# Rio Camera User Manual

## Models 1804, 1809, 1816



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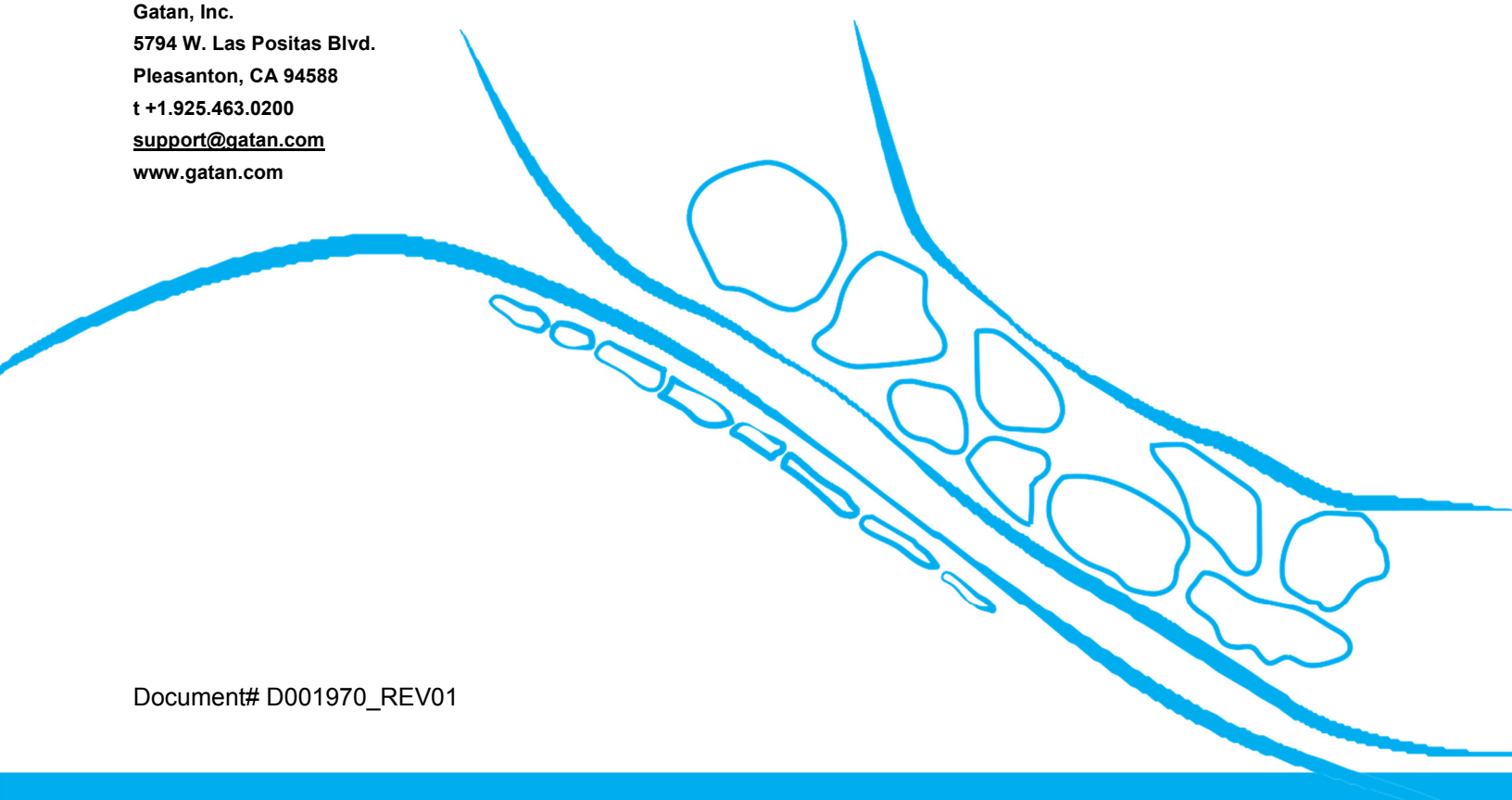

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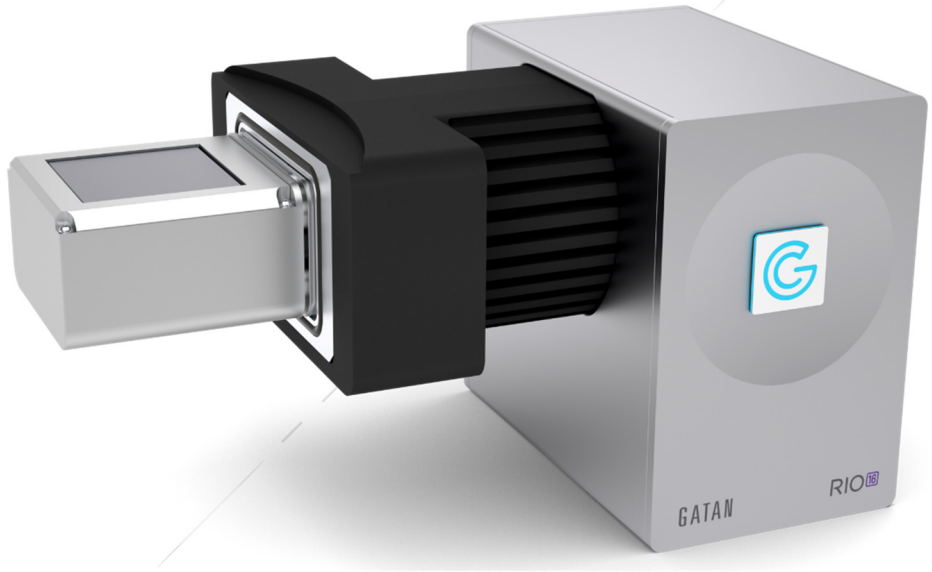
# 1 Introduction

## 1.1 Rio camera system

The Rio™ is the only CMOS camera that standardizes advanced performance for routine imaging and *in-situ* applications.




