)
- **Disk Space** and **Capture Time Avail** (Section 0.15.11)
- IS setup (Section 1.16.1)

1.15.2 K3 Camera palette

The **K3 Camera** palette is used to set conditions for capturing single images. The functionality of the controls is labeled in **Error! Reference source not found.** and described in the sections as indicated:

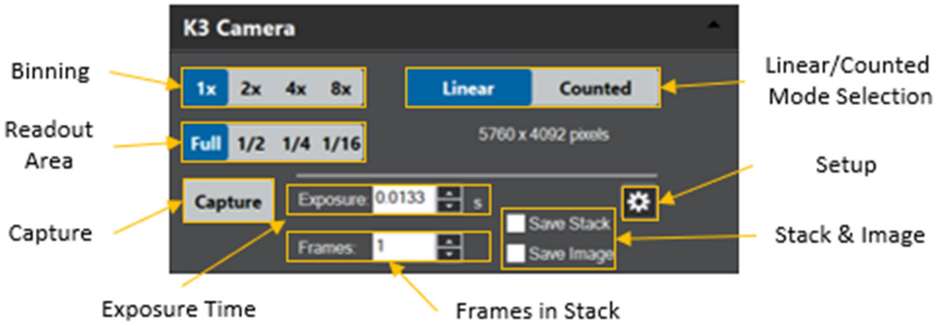


Figure 12: ***Camera Acquisition palette.***

- **Linear** / **Counted** mode selection (Sections 1.2 & 1.3)
- Binning (Section 1.15.3)
- Readout area (Section 1.15.4)
- **Exposure(s)** Time (Section 1.15.6)
- **Capture** (Section 1.15.7)
- **Saving Stack**, **Save Image**, and **Frames** (Section 1.15.8)
- Setup (Section 1.16.2)

1.15.3 Binning

There are numerous binning options available with the K3 IS. The binning options can be selected in either the **K3 In-Situ Acquisition** palette or the **K3 Camera** palette. Choosing binning in either of these acquisition palettes changes the binning selection in the other palette so that the selection for binning applies to the **Capture**, **View**, and **Record** functions. The specific binning options that are available depend upon whether the camera is operating in **Counted** or **Linear** mode.

The K3 IS sensor was carefully designed such that the PSF is slightly larger than the 5 µm physical pixel size. As a result, each incoming electron deposits signal in a small cluster of pixels. In **Counted** mode, the high-speed K3 IS electronics can recognize each electron event (at 1502 frames per second) and find the center of that event with sub-pixel precision. In **Linear** mode, the charge is integrated in each pixel.

Binning considerations

Higher binning does not reduce readout noise, as they often did in older CCD cameras. However, at very low dose rates, binning can reduce the effect of shot noise per pixel. Binning is recommended primarily to reduce the size of the data that is collected. A binning of 2x decreases the total data size by a factor of 4.

This can save significant hard drive space (e.g., if a bin x1 dataset would have been 400 GB, binning x2 saves 300 GB) as well as significant data transfer and processing time.

Counted (0.5x binned)

- Each primary electron's signal cloud is analyzed in the K3 IS processor to determine the electron's landing coordinates with sub-pixel accuracy; this technique extends the resolution beyond the number of pixels in the sensor. The net effect is a quadrupling of the effective number of pixels (pushing beyond the Nyquist information limit to even higher resolution), as well as a further improvement of the DQE (and MTF)
- Practically, this means that the field of view can be increased for the same end resolution allowing the researcher to capture much more data per image
- The electron-counted mode of the K3 IS camera replaces the analog signal from each primary electron with a discrete count

Counted (1x binned)

- Individual primary electrons are counted in-line in the K3 IS electronics on a pixel-by-pixel and frame-by-frame basis
- The electron-counted mode of the K3 IS camera replaces the analog signal from each primary electron with a discrete count

Counted (2x binned)

- Individual primary electrons are counted in-line in the K3 IS electronics, and then 2 x 2 groups of pixels are summed to form each image pixel on a frame-by-frame basis
- The electron-counted mode of the K3 IS camera replaces the analog signal from each primary electron with a discrete count

Counted (4x binned)

- Individual primary electrons are counted in-line in the K3 IS electronics, and then 4 x 4 groups of pixels are summed to form each image pixel on a frame-by-frame basis
- The electron-counted mode of the K3 IS camera replaces the analog signal from each primary electron with a discrete count

Linear (1x binned)

- The total integrated signal level for each pixel is assigned on a pixel-by-pixel and frame-by-frame basis

Linear (2x binned)

- The total integrated signal level for each pixel is captured, and then 2 x 2 groups of pixels are summed to form each image pixel on a frame-by-frame basis

Linear (4x binned)

- The total integrated signal level for each pixel is captured, and then 4 x 4 groups of pixels are summed to form each image pixel on a frame-by-frame basis

Linear (8x binned)

- The total integrated signal level for each pixel is captured, and then 8 x 8 groups of pixels are summed to form each image pixel on a frame-by-frame basis

1.15.4 Sensor readout area

The K3 IS sensor can be read out with the full sensor field of view or with 1/2, 1/4, or 1/16 of the full sensor height. When a sub-area is selected, the sub-area that is read from the sensor is centered on the physical sensor. Further, with sub-area readouts, images are cropped to be square, as shown in **Error! Reference**

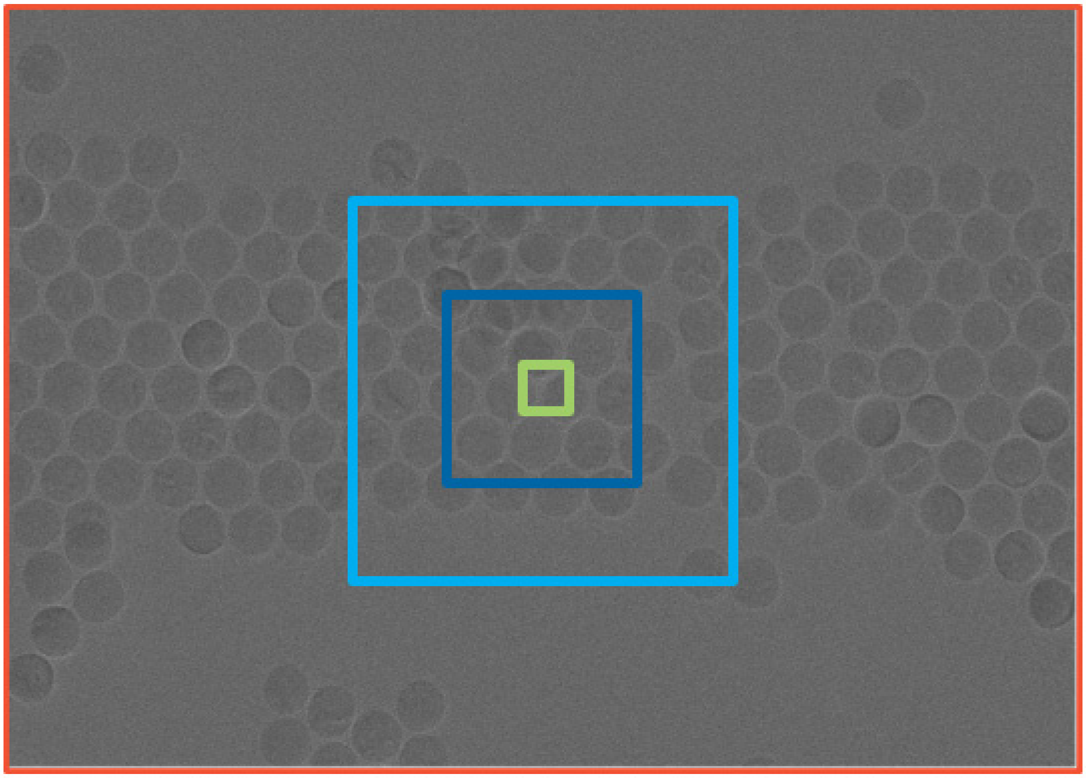


Figure 13: Sensor readout areas.

source not found.13.

The rate at which image frames can be stored to disk increases with decreasing readout area as discussed in Section 0.15.6.

The following tables provide the number of pixels per image for **Counted** and **Linear** modes, sensor readout area, and binning combinations for both the K3 IS (Model 1027) and K3 IS Base (Model 1026).

Table 4: Possible image sizes (in pixels) for Counted mode, K3 IS (Model 1027)

	0.5x Binning	1x Binning	2x Binning	4x Binning
Full Sensor	11520 x 8184	5760 x 4092	2880 x 2046	1440 x 1023
1/2 Sensor	4096 x 4096	2048 x 2048	1024 x 1024	512 x 512
1/4 Sensor	2048 x 2048	1024 x 1024	512 x 512	256 x 256
1/16 Sensor	512 x 512	256 x 256	128 x 128	64 x 64

Table 5: Possible image sizes (in pixels) for Linear mode, K3 IS (Model 1027)

	1x Binning	2x Binning	4x Binning	8x Binning
Full Sensor	5760 x 4092	2880 x 2046	1440 x 1023	720 x 511
1/2 Sensor	2048 x 2048	1024 x 1024	512 x 512	256 x 256
1/4 Sensor	1024 x 1024	512 x 512	256 x 256	128 x 128
1/16 Sensor	256 x 256	128 x 128	64 x 64	32 x 32

Table 6: Possible image sizes (in pixels) for Counted mode, K3 IS (Model 1026)

	0.5x Binning	1x Binning	2x Binning	4x Binning
Full Sensor	6912 x 8184	3456 x 4092	1728 x 2046	864 x 1023
1/2 Sensor	4096 x 4096	2048 x 2048	1024 x 1024	512 x 512
1/4 Sensor	2048 x 2048	1024 x 1024	512 x 512	256 x 256
1/16 Sensor	512 x 512	256 x 256	128 x 128	64 x 64

Table 7: Possible image sizes (in pixels) for Linear mode, K3 IS (Model 1026)

	1x Binning	2x Binning	4x Binning	8x Binning
Full Sensor	3456 x 4092	1728 x 2046	864 x 1023	432 x 511
1/2 Sensor	2048 x 2048	1024 x 1024	512 x 512	256 x 256
1/4 Sensor	1024 x 1024	512 x 512	256 x 256	128 x 128
1/16 Sensor	256 x 256	128 x 128	64 x 64	32 x 32

1.15.5 Dataset name

The K3 *in-situ* palette displays the name that will be applied to the next dataset that will be acquired with the **Record** button. The name for the dataset to be acquired can be set on the main **K3 In-Situ Acquisition** palette or in the **K3 IS Setup** dialog (*see Section 1.16.1*), but the location at which to store recorded data sets must be configured through the setup dialog.

IMPORTANT
*The dataset name and location must be specified through the **K3 IS Setup** dialog before the first dataset is recorded; otherwise, the DM software will warn that the folder location for* in-situ *data has not been configured.*

Individual images and frame stacks acquired with the **Capture** button are named using the **Auto Save Setup** dialog. This is discussed as part of the K3 Setup in Section 1.16.2.

1.15.6 Exposure(s)

The **Exposure(s)** field defines the time resolution for each image. There are **Exposure(s)** fields in both the **K3 In-Situ Acquisition** and **K3 Camera** palettes, but these fields are independent of each other. The **Exposure(s)** field in the **K3 In-Situ Acquisition** palette is used to set the time resolution for images in the live view and data streams acquired with the **Record** button.