### Advantages

- High sensitivity: Optimized scintillator and 1:1 fiber optic coupling. Gpixel CMOS sensor with 100% duty cycle and ultra-low readout noise.
- High resolution: 9 µm pixel, optimal for 30 − 200 kV operation
- Large field of view: Up to 16 megapixels (4k x 4k)
- High speed: Up to 20 frames per second (fps) at up to 4k x 4k resolution and up to 160 fps at 1k x 1k
- Guarantee optimal image quality with real-time drift correction and outlier removal using in-line data processing
- Increase productivity, even for novice users, with intuitive, built-in workflows to support and optimize recording modes

### *In-situ* option (model 1816 only)

- Flexibly trade-off resolution against frame rate − from 4096 x 4096 pixels at 20 fps to 1k x 1k pixels at 160 fps, always at 100% duty cycle
- Video buffer allows you to capture only the video you want; with post-event triggering and LookBack™ feature
- Never miss the start of an *in-situ* reaction again
- Tailor videos to your unique applications with powerful post-processing tools

## 1.2 Gatan Microscopy Suite interface

Launching Gatan Microscopy Suite® (GMS) version 3.3 brings up the following screen:

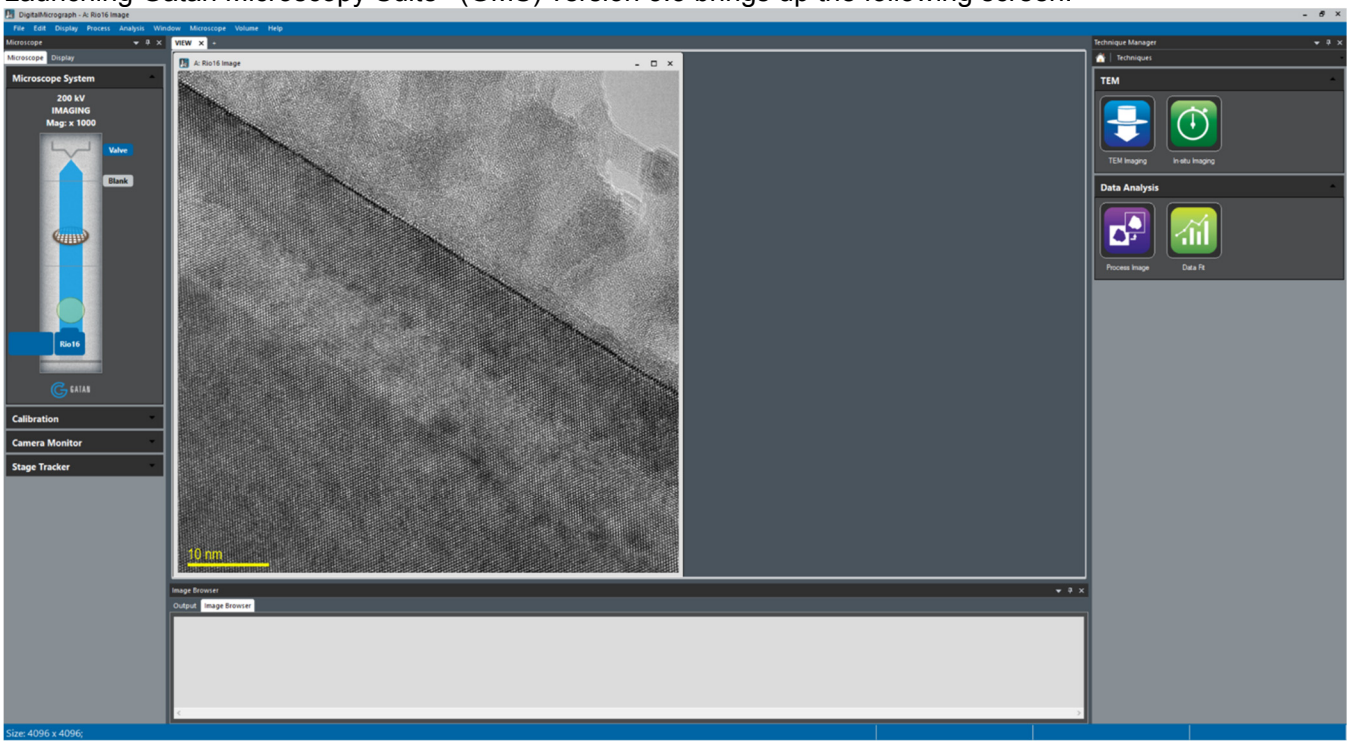



***Figure 1:*** *The GMS 3 interface, with the Microscope System panel on the left, the Workspace area in center, the Technique Manager panel on the right and the Image Browser and Output areas on the bottom.*

On the left: Microscope System panel, a schematic diagram of the microscope that graphically indicates:

- Whether the camera is inserted or retracted into the beam: Inserted shown
- The current choice of either imaging or diffraction mode: Imaging mode shown
- The presence of a sample: Grid shown
- The status of the viewing screen: Up is shown

In the center is the Workspace where images are shown.

At the bottom of the screen are two tabbed panels, one for text output and another showing recently acquired images.

On the right is the Technique Manager, with as many as five groups of techniques (Dependent on licenses):

- TEM: TEM Imaging; *In-Situ* Imaging; and TEM Tomography
- Scanning TEM (STEM): STEM Imaging and STEM Tomography
- Analytical TEM: Energy-filtered TEM (EFTEM) Map; EFTEM spectrum imaging (SI); STEM SI; and Spectroscopy
- Data Analysis: Analytical; Data Fit; and Process Image
- Custom

# 2 Operation of the Rio camera

**IMPORTANT**
*Before installing and operating this produce, and to avoid the risk of injury and potential hazards, read and review the Regulatory Pamphlet and follow all safety instructions.*

## 2.1 Rio camera imaging

Select TEM Imaging from the techniques Page. In the Camera palette, the top row shows binning choices which depends on the camera model that is installed (see table below):

| Camera Model | Image Size | | |
|---|---|---|---|
| | Bin x1 | Bin x2 | Bin x4 |
| Rio 16 | 4096 x 4096 | 2048 x 2048 | 1024 x 1024 |
| Rio 9 | 3072 x 3072 | 1536 x 1536 | - |
| Rio 4 | 2048 x 2048 | - | - |

*Note: Screenshots in this document are for model Rio 16.*

View mode allows the user to observe live (continuous) images on the screen and Capture mode is for the standard acquisition of TEM images and diffraction patterns. View exposure time defaults to minimum exposure available for the selected image size. Increasing the exposure time will result in summing two or more frames.

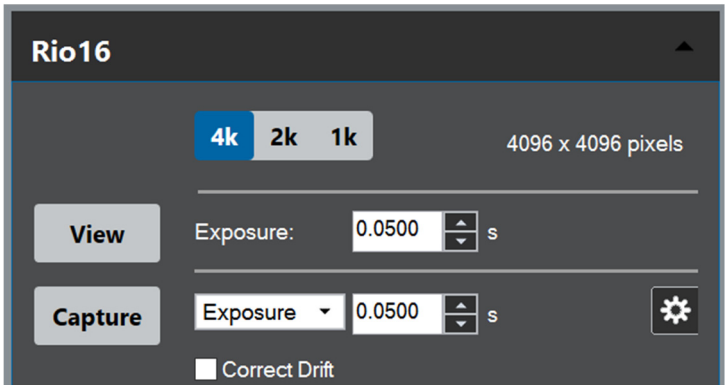



***Figure 2:*** *The Rio 16 camera palette.*

There are three different options available for image capture (Figure 3):

- Automatic: sums frames until it reaches a preset signal to noise ratio
- Exposure: sums frames for a user defined exposure time
- Target Dose: sums frames until it reaches a user-defined total dose (e/A$^2$)

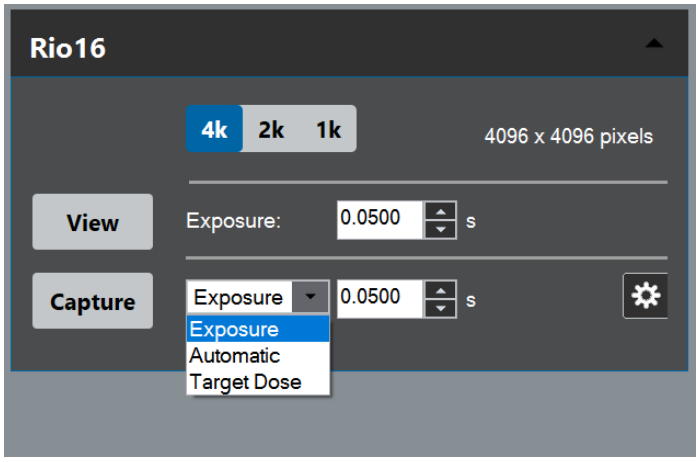



***Figure 3:*** *The Capture exposure control drop down list.*

The Capture control also has a tool button (the small gear to the right of the Capture button), and when clicked calls up the dialog shown in Figure 4. Options include:

- Drift Correction
- Trim Drift Corrected Image: means only the regions covering the same part of the sample image are retained in the alignment process, and the outer margin that is not used in the aligned, summed image is cropped
- Rotation: Software image rotation. When this feature is enabled, it will crop the viewed image to a lower size keeping the cropped image in a square format. The size is based on the maximum angle of rotation (45°).
- Remove outliers (statistical outliers in pixel intensity value).

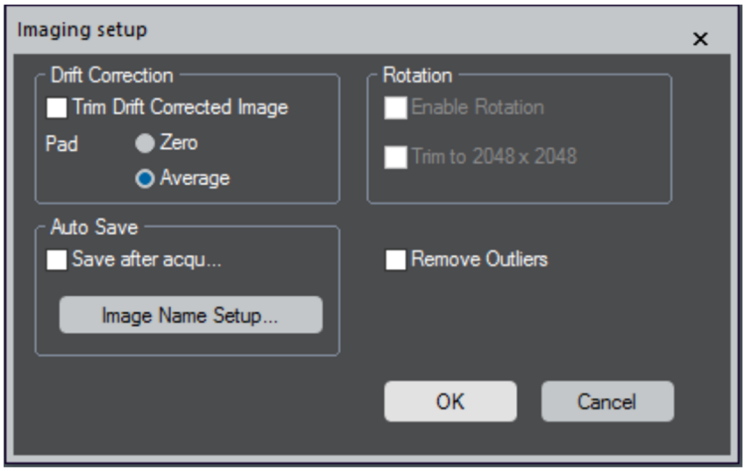



***Figure 4:*** *Clicking the Capture tool icon brings out these options in the imaging setup.*

An example of how effective drift correction can be seen in Figure 5.

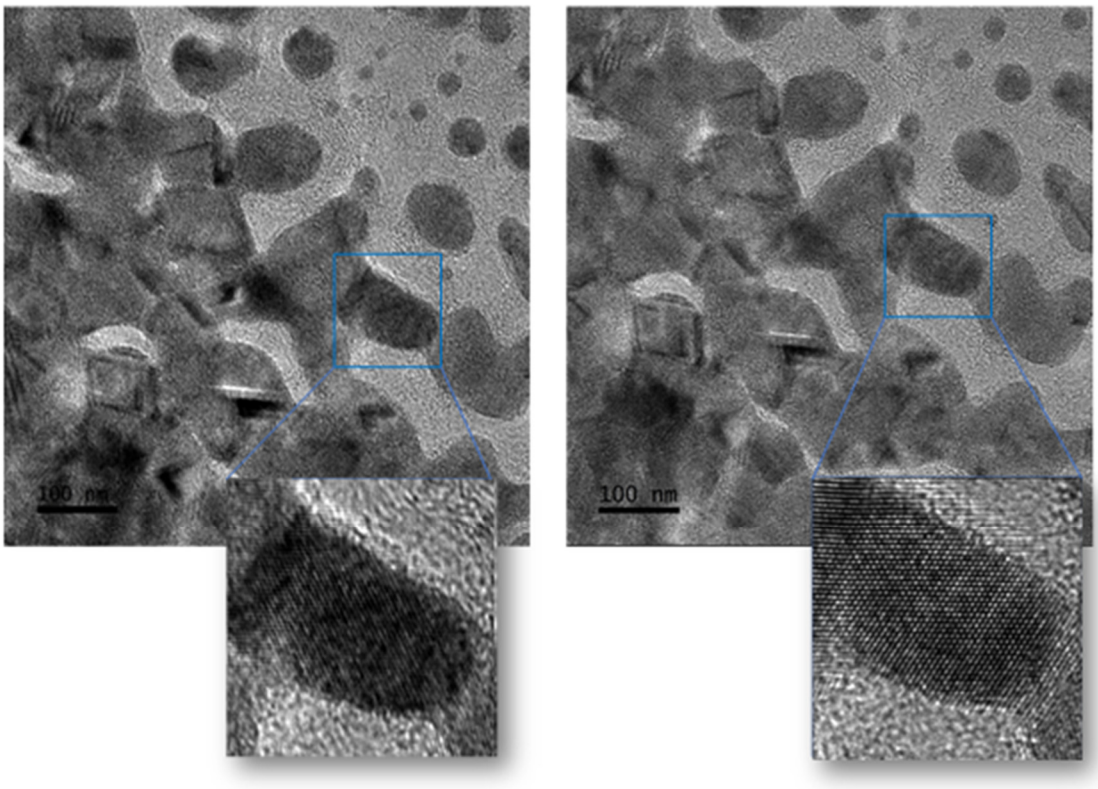



***Figure 5:*** *Comparison of high resolution of Au nanoparticle images acquired with drift correction on (right) and off (left).*

## 2.2 Rio camera in TEM *in-situ* mode (model 1816 only)

The TEM *In-situ* Imaging technique allows the capture of a series of images.