For single image acquisition (e.g., **Capture**), the value set for **Exposure(s)** is simply the duration used to acquire an image. The minimum **Exposure(s)** value depends upon the sensor area readout (Section 1.15.4), binning (Section 1.15.3), CDS (Section 1.16.2 – CDS mode subsection), and whether the camera is in the standard speed mode or high-speed mode (Section 1.16.2 – High Speed mode subsection). The following tables show minimum exposure times for image **Capture** across this range of possible camera configurations.

Table 8: Minimum Exposure time in seconds for capturing single images: CDS and Non-CDS, Standard Mode

	All binning options
Full Sensor	0.0133
1/2 Sensor	0.00677
1/4 Sensor	0.00349
1/16 Sensor	0.00104

Table 9: Minimum Exposure time in seconds for capturing single images: High Speed mode, Non-CDS

	Binning	
	Counted: 0.5x ***Linear: 1x***	**Counted: 1x, 2x, 4x** **Linear: 2x, 4x, 8x**
Full Sensor	0.0133	0.00333
1/2 Sensor	0.00677	0.00169
1/4 Sensor	0.00349	0.000874
1/16 Sensor	0.00104	0.000259

Table 10: Minimum Exposure time in seconds for capturing single images: High Speed Mode, CDS

	Binning ***Counted: 0.5x*** ***Linear: 1x***	Binning **Counted: 1x, 2x, 4x** **Linear: 2x, 4x, 8x**
Full Sensor	0.0133	0.00399
1/2 Sensor	0.00677	0.00203
1/4 Sensor	0.00349	0.00105
1/16 Sensor	0.00104	0.000311

For *in-situ* data acquisition (e.g., **Record**), the value of the **Exposure** is the same as the inverse of the frame rate that will be continuously recorded. Although only **Exposure** is available on the **K3 In Situ Acquisition** palette, the **K3 IS Setup** dialog (Section 1.16.1) can be used to enter both **Frame Exposure** and **Frame Rate**, wherein the setup dialog these two fields are linked. The following tables show minimum exposure times and maximum frame rates for continuous data acquisition (e.g., **Record**) across this range of possible camera configurations.

Table 11: Minimum exposure time in seconds and corresponding maximum frame rates for continuously recording data: CDS and Non-CDS, Standard mode

	All binning options (unless noted)	
	Min exposure time (s)	**Max frame rate (fps)**
Full Sensor	0.0333A, 0.0133B	30.1A, 75.1B
1/2 Sensor	0.00677	147.7
1/4 Sensor	0.00349	286.1
1/16 Sensor	0.00104	964.5

A: Counted:0.5x binning, Linear: 1x binning *B: Counted:1x, 2x, 4x binning, Linear: 2x, 4x, 8x binning*

Table 12: Minimum Exposure time in seconds and corresponding maximum frame rates for continuously recording data: High Speed Mode, Non-CDS

	Binning			
	Counted: 0.5x		Counted: 1x, 2x, 4x	
	Linear: 1x		Linear: 2x, 4x, 8x	
	Min exposure time (s)	Max frame rate (fps)	Min exposure (s)	Max frame rate (fps)
Full Sensor	0.0133	30.1	0.006[C], 0.00333[D]	166.9[C], 300.1[D]
1/2 Sensor	0.00677	147.7	0.00169	590.8
1/4 Sensor	0.00349	286.2	0.000874	1144.6
1/16 Sensor	0.00104	964.5	0.000259	3858.0

C: Counted:1x Binning, Linear: 2x Binning *D: Counted:2x, 4x Binning, Linear: 4x, 8x Binning*

Table 13: Minimum Exposure time in seconds and corresponding maximum frame rates for continuously recording data: High Speed Mode, CDS

	Binning			
	Counted: 0.5x		Counted: 1x, 2x, 4x	
	Linear: 1x		Linear: 2x, 4x, 8x	
	Min exposure time (s)	Max frame rate (fps)	Min exposure time (s)	Max frame rate (fps)
Full Sensor	0.0133	30.1	0.006[E], 0.00399[F]	166.9[E], 250.4[F]
1/2 Sensor	0.00677	147.7	0.00203	492.6
1/4 Sensor	0.00349	286.2	0.00105	952.4
1/16 Sensor	0.00104	964.5	0.000311	3215.4

E: Counted:1x binning, Linear: 2x binning *F: Counted 2x, 4x binning, Linear: 4x, 8x binning*

For most combinations of Sensor Area readout, Binning, and CDS, the minimum exposure times for Recording data are the same as the minimum exposure times for capturing single images. There are some exceptions due to hardware bandwidth limitations, and these exceptions are indicated by the footnotes in the tables above.

1.15.7 Capture

Single images can be acquired using the **Capture** button on the **K3 Camera** palette. This function is linked to the **Exposure** field in the **K3 Camera** palette. After pressing **Capture**, the image acquired by the K3 is displayed within the DM software.

1.15.8 Saving single images and frame stacks

The **K3 Camera** palette presents three options to configure how captured images are saved to disk.

IMPORTANT
*For best performance, the **Save Stack** mode should use the dedicated solid-state RAID array to store data. Use the K3 Camera palette **Setup** dialog to configure the location to where this mode will save the data.*

Frames

The **Frames** option allows an image to be captured as a sequence of subframes (e.g., image stack). In the standard speed mode, the K3 IS can save from 1 up to 75 full-sensor frames per second. In high-speed mode, the K3 can save from 1 up to 300 full-sensor frames per second.

The **Frames** option is used to fractionate the dose allotted to a single image across the specified number of frames. For instance, a 20 e^-/pixel dose in 1 s can be distributed over twenty 50 ms images, each with 1 e^-/pixel.

The frames in an image stack can be viewed independently and used for different options such as frame alignment and drift correction or even with advanced processing such as local drift correction within sub-regions of a single frame. Additional information about the drift correction of stacks is given in Section 1.18.1.

Setting the number of frames also has an impact on data format saturation, as described in Section 1.21.

Save Stack

The **Save Stack** option is used to write to disk the stack of subframes specified by **Frames**. When **Save Stack** is selected, the total exposure time for the entire series is set by the **Exposure** time in the **K3 Camera** palette. If **Save Stack** is not selected, then a stack of frames will not be saved to disk.

Save Image

The **Save Image** option is used to write to disk a single frame. If **Frames** is >1, then the saved image is created from the summed stack of subframes specified by **Frames**. If **Save Image** is not selected, then an image will still be displayed within DM, but an image will not be saved automatically to disk.

IMPORTANT
The name of images and frame stacks is configured through the K3 Camera Setup dialog, as discussed in Section 1.16.2.

Open Stack

Once a stack of frames has been collected, the data can be opened and processed within DM by right-clicking in the main workspace area of DM and selecting **Open Series.**

1.15.9 View, Record

The **View** button is used to start a live view with the K3 IS. The **View** function does not save data to disk but rather simply displays images within DM. If the Exposure is a small number, the rate at which data is updated within DM is limited by the refresh rate of the graphics card in the K3 IS computer system. However, if **Exposure** is set to a large enough value, then the live view will be updated by the time resolution indicated by **Exposure**.

The K3 IS can acquire high-quality images at very low dose rates, so to improve the contrast of the live display, there is an option within the K3 IS Setup window to average live view frames (see Section 1.16.1).

The **Record** button is used to save data continuously to disk. Data is saved with a time resolution equal to the **Exposure** value.

IMPORTANT
The dataset name and location must be specified through the K3 Setup dialog before the first dataset is recorded; otherwise, DM will warn that the folder location for in situ data has not been configured.

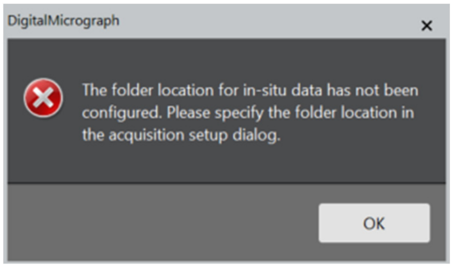


Figure 14: ***DM warning encountered if a dataset location has not been specified before trying to record data.***

Once a continuous stream of data has been recorded, the data can be opened in DM by right-clicking in the main workspace and selecting **Open InSitu Dataset….** InSitu Datasets can be processed with the In Situ Player. Numerous tutorial videos and a webinar on the IS player can be found on Gatan's YouTube channel.

1.15.10 LookBack Buffer

LookBack is a feature that allows continuous data capture to include a buffer of data that precedes the moment at which the **Record** button is clicked. The duration of the **Lookback Buffer** is configured through the **In-Situ Setup** dialog. The LookBack feature is useful both to prevent missing the start of an *in-situ* reaction and to reduce the total data storage needed to capture a reaction.

1.15.11 Disk space and capture time avail

DM monitors and reports the amount of disk space available on the drive where continuously captured data will be saved - this destination is configured through the **In Situ setup** dialog.

1.16 K3 setup dialog

There are two setup dialogs used to configure options for the K3. The options within these two dialogs are described in the following two sections.

1.16.1 K3 In-Situ setup dialog

The K3 **In Situ setup** dialog allows the user to set **Frame Exposure**, **Frame Rate**, **Look-back Duration**, the **Folder Path,** and **Dataset Name**, as well as to select whether to display the live view as an exponentially weighted running average.

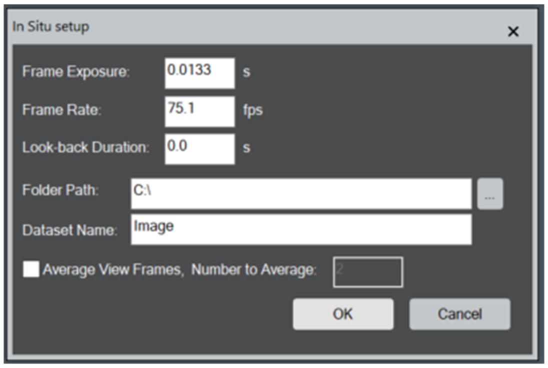


Figure 15: ***K3 In Situ setup window.***

Frame Exposure and Frame Rate

The **Frame Exposure** field in the setup dialog serves the same function as the **Exposure** field in the main K3 In Situ setup window - define the time resolution for each image to be acquired. In this window, the **Frame Exposure** field is linked to the **Frame Rate** field. Values can be entered into either field, but upon entering a value into one of the fields, the other field is automatically updated with the reciprocal value.

The minimum exposure times (and therefore the maximum Frame Rates) for the K3 IS depend upon the Sensor Area readout (Section 1.15.4), Binning (Section 1.15.3), CDS (Section 1.16.2 – CDS mode subsection), and whether the camera is in the Standard speed mode or High-Speed mode (Section 1.16.2 – High-Speed Mode subsection). The minimum exposure times / maximum frame rates are provided above in Table 11,

Table 12, and Table 13.

Look-back Duration

Look-back Duration can be set to have a value between 0 s and 30 s. The LookBack feature enables data to be continuously buffered (for an amount of time specified by the **Look-back Duration**) and then stored to disk as part of the continuous data stream acquired once the **Record** button has been selected. This feature is useful so that data can be acquired after a change in the sample has been detected but without failing to record that event completely.

IMPORTANT
When LookBack is enabled, the buffered data is added to the beginning of each captured dataset. Capturing data at full resolution and 75 fps, the maximum LookBack duration of 30 s can add ~100 GB (2250 image frames) of data to every video captured. To minimize the amount of unwanted data stored to disk, it is recommended to set the ***Look-back Duration*** *to less than 10 s.*

Folder Path and Dataset Name

The **Folder Path** and **Dataset Name** specify where to store continuously captured data and the name for the dataset.

IMPORTANT
To maximize system performance, it is recommended to save data only to the high-speed RAID provided with the K3 IS. If an alternative location is selected, then it is recommended to run the ***Drive Performance Test*** *(****Camera Monitor → QuickScan → Run Drive Performance Test****) on the destination disk drive to ensure that the destination disk drive can sustain a data rate >3.5 GB/s.*

Average View Frames

The K3 IS enables counted data collection at very low dose rates and high frame rates. In both cases, the amount of signal in a single frame can be very low, making it difficult to see details of the sample in the live view display. Simply increasing the exposure time (decreasing the framerate) can increase the contrast in the view, but this sacrifices the temporal resolution of the final data.

To maintain a high framerate to disk but also increase the signal and contrast in the live view, it is possible to display a live view that is the average over several frames. Gatan implements an exponentially-weighted running average of the data shown in the live view when the user checks the **Average View Frames** checkbox. This has no impact on any data saved to disk by the camera in *in-situ* mode or standard imaging mode.

The user can specify how many frames to average. A larger value will increase visibility more but will also lead to more blurring of anything that is moving or changing. A value of 10 frames is the recommended starting point.

There are two important points to consider when averaging frames for the live view. First, the framerate of the data sent to the live view window (and the averaging algorithm) can vary and is often much lower than the framerate of the data captured by the camera and stored to disk. This means that a live view frame average of 10 frames when collecting data at 100 fps does NOT average over 0.1 s. Second, the data type of the live view window is changed from an integer data type to a real data type when **Average View Frames** is selected. This change to the live view window does not change the data type of the data saved to disk.

1.16.2 K3 Setup dialog

The **K3 Setup** dialog allows the user to set the path where individual images will be saved. Also, the **K3 Setup** dialog allows the user to select the type of corrections to be applied to the acquired data and whether to correct defects. Finally, the **K3 Setup** dialog allows the **CDS Mode** and **High Speed Mode** to be selected. By default, the camera operates in non-CDS mode (e.g., **CDS Mode** unchecked) and Standard mode (e.g., **High Speed Mode** unchecked).

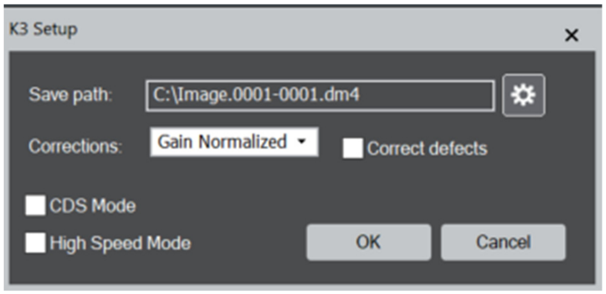


Figure 16: K3 Setup window.

Save Path

The save path is configured through a setup sub-dialog that allows the **File Directory** to be selected, the **File Name** to be constructed using parameters or from a string, and the **File Content and Format** to be specified.

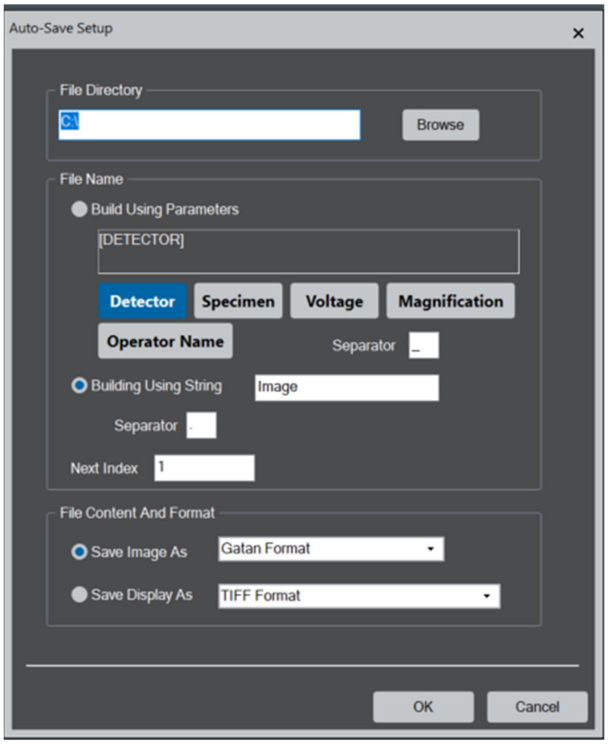


Figure 17: ***Auto-Save Setup dialog.***

Corrections

The K3 provides the option to automatically apply various corrections to images. The corrections that are applied make use of the dark and gain references discussed in Section 0.17, and the available corrections are dependent upon the imaging mode of the camera.

Table 14: Mode-dependent corrections

Camera mode(s)	Available corrections
Counted, Standard Mode; CDS and Non-CDS	• Unprocessed • Gain Normalized
Counted, High-Speed Mode; CDS and Non-CDS	• Unprocessed (the *only option in this mode*) • Gain Normalized (*disabled in this mode*)
Linear, Standard and High-Speed Modes; CDS and Non-CDS	• Unprocessed • Dark Subtracted • Gain Normalized

CDS Mode

The K3 IS camera can run the camera in CDS mode. When CDS Mode is selected, each sensor pixel is read twice and reset between reads. The first pixel read records the signal of interest, and the second read of the reset pixel serves as a reference. This double, pixel-by-pixel sampling reduces noise, resulting in a significantly higher DQE across all spatial frequencies. However, the double pixel reading also results in an output of 751 raw frames per second from the sensor. This impacts the minimum exposure time for single image capture and the maximum frame rates available for continuous data recording (compare

Table 12 with Table 13). Also, as a result of the double pixel reads, the dose rate should be set to half of the dose rate used when CDS mode is not used (see Section 0.19).

High Speed Mode

For most applications, the standard speed mode of the camera is enough for capturing high-quality, time-resolved data in Counted mode (see Standard Mode framerates in Table 11). However, in some circumstances, higher framerates may be desired, and this can be achieved by checking the **High Speed Mode** checkbox in the **K3 Setup** dialog as described in Section 1.16.2.

IMPORTANT
*The checkbox to enable **High Speed Mode** is found in the **K3 Setup** dialog, not in the **In Situ Setup** dialog*

There are several points to consider when choosing between standard and high-speed mode.

1. **High Speed Mode** disables the counted gain correction (Section 0.17.2), which will lead to slightly lower image quality.
2. **High Speed Mode** provides increased frame rates for Counted mode with bin x1, 2, and 4, and with Linear mode bin x 2, 4, and 8. **High Speed Mode** does not increase the achievable framerates in **Linear** mode with 1x binning or **Counted** mode with 0.5x binning (compare Table 11 - Table 13).
3. If the dose rate is low, then faster frame rates might not be optimal. At very low dose rates, multiple frames often need to be accumulated to provide enough contrast to form a useable image. So, in these conditions, a single high-speed frame might not be useful. For example, if a uniform dose rate on the camera is set to 10 e/pix/s, but the frame rate is set to 300 FPS, then >96.7% of pixels in the individual frames will have a value of 0, and <3.3% will have one or more electron counts in them. **High Speed Mode** should, therefore, be used only when the dose rate on at least some part of the camera will be high enough to facilitate subsequent data processing and analysis.

1.17 Reference images and corrections

Before corrected images can be acquired, both a dark reference and the gain reference for the desired mode of data acquisition must be prepared. To acquire these reference images, the **User Mode** must be set

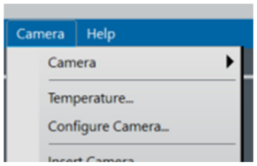


Figure 18: ***Camera drop down menu to prepare dark and gain reference images.***

to **Power User** so that the **Camera** pull-down menu is available (see Section 1.25.1). Dark and gain references can be prepared using the respective menu options, as shown in **Error! Reference source not found.**, where **Prepare Dark Reference** is shown as being highlighted.
All reference images are stored on the K3 IS computer at C:\ProgramData\Gatan\Reference Images.

1.17.1 Dark reference

The dark reference image collection is easy and very fast (10 s in full sensor readout mode). It is recommended that dark reference images be collected several times per day. The K3 IS will automatically blank the beam during dark reference acquisition if the shutters are configured. There is no need to blank the beam manually, lower the screen, or cover the viewing chamber, since the camera will blank the beam automatically using the configured TEM shutter (Section 1.12), and visible light does not excite the direct-detection sensor as it would a scintillator-based camera.

1.17.2 Gain reference

Gain correction normalizes the response of each pixel to an electron strike. With the K3 IS, gain reference images can be acquired for all combinations of **Linear**/**Counted**, **CDS**/**Non-CDS**, and **Sensor Readout Area**. It is usually sufficient to collect gain reference images once per week, ideally after an annealing cycle (Section 1.18.2) before capturing any data.

IMPORTANT
Clicking on the Prepare Gain Reference menu item in ***Error! Reference source not found.*** *will result in gain references being acquired only for the current camera mode. To prepare gain references for other/all modes quickly, hold the Alt key while clicking the menu item. This will bring up the dialog shown in* ***Error! Reference source not found.***.

.

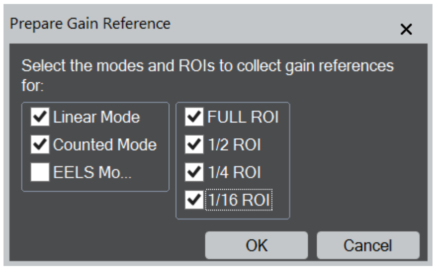


Figure 19: ***Prepare Gain Reference dialog (accessed via alt-click).***

When collecting gain normalized data with the K3 IS, the reference images for the camera state must exist; so, if the relevant reference images have not yet been acquired, then the user must first collect them. If the reference images already exist, the relevant image will be loaded from the DM reference image directory into the camera processor. Gain corrected imaging in Counted mode makes use of the corresponding **Linear Mode** reference images (e.g., all other camera options being the same). Therefore, when collecting gain references, first collect a **Linear Mode** high-beam level gain reference for the camera mode(s) of interest.

The gain reference collection process starts the automatic collection of a new 10 s dark reference image. The user is then presented with a series of dialog boxes.

You may be asked if it’s OK to insert the camera or switch from another mode if the camera is currently running a live view. Click **Yes**.

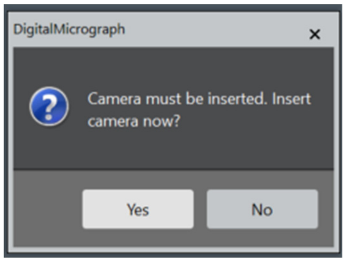


Figure 20: ***Camera insertion dialog (during gain reference).***

A dark reference is automatically acquired and saved to the processors

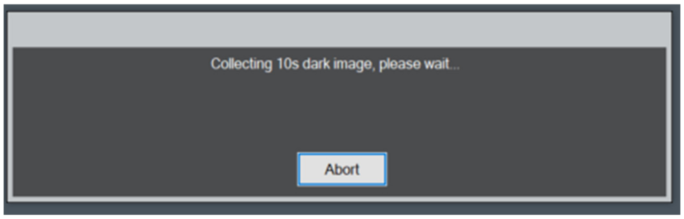


Figure 21: ***Dark reference dialog.***

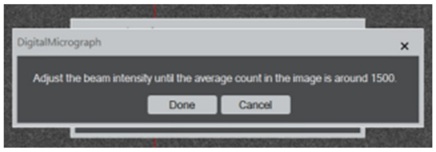


Figure 23: ***Gain reference dose setup.***

As part of the gain reference process, the user must adjust the beam conditions to achieve the required beam intensity. Short exposures are used during the gain reference process, so the requested dose rate is a fraction of the total dose that will be used to collect the reference image.