The *In-Situ* Acquisition palette has four binning options: 1x (4k), 2x (2k), 4x (1k), and 8x (512), which allows for frame rates of 20, 80, 160, and 160 fps respectively.

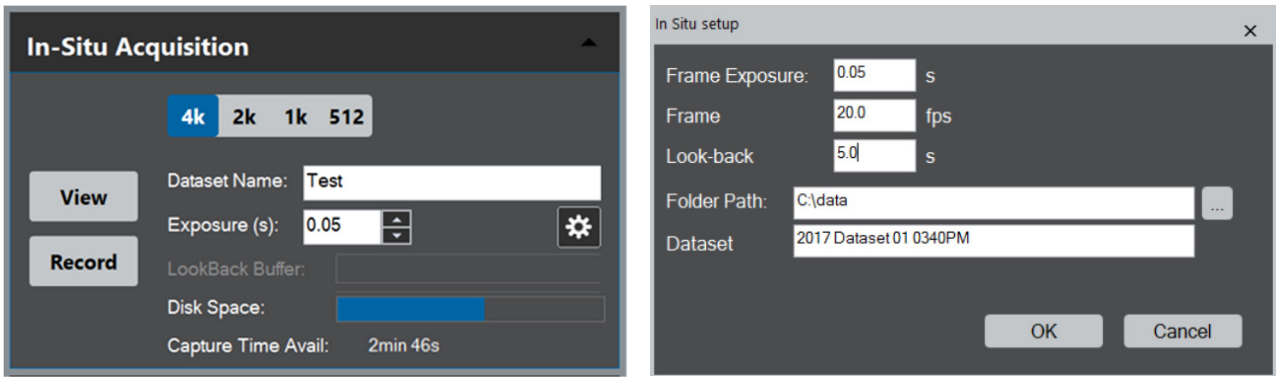



***Figure 6:*** *The In-Situ Acquisition control palette, on the left, and In Situ setup dialog, on the right.*

To record an *in-situ* dataset:

1 Open the *In Situ* setup window.
   a. In Folder Path, enter desired header name for collected files and the folder where they will be stored.
   b. In Dataset Name, enter the filename of the image series.
   c. Set the Look-back Duration (maximum time shown on the graphical user interface).
   d. Click OK to use these settings.
2 Click on Record button to begin capturing data to the PC.
3 Press the Stop button when your reaction is finished.
4 To take the next dataset, click on Record again.

Once the *in-situ* data is captured, use the *In-Situ* Player to process it:

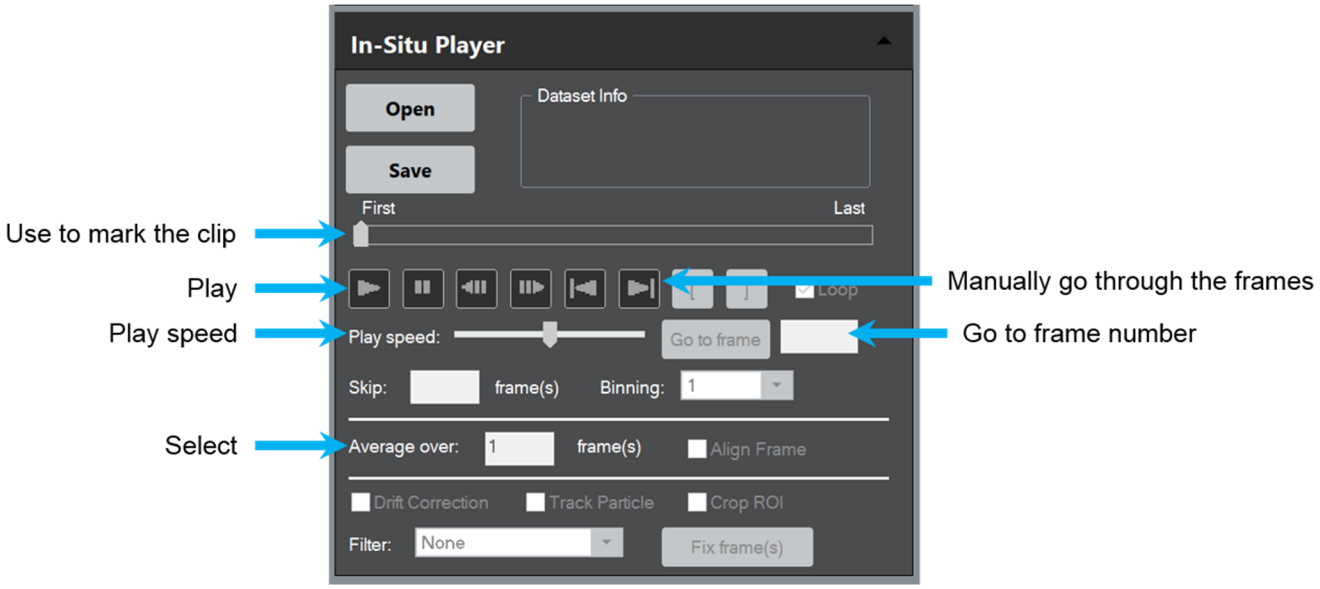



***Figure 7:*** *The In-Situ Player palette options for playback and post process of data.*

Click on Open and browse to the dataset. Once the data is open, you have multiple options for previewing and post-processing:

- Automatically playback the data set by selecting the Play speed and clicking on Play

*Note: Play speed setting here is only for the playback display and does not affect the saved dataset frame rate.*

- Manually move through the frames:
- Use the slider and move it to left and right to view a specific frame
- Set the step size (#frames) in Next/Prev step section and then click on the arrows
  - Setting to 1 means each frame in the dataset will be played
  - Setting it to 3 means playback frames will correspond to every 3rd frame
  - Specify the Frame/Time of interest and click on Go To

*Note: The same step size selected here will be used when data is saved.*

- Crop:
  - To crop in time, use the left and right brackets to specify the section of the frames you are interested in
  - To crop in space, use the ROI tool and select the region on the displayed clip. Then check Crop ROI. Once the data is Saved, DigitalMicrograph® (DM) automatically crops each frame to this specified region of interest (ROI).
- Binning: To improve the signal-to-noise ratio (SNR) or to save disc space you can bin these frames by 2, 4, or 8.
- Average over frames: determines how many frames from your dataset will be averaged to produce one frame of playback

Setting to 1 means no averaging while setting to 3 means that the $n^{th}$ frame of playback is the average of the $(n-1)^{th}$, $n^{th}$, and $(n+1)^{th}$ frames from the original dataset.