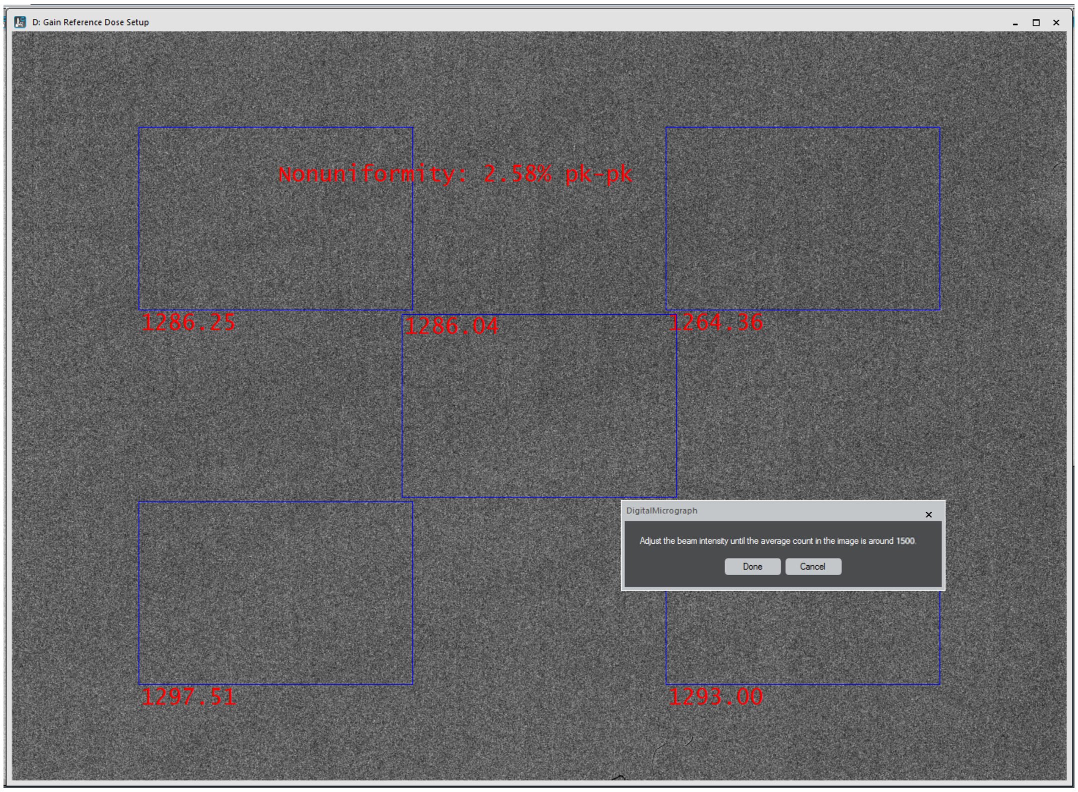


Figure 22: ***Gain reference dose target.***

Click **Done** once the dose rate and beam conditions have been adjusted. The gain reference image will then be collected, and a progress window will update. During this reference image process, the DM software also calculates a pixel mask to correct for defective pixels. A notification appears once the gain reference acquisition has been completed.

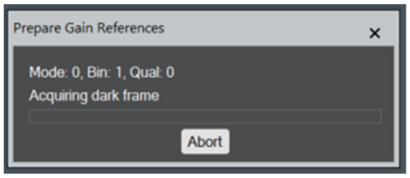


Figure 24: ***Gain reference progress window.***

The default acquisition time for gain reference image collection is set to achieve a specific total dose on the sensor.

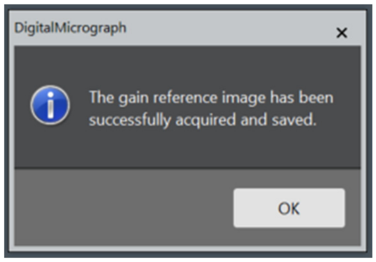


Figure 25: ***Gain reference complete dialog.***

IMPORTANT
The default gain reference beam level for Linear mode is more than 10x brighter than the typical Counted mode level. if you have been operating in counted modes you have to brighten the beam considerably to take linear gain reference images.

1.17.3 Defect correction

Defect pixel mapping and removal are performed automatically as part of the reference acquisition process and do not require specific attention. Regular updating of references allows continued high performance of the camera as the sensor ages.

Defect correction removes poorly performing pixels, which contribute to fixed pattern noise such as hot, dark, or noisy pixels. The number of pixels masked in this way is very small (typically <1 in 100,000).

There are a couple of ways to perform defect correction with the K3 IS camera:

1. Pixel defect map: These maps are images generated during gain reference acquisition and are stored on the DM computer at C:\ProgramData\Gatan\Reference Images. The last two digits of the name of the pixel defect map images indicate the mode for which the defect map is applied:

Table 15: Mode designation for pixel defect map naming

Mode	Saved as
Standard Linear	Pixel defect map.m0
Standard Counted	Pixel defect map.m1
CDS-Linear	Pixel defect map.m2
CDS-Counted	Pixel defect map.m3

2. User-defined pixel defect map: User-generated defect map. To edit this, use the **Defects** text box located in the camera configuration dialog. This uses the same convention as other Gatan cameras, as indicated in the following examples:

- 2 row defects: *r1000-1001*
- Single column defect: *c52*
- 25 column defects: *[0,100,2880,124]*
- Cluster(11x21) defect: *[200,500,210,520]*

1.17.4 Unprocessed vs. gain corrected images

K2 camera users frequently requested that Gatan minimize the size of files saved. For K3 IS camera, the gain correction process has been adjusted to try to help minimize file size.

Traditionally, gain correction involves the conversion of raw counted images (integers) to gain corrected images (floating point), which correct for small (typically 1 – 3%) variation seen in the pixel response. Conversion of an 8-bit integer to a 32-bit floating-point image results in an increase in file size by a factor of 4x.

With the K3 IS camera, gain correction no longer requires conversion of the file types from an 8-bit integer to a 32-bit floating-point. During the gain correction, each count is scaled by 32x on average while keeping the file type the same.

The unprocessed defect-corrected image can be saved without gain correction, allowing for user-defined post-acquisition gain correction.

1.18 Image quality

To check that the gain reference acquisition was successful, after completing reference image acquisition, acquire a flat-field image under dose rate conditions like those used in the reference image process. Then use DM to compute the FFT of this flat field image. A well-corrected image will show uniform intensity with no defects, and the FFT will look like that of white noise.

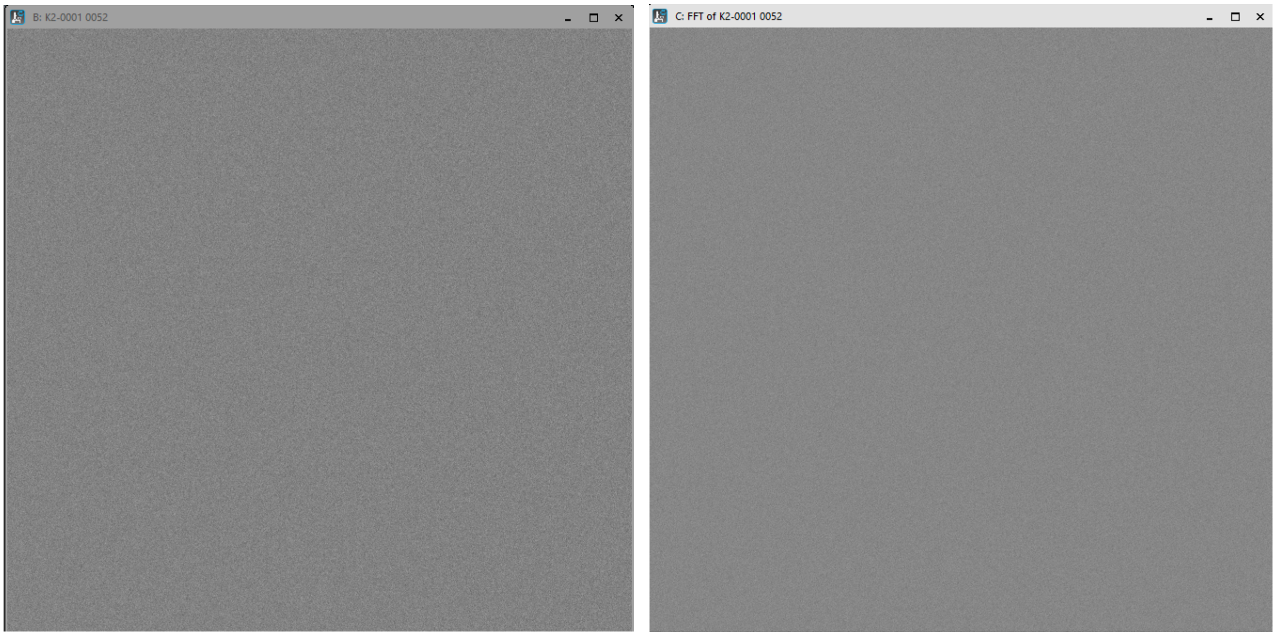


Figure 26: ***Example of a well-corrected image showing uniform intensity (Left) and a white noise FFT (Right).***

1.18.1 Drift correction

The spatial resolution of images of real samples is often degraded due to drift – whether induced from the TEM, sample holder, or sample itself.

Drift correction of a series of frames, followed by summing together the aligned frames, can significantly increase the signal-to-noise of data collected at low dose rates or high frame rates.

There are several ways to correct drift when collecting data with the K3 IS.

- Live drift correction with GPU (Section 1.29).

  Live drift correction only applies to the capture of single images or stacks of images, not to in-situ video acquisition.

- Post-acquisition drift correction of stacks

Existing free tools in DM enable drift correction of stacks collected with the K3 IS. Combined with the volume tools, this enables the production of drift-corrected single images. These tools are powerful and flexible, and a full description is beyond the scope of this manual. Documentation for these tools can be found in the DM help files in the sections Image Alignment, and Volume.

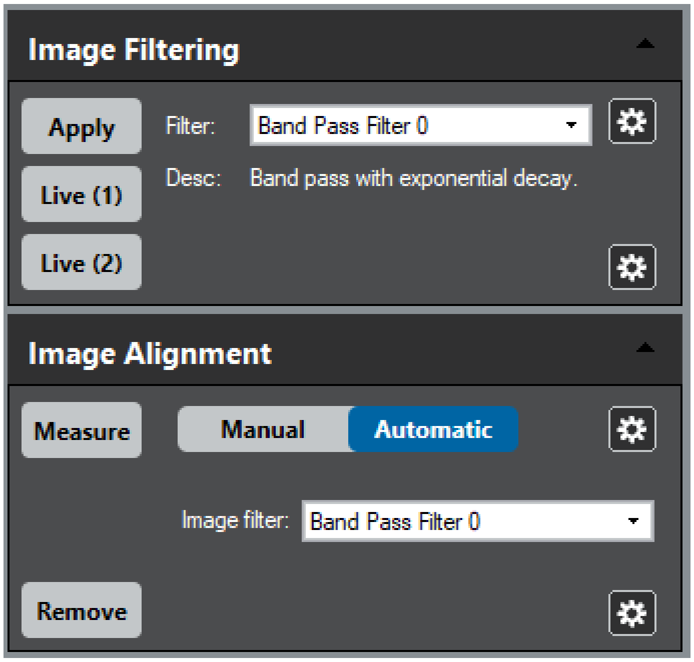


Figure 27: ***Free image alignment tools for stacks in DM.***

- Post-acquisition drift correction of *in-situ* datasets

DM includes a free in-situ player, which has been expanded since version 3.4.1. The **In-Situ Player** and **In-Situ Editor** enable drift correction of *in-situ* video datasets. Alignment can be performed on the full frames. Alternatively, if an ROI is placed on the data, a specific region of interest can be tracked over time. Filters, such as bandpass filters, and edge-detection filters, can be applied to the data during drift correction to improve results. These filters are the same user-adjustable filters that can be applied in the drift correction of stacks.

If the **Apply processing while playing** checkbox is selected, drift correction will be applied as the data is played back. This will result in a reduction of the display update frequency but can help to quickly judge if the current settings are likely to produce a good result. To produce a drift corrected dataset for further processing and analysis, export the data as an *in-situ* dataset.

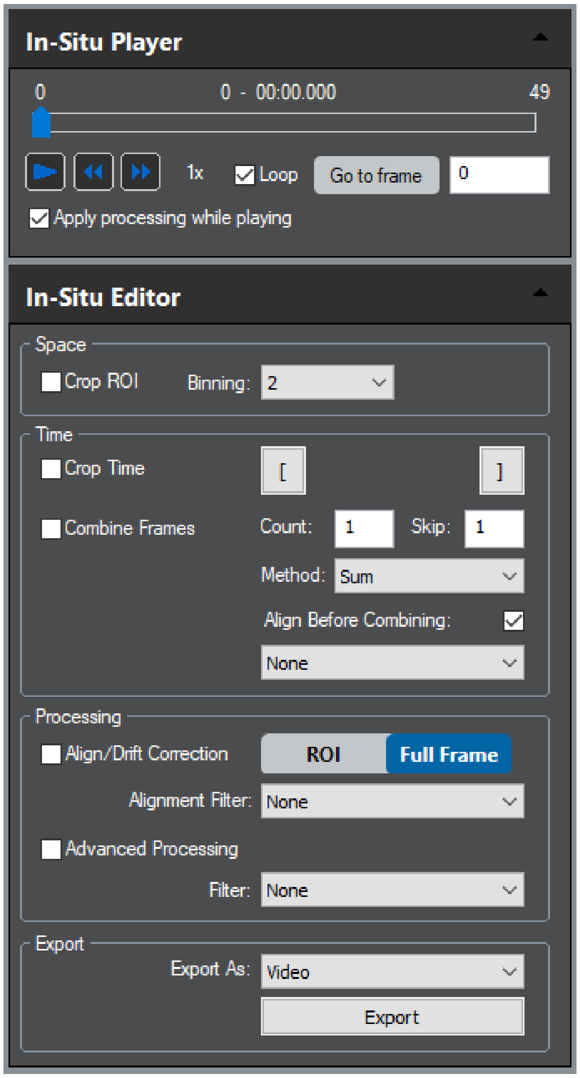


Figure 28: ***In-Situ Player and In-Situ Editor.***

IMPORTANT
A full description of the **In-Situ Player** and **In-Situ Editor** *is beyond the scope of this manual, but tutorial videos and a webinar on the* **In-Situ Player** *can be found on Gatan's YouTube channel.*

1.18.2 Anneal cycle

Regular annealing (heating to +50 °C) of the sensor helps maintain the top performance of the sensor by reducing background levels and levels of surface contamination. Annealing can also help to repair some radiation damage. We recommend that an anneal cycle be performed each time the microscope does a cryo-cycle. This can be done as often as every evening or less frequently (e.g., once a week) during extended periods of data collection.

To perform the annealing cycle:

1. In the **Camera** menu, select **Temperature.**
2. Select a set point of **+50 °C** and click **OK**.
3. Use the **Options** button to select a duration other than the current settings. Usually, an annealing cycle that lasts overnight is enough.
4. Click the **Start** button to begin the cycle. The camera cools down after it has completed annealing to whatever the setpoint was when the annealing cycle was started. If that was -20 °C, then after the annealing cycle is complete, it cools back down to -20 °C.

IMPORTANT
It is not necessary to interrupt extended data-taking to perform the anneal cycle; however, it is recommended to anneal the sensor about every 7 days.

WARNING
DigitalMicrograph software MUST CONTINUE RUNNING during the annealing cycle.

1.19 Optimum Dose Rates

For the K3 IS camera, the dose rate (instead of the total dose) becomes the first concern in getting good images. The ideal dose rate ranges are shown in the table below. These dose rates are given in terms of physical sensor pixels, while the dose rate monitor reports the dose in terms of image pixels. Binning must be considered to set up imaging conditions properly when using the values shown in the dose rate monitor.

IMPORTANT
In Counted mode, counting efficiency starts to sharply decrease above the suggested dose rates.

Table 16: Ideal dose rates in different sensor readout modes

Ideal dose rates on camera (e^-/pix/s)		
Sensor readout mode	**Standard**	**CDS mode**
Full Sensor	8 – 40	4 – 20
1/2 Sensor	16 – 79	8 – 39
1/4 Sensor	30 – 152	15 – 76
1/16 Sensor	103 – 514	51 – 257

5 Dose rate

1.20 Dose rate monitor

The dose rate monitor provides two measures of the electron beam, which are both important.

The dose rate monitor calculates its values based on the entire image unless a rectangular ROI is placed on the image. If a rectangular ROI is present, only the pixels inside the ROI are used in the calculation.

To get the most accurate measure of the dose rate incident on the sample, place an ROI in a vacuum region, where the beam does not pass through the sample or support films.

The dose rate on the detector is reported per *image* pixel, not per *sensor* pixel, as specified in Section 1.19. The total dose at the sample is reported per exposure. When the active image is the live view or an in-situ dataset, this exposure is the view exposure time. When the active image is a captured single frame, the exposure is the capture exposure.

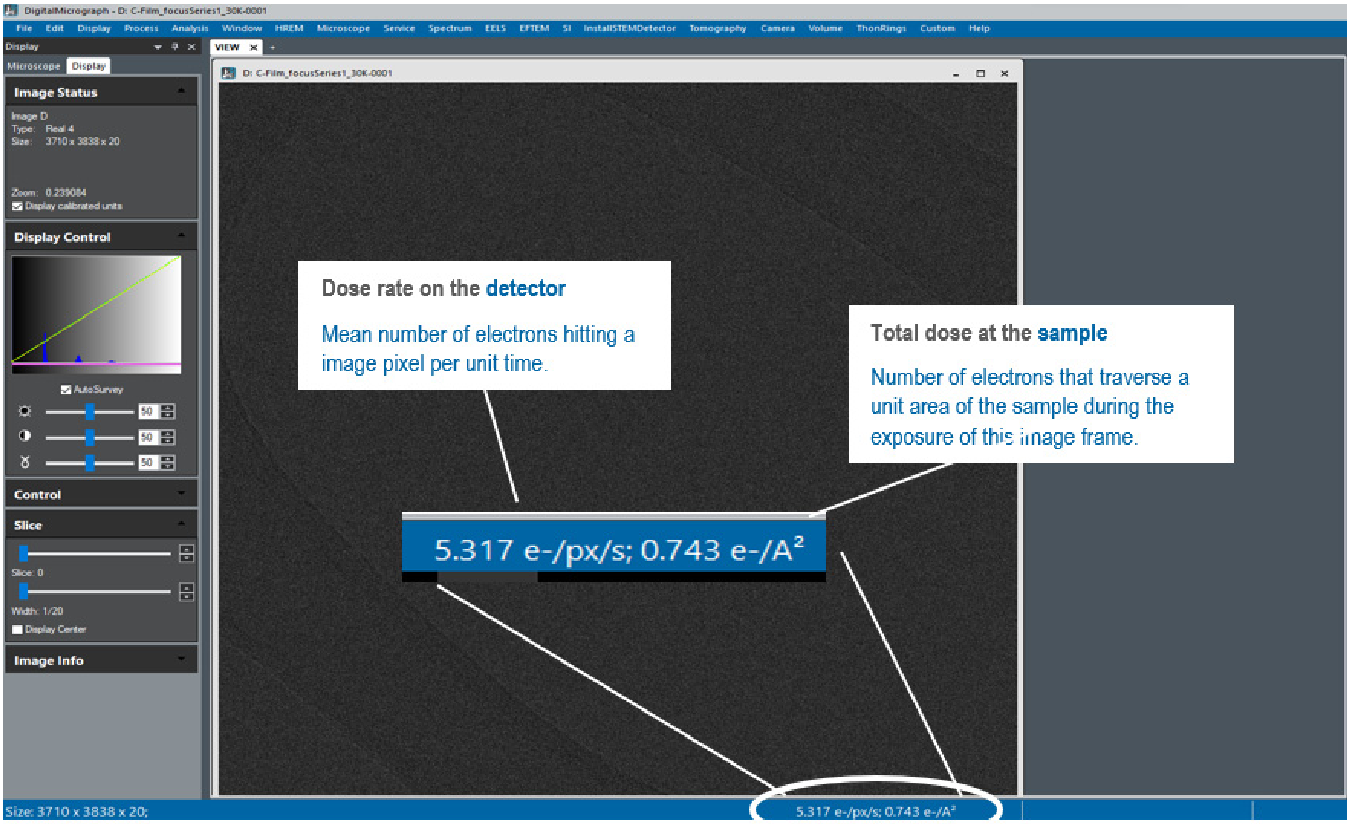


Figure 29: ***Dose rate monitor.***

1.21 Saturation

There are three types of saturation relevant to the K3 IS camera:

1. Sensor saturation

   While it is possible to saturate the sensor, this is unlikely when working in the Counted mode as the sensor itself can handle a dose rate that is 375x higher than the maximum recommended in Counted mode (see section 1.19 for recommended counted dose rates).

2. Counting algorithm saturation

   Coincidence loss can result in saturation of the counting algorithm if the dose rate is set too high. Coincidence loss rises continuously for doses above 0, but at the maximum recommended dose for counted Full Sensor Readout mode, it is only ~10%.

   If the dose rate in the dose rate monitor is too high, either increase the magnification or reduce the beam intensity on the sample.

3. Data format saturation

   It is relatively easy to saturate the 1-byte data format used for IS mode. Since there is a factor of 32 used in the counted gain correction (section 1.17.4), only 8 electrons are required per pixel per image frame to saturate the 1-byte data format, which only stores values from 0 to 255. This type of saturation is indicated to the user by the saturation monitor to the right of the dose rate monitor.

   If the data format is saturated, try to reduce the **Exposure** for individual frames, increase the magnification, or reduce the beam intensity on the sample. Reducing the exposure time for individual frames is performed differently for in-situ recording versus single-image capture. For in-situ recording (or for the live view) reduce the live view **Exposure**. For single-image capture, increase the **Frames** parameter. Unless the **Save Stack** checkbox is checked, increasing this number will not affect the amount of data stored to disk and will only affect saturation, as seen below. If none of these are viable options, and data quality can be reduced, turning off the counted gain normalization (Section 1.16.2) is a last-resort option to eliminate data format saturation.