Note: If you check Average Frame Alignment, with averaging over 3 frames, every 3 frames are aligned before they are averaged to replace the $n^{th}$ frame. This is very useful in cases where the sample drifts in between frames.

**Examples**

If you set Averaging to 3 and the step size is 1: the first frame will be 1/2/3; then the $2^{nd}$ frame will be frames 2/3/4; and then the third frame will be 3/4/5, and so on.

If you set Averaging to 3 and step size of 3: the first frame will be 1/2/3; then the $2^{nd}$ frame will be 4/5/6; and then the third frame will be 7/8/9, and so on.

*Note: the same Average settings selected here will be used when data is saved.*

- Drift Correction: Compensate for continuous drifting effects in the sample, allowing a drifting sample to appear stationary in processed images

  For example, if you set Average Over 3 frames, each 3 frames will be averaged, and then averaged frames will be drift corrected. This is especially helpful when the dose in each frame is too low to do drift correction correctly.
- Track Particle: The user draws an ROI on a feature that is moving in the original dataset, DM will drift correct for the selected feature and crop to this specified ROI to keep this feature in the center of the frame in processed images
- Filter: Most of the processes explained above are done using cross-correlation between consecutive frames. In some cases, application of imaging filter may improve the results
- Fix planes: Can be used to replace one or multiple frames with a weighted average of their neighboring good frames

## 2.3 Power on sequence for Rio

1. Use the power switch on the camera head to turn it on, wait 20 seconds before moving to next step.
2. Launch DM.
3. Camera monitor will flash as the camera is powered on.
4. Wait for temperature to stabilize (camera monitor window).

## 2.4 Power off sequence for Rio

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

1. Warm the camera up to room temperature (for instructions see next section).
2. Close DM; camera will be automatically retracted.
3. Switch the camera off using the power switch toggle on the camera head.

4 Turn off the PC if desired.


***Figure 8: Power switch toggle is located on bottom of the camera head.***

## 2.5 Setting camera temperature

For normal use, the camera's image sensor must be cooled, and this is done by a menu choice shown below in Figure 9 (must be in Power User mode, select user mode under DM Help menu).

In the Camera menu, select Temperature and enter the set point value. The typical operating temperature is +10 °C. If the camera chamber needs to be vented, set this to +20 °C, wait for the warm-up to complete before venting.

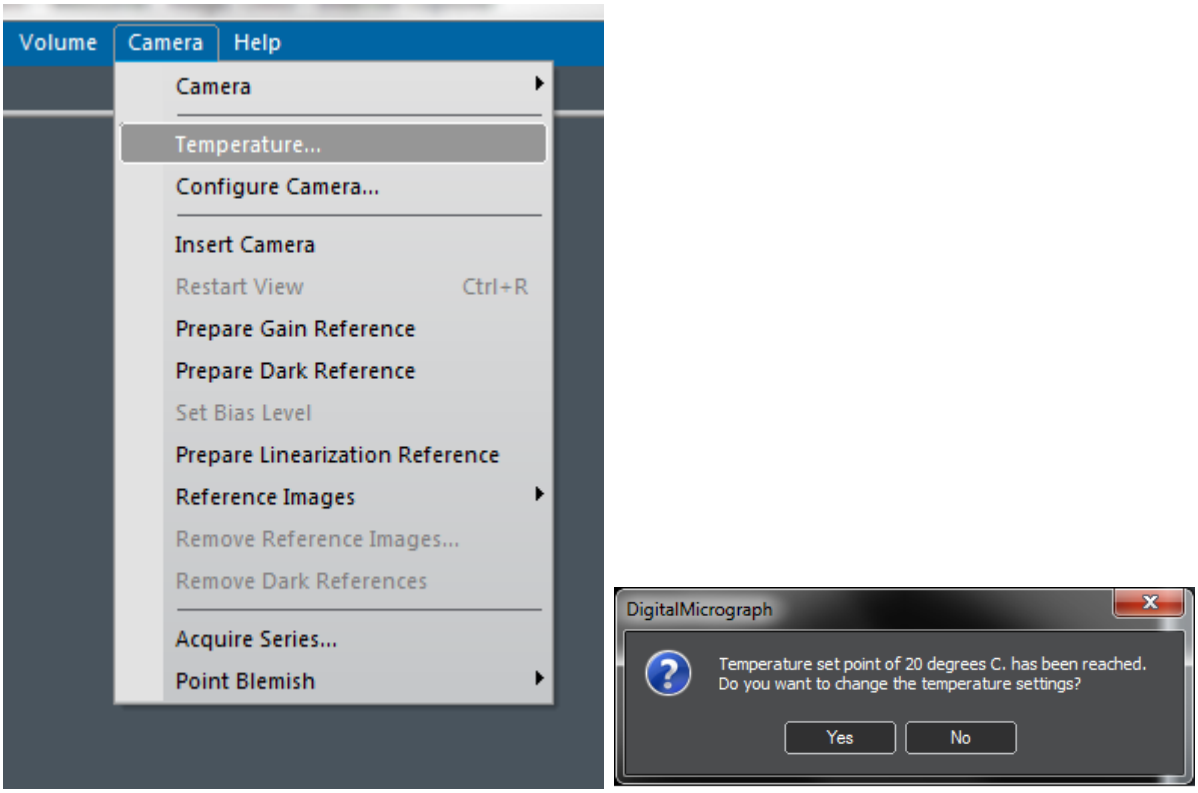



***Figure 9:*** *Camera menu with the Temperature choice highlighted, and the dialog.*

## 2.6 Obtaining reference images

Before images can be acquired, both the Dark Reference and the Gain Reference must be prepared. These are accomplished by menu choices shown in Figure 10 and 11.

### 2.6.1 Prepare dark references

Select the camera menu and click on Prepare Dark. Follow the instructions on the screen (shown below). The software will automatically collect reference images for all binned modes. Click ok when dark reference acquisition is done.

*Note: You can collect the dark reference images for only your currently-selected binning mode if the shift key is held down on the keyboard when Prepare Dark is clicked.*

### 2.6.2 Prepare gain references

Select the camera menu and click on Prepare Gain. Follow the instructions on the screen (shown below). The software will automatically collect reference images for all binned modes. Click ok when gain reference acquisition is done.