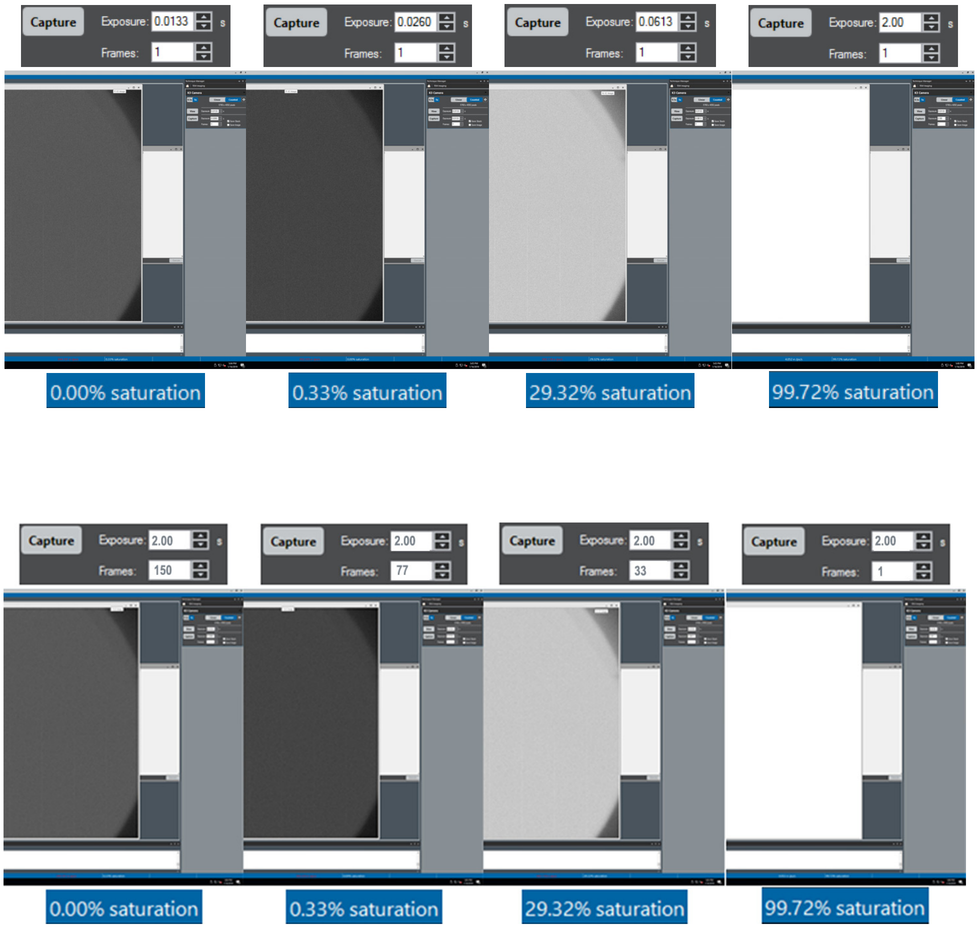


Figure 30: Data format saturation can be reduced by increasing the number of frames.

1.22 Dose rate optimization: Field of view and magnification

It is important to understand the relationship between the dose rate on the camera, dose rate on the sample, and magnification. As implied by the dose rate monitor design (Section 1.20), two dose rates are important for collecting high-quality data with the K3 IS. Sometimes a balance must be struck between the two.

- Dose rate on the camera

  Maintaining a dose rate on the camera within the ideal range is essential to acquiring good data (Ideal ranges can be found in section 1.19)

- Dose rate on the sample

  Maintaining a low dose rate (or sometimes a low total dose) on the sample can reduce deleterious effects of the electron beam and is required for imaging some classes of materials. The total dose depends on the duration of observation as well as the dose rate. The total dose specified in the dose rate monitor is per exposure, as defined by the exposure time. The accurate calculation of this sample dose depends on the correct magnification calibration (Section 1.26).

The parameter linking these two dose rates is the magnification. If the dose rate on the sample is maintained at a constant value, increasing the magnification results in a lower dose rate on the camera. Conversely, if the dose rate on the camera is actively maintained at a fixed value, increasing the magnification requires increasing the dose rate on the sample.

IMPORTANT
The optimal magnification for K3 IS is usually lower than for other cameras. For example, a suitable magnification on the K3 IS will be ~3x lower than the comparable magnification used for a Gatan OneView® IS camera mounted in the same physical position.

1.23 Radiation hardness

To extend the life of the sensor, minimize exposing the CMOS sensor to the electron beam when the camera is not in use.

Based on the results to date, the life of the K3 IS detector is estimated at 5 billion e^-/pixel. Considering exposure rates of 100 e^-/pixel/s, if the sensor were exposed continuously for 24 hours a day every day, the expected lifetime would be well over one year. The camera employs a pixel design that is 10x more resistant to incident electron damage as compared with other direct-detection sensors. The extremely short exposure time coupled with the event discrimination of Counted mode confers an additional approximately 10x immunity to radiation.

The sensor can sometimes be marked by a bright beam. Annealing, followed by new dark and gain references, should be able to remove the resulting minor beam spots. Use caution with intense beams and bright spots, like those in diffraction patterns. Leaving the sensor exposed to the beam when changing magnification levels or spot sizes can also create brief and intense spots.

To prevent actual damage to the camera, it will automatically retract if the beam intensity is too high. This is called dynamic sensor protection (DSP). The thresholds for this automatic retraction can only be set (or DSP turned off completely) with DM in service mode, which requires assistance from a service engineer or applications expert. If you believe your DSP setting is causing problems, contact Gatan service.

6 DigitalMicrograph software

DigitalMicrograph is an application used for acquiring, visualizing, analyzing, and processing digital image data, primarily within the context of electron microscopy. It is used to control the K3 IS as well as to manage the data collected with the camera.

1.24 Installation

DM software requires valid licenses for instrument control and to enable many analysis features. Installation is a two-step process. First, install DigitalMicrograph licenses using the Gatan license USB or downloaded license files. Installation instructions are included with the licenses. Then, install the DM application software using the DM installer USB or downloaded *.zip file. Installation instructions are included with the USB or can be found on the Gatan website. Make sure the K3 IS direct detection camera is selected in **Camera Hardware** and TEM.

WARNING

Do not add any additional PCI cards to PC

Do not modify PC configuration

For optimal performance, do not run anti-virus applications during operation of the camera

For optimal performance, do not load third-party applications other than those recommended or verified by Gatan

---

1.25 Overview

When DM software is launched, you will see five primary areas on the screen

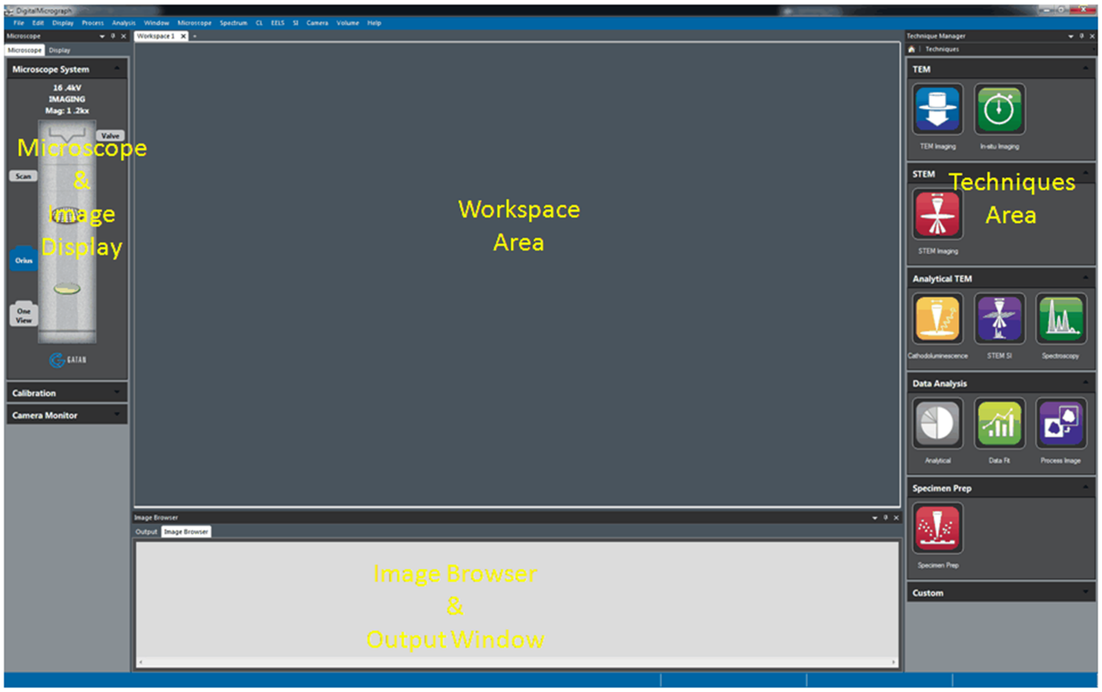
1 The **Menu Bar** at the top contains the **File, Edit, Display, Process, Analysis, Window, Microscope,** and **Help** menus. In these menus are all the controls for operating the application.

2 The main **Workspace** area is in the middle. This is where images and text windows will be displayed. This area can be separated into multiple workspace tabs, which allow better grouping of data.

3 The **Microscope** system and the image **Display** panel are on the left side. The **Microscope** tab shows a diagram of the microscope and can be used to control the microscope system.

4 The **Image Browser** and **Output** panel are on the bottom. The **Image Browser** window displays thumbnails of all images, which can be used to find and organize data. The **Output** window is used for displaying text.

5 The **Techniques Manager** is on the right side. This panel will show the different techniques used to acquire and analyze data. The techniques shown will depend on the licenses purchased, so will be different on different installations. To start acquiring data from a camera, select one of the techniques, e.g., **In-situ Imaging**.



To configure/choose your palettes, go to **Window/Floating Windows**. Then select the windows/palettes you would like to display. The checked items are displayed in the DM software configuration.

Within the **Workspace** area, DM software presents all of its information through the use of windows. Each window contains a set of related information.

Image document windows

Image document windows contain a visible representation of a piece of paper. Images can be placed on this page. Other objects such as lines, boxes, and text can also be placed on this page. You can open, save, and print image document windows.

Many aspects of images and objects placed on pages can be controlled using palettes. Palettes are in the control panels (**Display Panel** and **Techniques Panel**), which are by default docked on the left and right side of the DM application window.

Text document windows

Text document windows contain text, but their main purpose within DM is that they display DM or Python scripts. You can open, save, and print text document windows.

Plug-ins

DM software can be extended to support acquisition devices using plug-ins. Plug-ins are placed in a folder named **PlugIns**.

Scripts

DM software can also run simple programs (called scripts) which carry out automated tasks. See Section 0.32 for more information specific to the use of scripts with Digital Micrograph.

Help

DM includes extensive documentation from the **Help** menu. Consult the **Help** files for more information on DM, scripting, and for the hardware and analysis features for which licenses are installed.

1.25.1 User modes

DM provides 3 different user modes: Regular, Power User, and Service. Regular and Power User modes are intended for customer use, and Service mode is reserved for Gatan Service personnel.