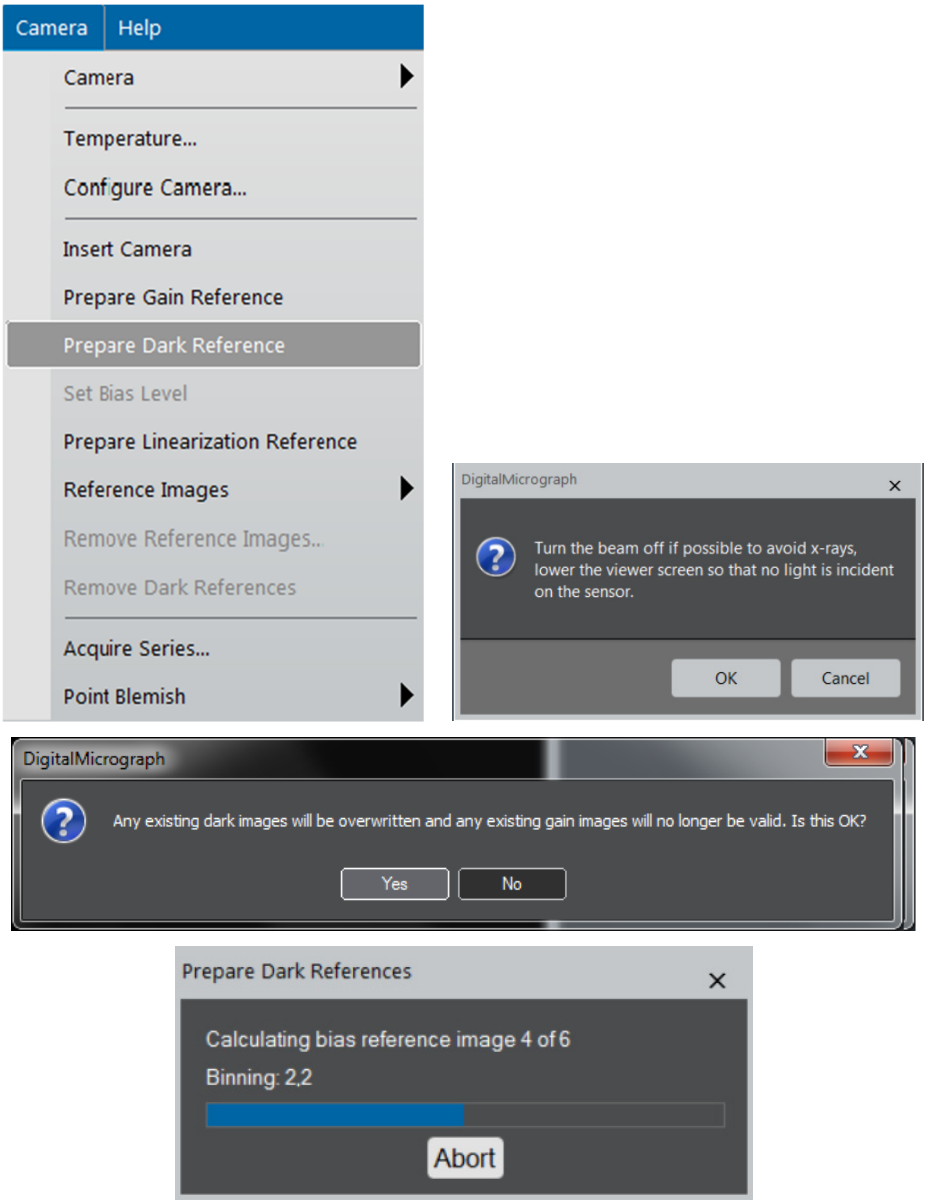



***Figure 10:*** *Preparing the dark reference.*

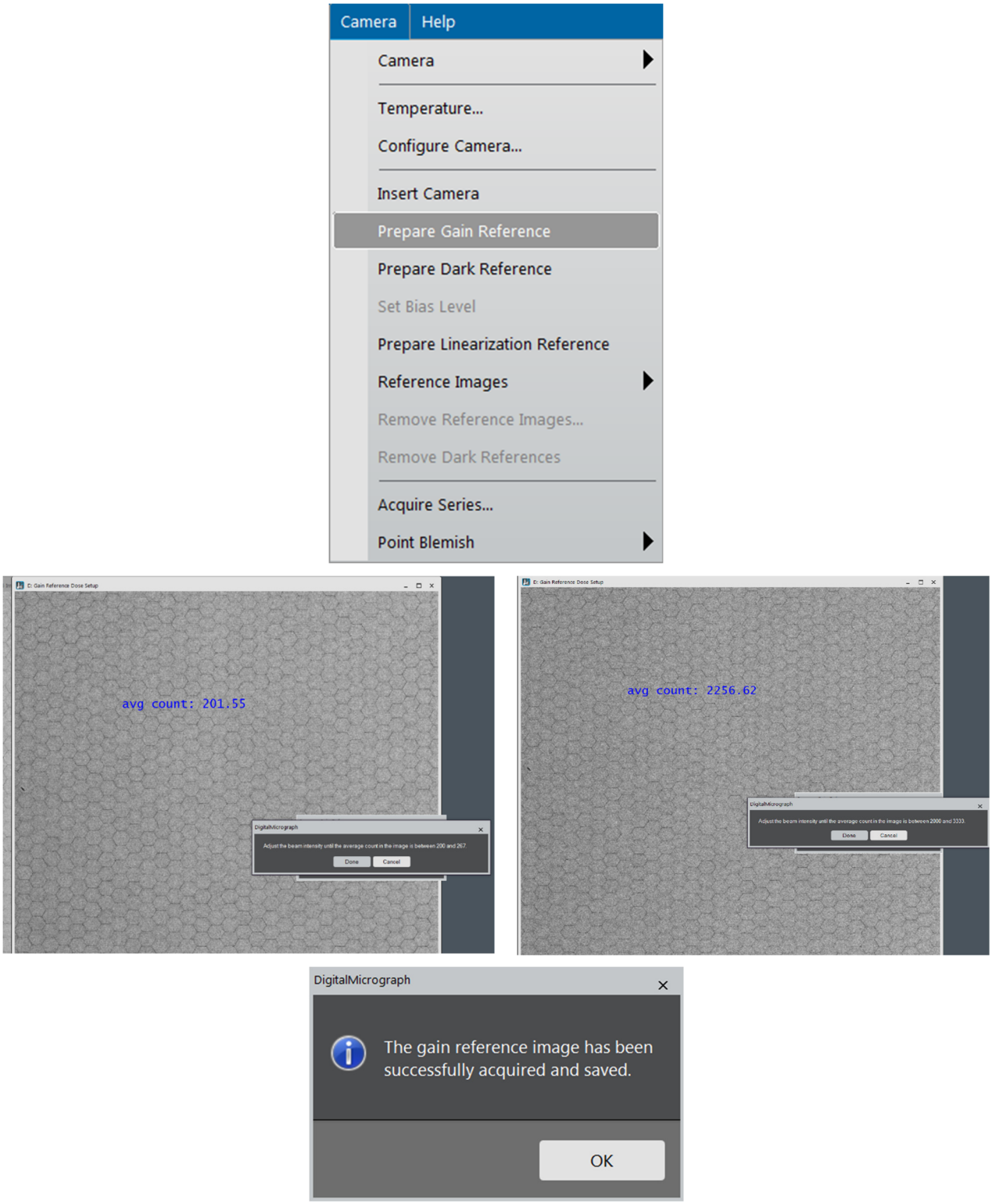



***Figure 11:*** *Preparing the gain reference.*

# 2.7 Care of detector

## 2.7.1 Temperature

Typical operating temperature is +10 °C. Monitor the temperature; the Camera Monitor palette indicates the camera temperature.

Check the flow of cooling water periodically. If the flow rate of the cooling water deviates significantly from the originally set value of ~15 – 30 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.

### Annealing

Monthly camera anneal is recommended. Set the camera temperature to 35 °C and run the anneal cycle for 4 – 8 h, as convenient, for example overnight.

## 2.7.2 Insertion indication

The blue and white Gatan logo on the side of the camera lights up continuously when powered. When the camera is retracted the orange, physical flag will out on the back side of the camera head (shown below).

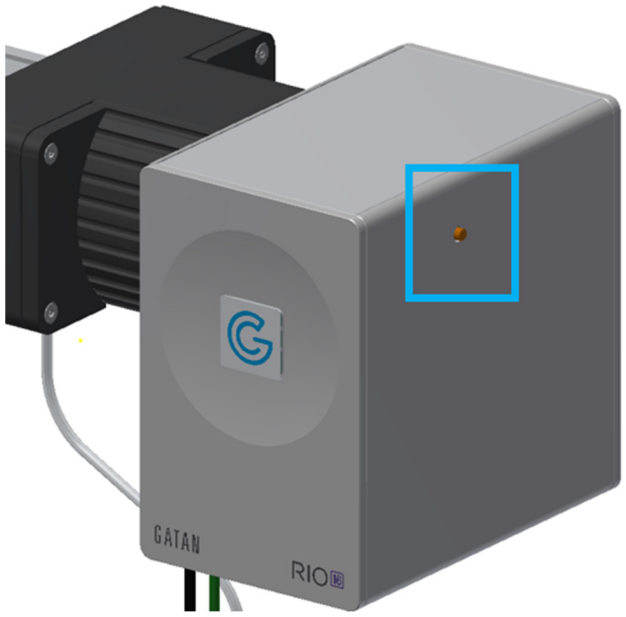



***Figure 12:*** *Rio camera flag shown above in retracted position.*

# 2.8 Magnification correction/calibration

The displayed nominal magnification on TEM is for photographic film and has an accuracy of 5 – 10%. The Rio camera is located on a different plane (height wise) respect to 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 and use the FFT method.

It is very important to make sure 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 software 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.

## 2.8.1 Low magnification

Record an image of a cross grating replica, such as shown in Figure 13.

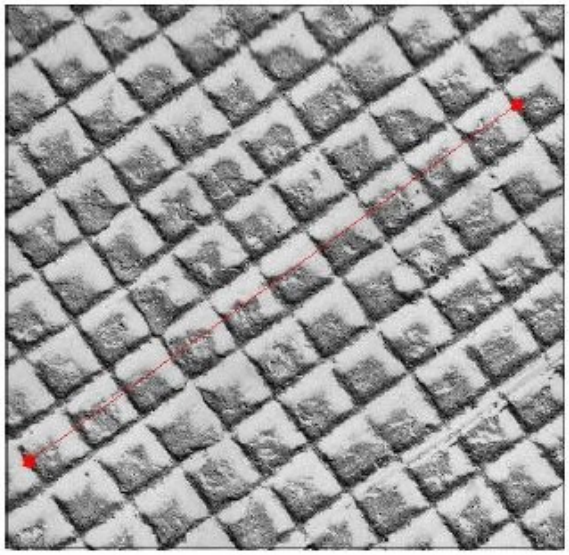


***Figure 13:*** *Example of marking a known distance during magnification calibration.*

1 Choose Microscope > Calibrate Image.

2 Follow the instructions on screen, as shown in Figure 14, and a red line will appear on the image.

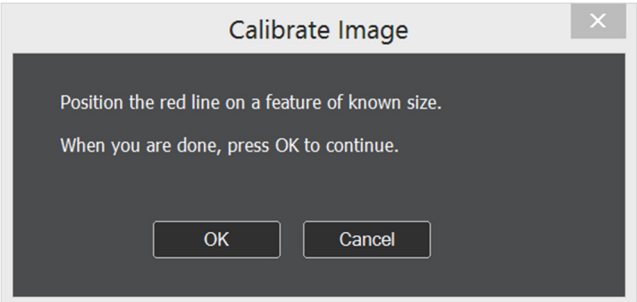



***Figure 14:*** *Magnification calibration instructions.*

3 Position the red line on a feature of known size.

4 Press OK on the Calibrate Image window.

5 Enter the correct distance for the selected feature (for example 10 line pairs of cross grating sample where the distance = 10 x 0.463 µm) in the Calibration window and select the units.

6 Select the distance marked in the previous figure to perform the magnification calibration, in the Calibration dialog, as shown in Figure 15.

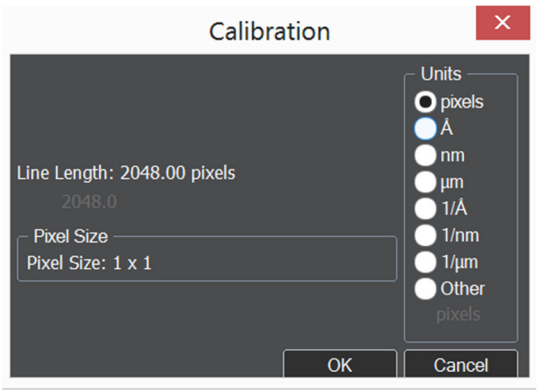



***Figure 15:*** *Calibration dialog.*

7 Press OK.

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

9 To view the magnification table, select Microscope > Calibrations.

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

## 2.8.2 High magnification

Record a lattice image of the crystalline sample.