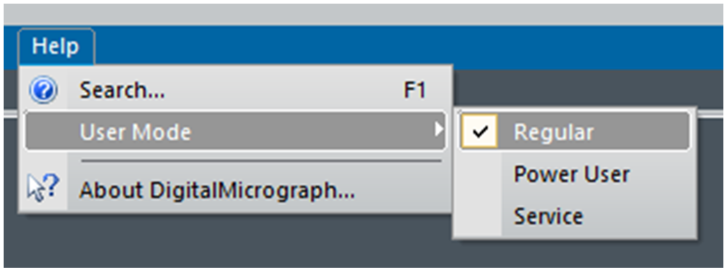


Figure 31: User Mode selection is available from the DM Help menu.

The main difference for the K3 IS between **Regular** and **Power User** modes is that the **Camera** dropdown menu is not available in **Regular** user mode but is available in **Power User** mode. The **Camera** menu is used for advanced camera setup and functions, including:

- Collecting references (Section 1.17)
- Changing the camera temperature (Section 1.11)
- Setting insertion, retraction, and auto retraction delays (Sections 1.7 and 1.9)
- Configuring the behavior of the TEM shutters (Section 1.12).
- Submitting software bugs (Section 1.34).

1.25.2 Context-aware tools

The most common commands for working with images are accessible from the image context menu by right-clicking on an image and/or the **Workspace Area**.

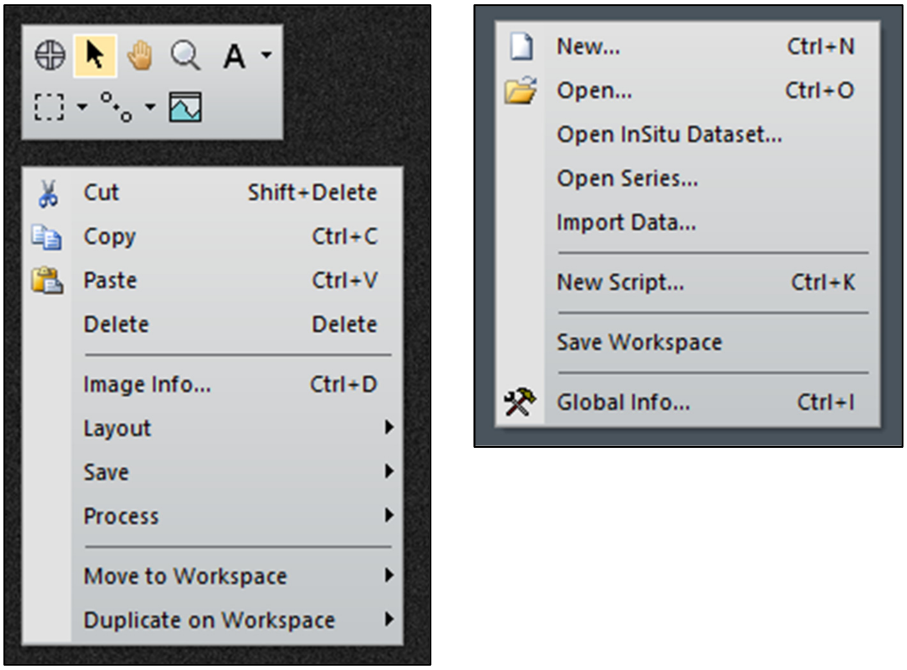


Figure 32: Image context menus.

1.26 Magnification correction/calibration

The displayed nominal magnification on most TEMs is for either the viewing screen or for photographic film and has an accuracy of 5 – 10%. The K3 IS camera is located on a different plane (height-wise) than the film camera. Consequently, the magnification must be calibrated. The calibration is done using reference calibration samples.

At low magnifications:

- Use a cross grating sample or any sample with known spacing

At high magnifications:

- Use graphite or any crystalline samples with known lattice spacing
- Use the Calibrate Image from Diffractogram method

It is essential to make sure that the DM software correctly reads the TEM magnification. If the communication between the computer and the TEM is established, the magnification is read automatically. Otherwise, make sure DM is set to prompt the user to enter a value for TEM magnification every time an image is to be acquired. This can be set by choosing the **Global Microscope Info** window under the **Microscope** menu.

1.27 Low magnification

Record an image of a cross grating replica.

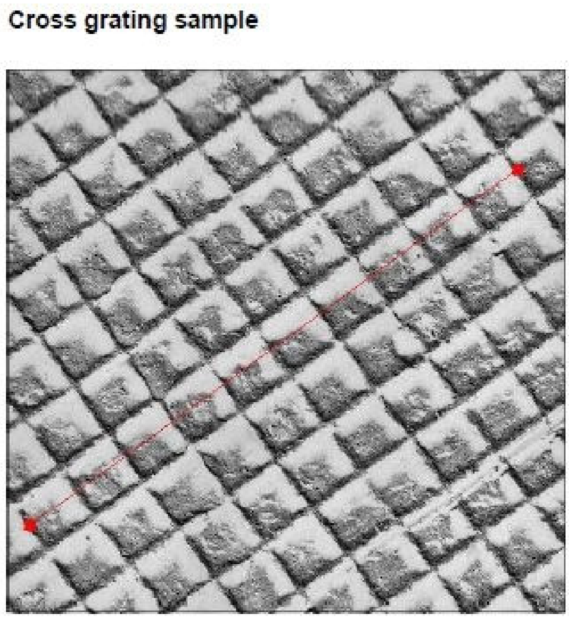


Figure 33: Example of marking a known distance during magnification calibration.

1 Choose **Microscope** / **Calibrate Image**.

2 Follow the instructions on the screen.

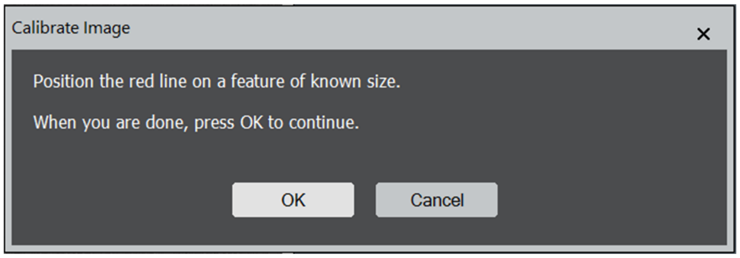


Figure 34: Magnification calibration instructions.

A red line appears on the image.

3 Position it on a feature of known size.

4 Press **OK** on the **Calibrate Image** window.

5 Enter the correct distance for the selected feature (e.g., 10 line pairs of a cross-grating sample where the distance = 10 x 0.463 µm) in the **Calibration** window and select the units. Select the distance marked in the previous figure to perform the magnification calibration.

6 Press **OK**.

7 Click **Yes** to complete the calibration.

8 The calibration can be checked on the calibration table containing pairs of values, the nominal microscope magnification, and the calibrated value.

9 To view the magnification table, select **Microscope** / **Calibrations**.

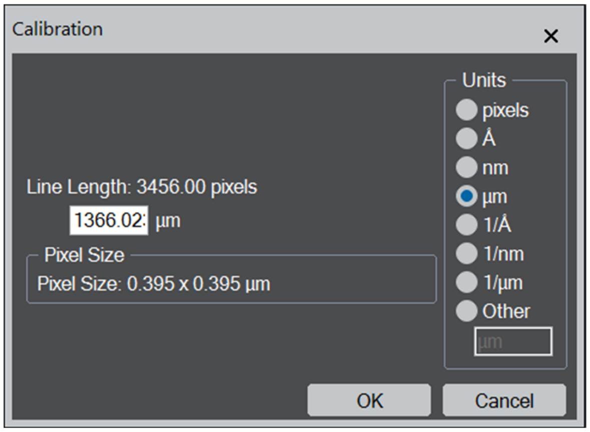


Figure 36: Calibration settings.

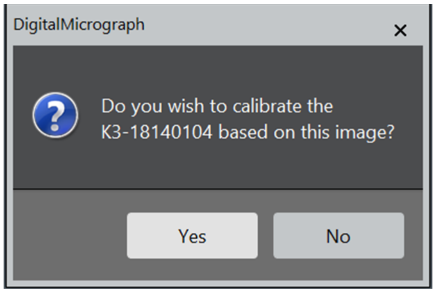


Figure 35: Calibration confirmation.

The microscope calibration dialog shows the table of magnification calibrations stored for the current imaging device and microscope operating condition.

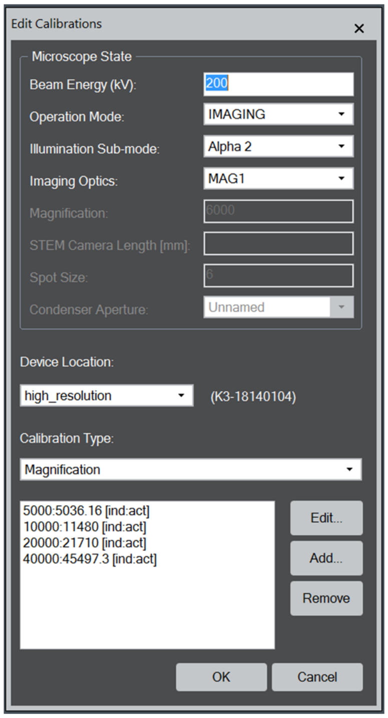


Figure 37: Microscope Edit Calibrations dialog.

1.28 High magnification calibration

Record a lattice image of the crystalline sample.

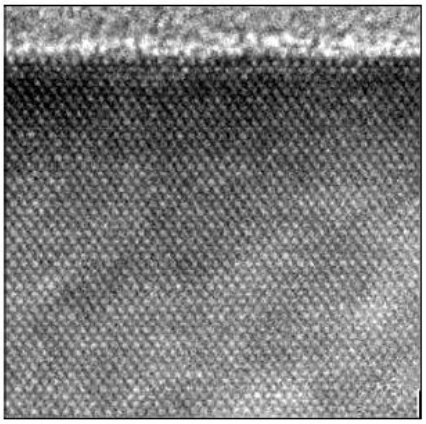


Figure 38: High-resolution image of sample to be used in the magnification calibration.

1 Select **Microscope** / **Calibrate Image** from Diffractogram.
2 To calculate the diffractogram, follow the on-screen instructions.
3 A red line appears on the diffractogram, indicating the distance between peaks.
4 Position the endpoints of the red line on two symmetrical diffraction peaks.

Diffractogram

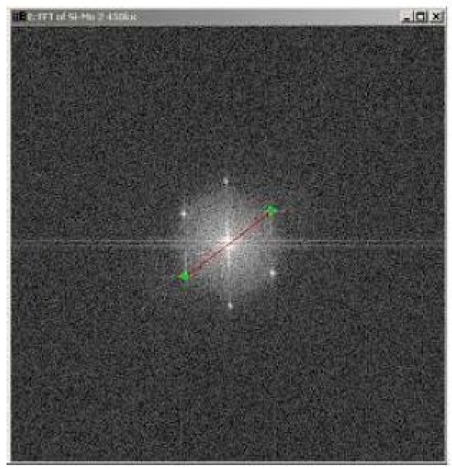


Figure 39: Distance between peaks in the calculated diffractogram.

5 Press **OK** to specify the reciprocal unit and the d-spacing (in the corresponding real units) in the next window.
6 Read the calibration instructions and click **OK**., then enter the known spacing between peaks in the magnification calibration in the **Calibration Settings** window.