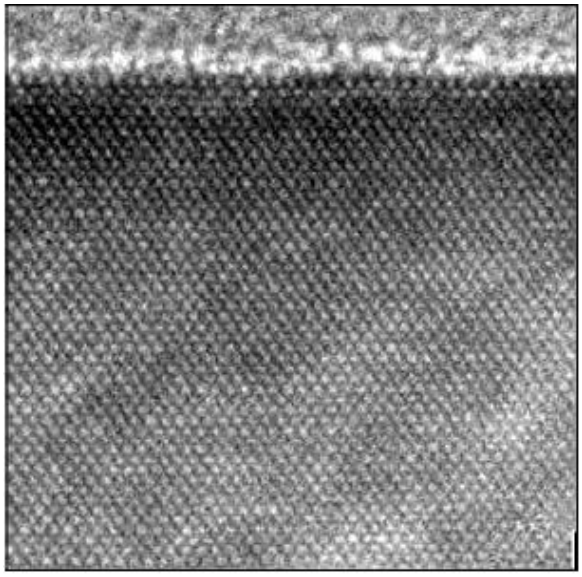


***Figure 16:*** *High-resolution image of sample to be used in the magnification calibration.*

1 Select Microscope > Calibrate image from Diffractogram.

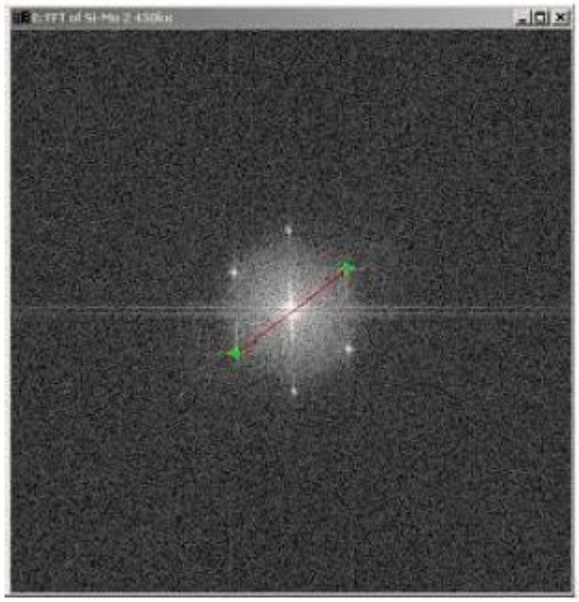


***Figure 17:*** *Distance between peaks in the calculated diffractogram.*

2 To calculate the diffractogram, follow the on-screen instructions.

A red line appears on the diffractogram indicating the distance between peaks. Position the endpoints of the red line on two symmetrical diffraction peaks.

3 Press OK to specify the reciprocal unit and the d-spacing (in the corresponding real units) in the next window.

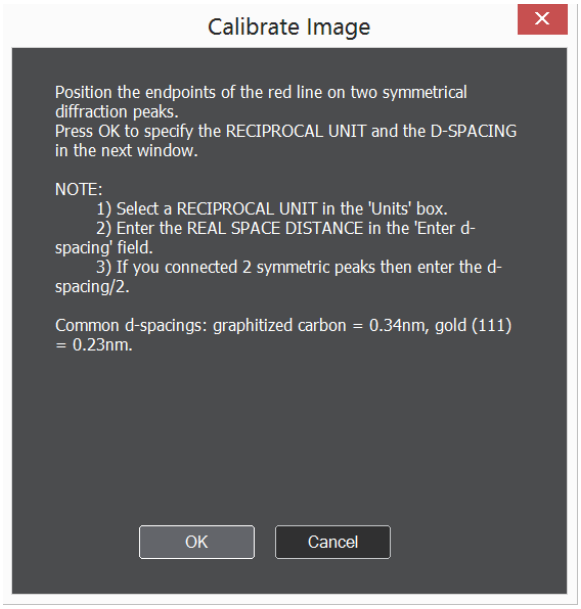



***Figure 18:*** *Calibration instructions.*

4 Read the calibration instructions and click OK, then enter the known spacing between peaks in the magnification calibration in the Calibration settings window.

## 2.9 Compatibility with other Gatan cameras and GIFs

All Rio cameras are fully supported within Gatan's industry-leading software platforms, DM and GMS. GMS software gives you access to a wide range of additional applications to make electron microscopy easier and more efficient.

The Rio 9 and Rio 16 cameras are retractable, which allows simultaneous installation of other cameras, Gatan imaging filters (GIFs) or electron energy loss spectrometers, to cover the entire range of imaging requirements.

# A Specifications comparing Rio camera models

| Camera Model | Rio 16 | Rio 9 | Rio4 |
|---|---|---|---|
| TEM Operating voltage (kV) | 30 – 200 | | 30 – 120 |
| Sensor active size (mm) | 36.9 x 36.9 | 27.8 x 27.8 | 18.5 x 18.5 |
| Sensor size (pixels) | 4096 x 4096 | 3072 x 3072 | 2048 x 2048 |
| Pixel size (µm) | 9 | 9 | 9 |
| Full sensor read-out speed (fps) | 20 | 15 | 15 |
| Image display on monitor (fps) | 20 | 15 | 15 |
| Recording modes | Image<br>Video (*in-situ* option) | Image | Image |
| Image formats | 1:1 (4k, 2k, 1k) | 1:1 (3k, 1.5k) | 1:1 (2k) |
| Full resolution frame rate, including *in-situ* for Rio16 (fps)<br>4k x 4k<br>3k x 3k<br>2k x 2k<br>1.5k x 1.5k<br>1k x 1k | <br>20<br>–<br>80<br>–<br>160 | <br>–<br>15<br>–<br>60<br>– | <br>–<br>–<br>15<br>–<br>– |
| Dynamic range frame accumulation | ≥16-bit | ≥16-bit | ≥16-bit |
| Mounting Position | Bottom and side, retractable | Bottom and side, retractable | Bottom, non-retractable |
| GIF compatible | Yes | Yes | No |
| Acquiring reference images (gains & darks) | Directions are the same for all cameras | – | – |
| Drift correction | Yes | Yes | Yes |