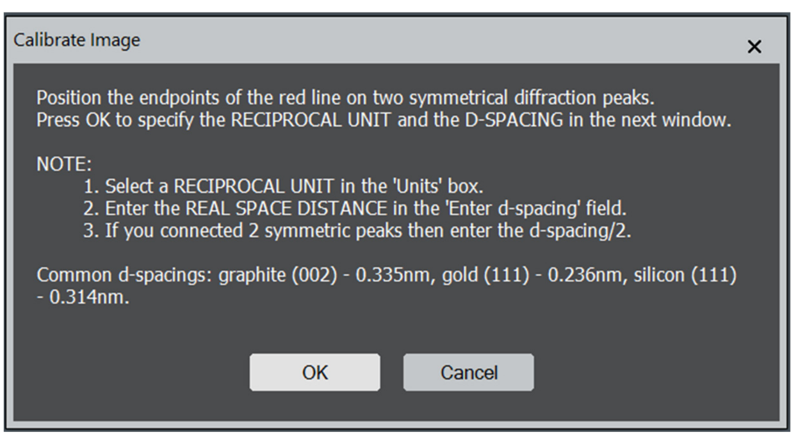


Figure 40: Calibration instructions.

7 Advanced topics

1.29 Data rates, storage, and transfer

The K3 IS computer is configured with a high-speed RAID storage system. The RAID is available as the X: drive within Windows Explorer and for best performance should be the destination for data capture. The RAID system provides

- >30 TB of RAID 0 solid-state storage
- ~120 min of data storage when capturing at the largest field of view and fastest frame rate
- >28 Gbps max write speed

The data size of a single, **Counted Mode**, the full field of view, bin x1 image is >92 MB. Single images are saved with a 4-byte Integer data type.

When continuously capturing counted images, the data is saved with a 1-byte integer data type. As a result, Counted mode, the full field of view, bin x1 images captured at 75 fps leads to a data rate of 13.2 Gbps being written to the RAID.

The K3 IS computer system provides USB-3 ports for transferring data to portable hard drives. The transfer speed may vary between the different USB ports, but data transfer rates of over 250 MB/s (2000 Mb/s) are possible.

Generally, portable hard drive transfer rates are limited by the drive write speed and not by the USB transfer speed unless using an SSD as the destination drive.

The K3 IS computer system also provides a Gigabit Ethernet port to transfer data. Contact Gatan service to enable the use of the ethernet connection.

1.30 GPU Option

There is an optional GPU available for the K3 IS camera. With this option, the camera can automatically drift-correct single images during acquisition. An advanced algorithm handles low-signal short-exposure frames from the high-speed acquisition. An example of data collected with and without the GPU-enabled drift correction.

When the GPU option is installed, there are two changes to the standard K3 interface:

- A drift correction checkbox is made available in the **K3 Camera** palette
- The **K3 Setup** dialog is modified to configure the drift correction algorithm

The GPU live drift correction cannot handle an arbitrarily large series of frames. The software displays a warning when the current settings would create too much data for the GPU to handle. If this warning appears, decrease the number of frames or increase the binning to reduce the data size until the warning disappears.

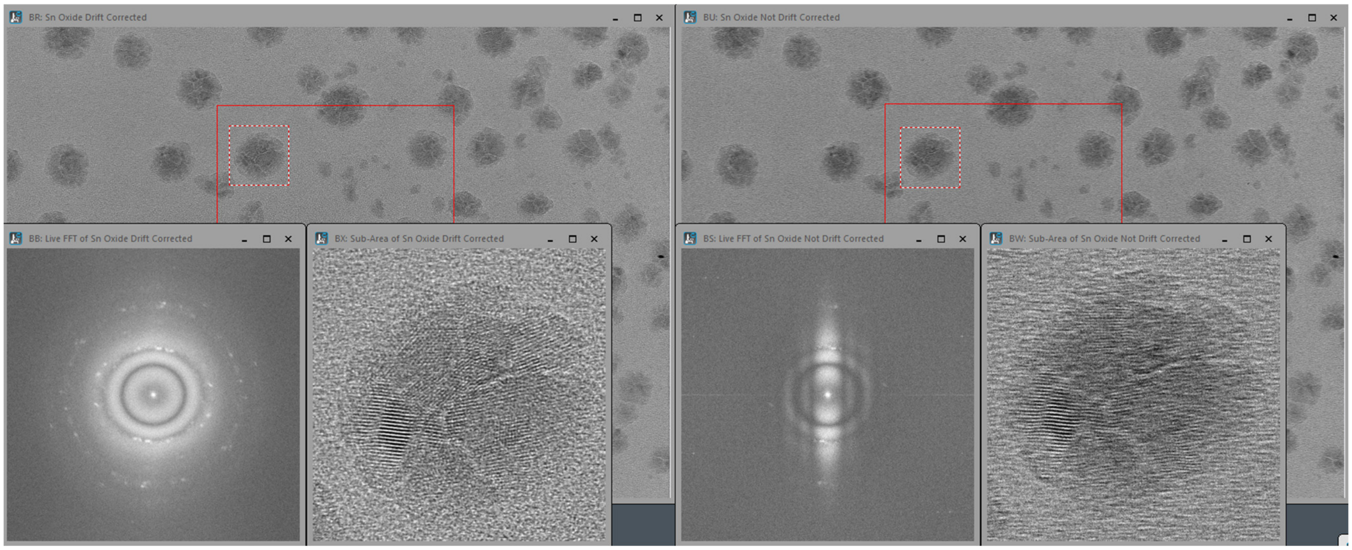


Figure 42: Left: Image captured with GPU live drift correction.

Figure 43: K3 Camera Setup dialog with optional GPU license.

Figure 41: K3 Setup dialog with optional GPU.

1.31 STEMx

The Gatan STEMx® system offers 4D scanning transmission electron microscope (STEM) diffraction data collection through hardware synchronized beam control with Gatan's *in-situ* cameras (K3 IS, OneView® IS, and Rio™ IS). There are many advantages to collecting 4D STEM data through DM software with the K3 IS camera, including:

- High-speed data acquisition for detail-rich mapping using direct hardware communication and synchronization
- Minimized artifacts associated with specimen damage and system drift - due to the high-speed, shorter duration of each experiment
- All camera functions are still available in addition to the 4D STEM data collection mode

This allows the K3 IS camera to function as both a data collection tool for 4D STEM and also be used to collect low dose, high-framerate, electron-counted images.

- Simultaneously acquire multiple signals produced in the electron microscope as part of a spectrum image
- Easy to use interface: 4D datasets are collected, stored, and processed in a single application (e.g., DM software)

More information on STEMx can be found on the Gatan website.

1.32 Scripting

Commercial software is great for standard data acquisition and processing techniques, but there is a vast spectrum of techniques and applications which are not all supported equally. Researchers are also constantly developing new methods uniquely tailored to specific experimental designs and the questions they are seeking to answer. Scripting provides nearly infinite flexibility for data acquisition, processing, analysis, and visualization and can be rapidly prototyped and tweaked as needed, and DM software supports two integrated scripting environments.

1. DM scripting
   - Scripting language native to DM
   - Supports user-written routines from simple macros to large applications
   - Custom syntax (C++ style, Object Oriented Programming)
   - Recently added greatly improved editor, debugging, and documentation

See **DM Help** for more info about DM scripting.

2. Python
   - Embedded within DM as an alternative scripting language
   - Includes support for Python libraries, including:
   - NumPy, SciPy, and Matplotlib
   - Machine Learning and Deep Learning using TensorFlow, Scikit-Learn

- Spectral analysis with HyperSpy….

See **DM Help** for more info about Python Scripting within DM software.

For the K3 IS camera, scripting can be used to capture still frames. It can also be used to start and stop in-situ acquisition using the acquisition parameters currently specified in the UI. This can be useful for roughly synchronizing the acquisition of data with other actions in the Gatan software as well as with equipment not controlled directly by DM.

A short example DM script for generating a 2-button UI to acquire an in-situ video dataset using a Gatan IS camera is given below. Additional scripts and scripting resources can be found on Gatan's website.

```
//$BACKGROUND$
class ISAcquireDialog : UIFrame{
 TagGroup CreateDLGTagGroup( object self ){
        // Dialog building method
        TagGroup DLGtgs, DLGItems
        DLGtgs = DLGCreateDialog( "IS Acquire Dialog", DLGItems )
        TagGroup Button1 = DLGCreatePushButton( "Start", "Act1Meth" )
        TagGroup Button2 = DLGCreatePushButton( "Stop", "Act2Meth" )
        DLGItems.DLGAddElement( Button1 )
        DLGItems.DLGAddElement( Button2 )
        DLGtgs.DLGTableLayout( 2, 1, 0 )
        return DLGtgs
 }
 object LaunchAsModelessDialog( object self ){
        // Main call. Create and display the modeless Dialog
        self.init( self.CreateDLGTagGroup() )
        self.Display( "IS Acquire" )
 }
 // Methods invoked by buttons
 void Act1Meth( object self ){
        Result( "\nStart Recording.\n" )
            CM_InSitu_StartRecord()
 }
 void Act2Meth( object self ){
        Result( "Stop Recording.\n" )
        CM_InSitu_StopRecord()
 }
}
Alloc(ISAcquireDialog).LaunchAsModelessDialog()
```

8 Troubleshooting

1.33 Software updates

The K3 IS computer is configured and tested with the DM software installed. Updates to DM are regularly made available to add features and to fix reported bugs. Check the Gatan software website for the latest patches and update the DM software and camera firmware as required.

1.34 Reporting bugs

Users can report bugs found in software by clicking the camera monitor settings button. This button is only visible in power user mode (Section 1.25.1)

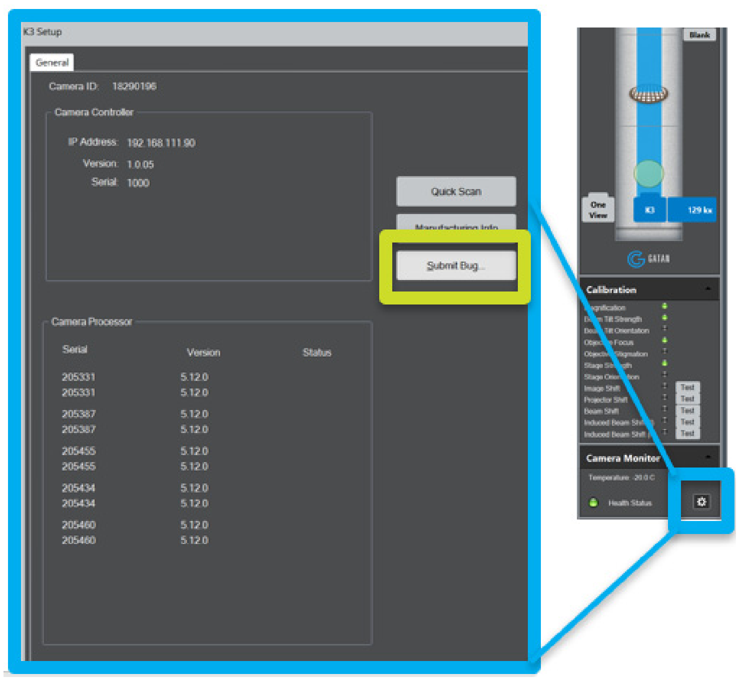


Figure 44: Software Bug Submission in Camera Monitor Settings

- Submissions are reviewed weekly by the Gatan software team
- For critical issues that make camera un-useable, contact the Gatan service team
- http://www.gatan.com/contact