ThermoFisher SCIENTIFIC

Tomography STEM

for STEM mode
User Manual

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thermo scientific

Contents

1 Introduction

1.1 Introduction to Tomography STEM

The Thermo Scientific Tomography STEM software provides the functionalities to prepare and execute the automated acquisition of one or more *Tilt Series*. A Tilt Series is a set of images of the same specimen feature, viewed at different tilt angles. The Tilt Series images are processed by reconstruction software to create a 3D reconstruction of the specimen feature.

The Tomography STEM software aims to:

- Enable fast, reliable, automated Tilt Series acquisition.
- Produce high quality Tilt Series images for successful reconstruction of the largest achievable volume.

  This requires maximum stability of the position and focus of the feature of interest at each Tilt Series angle.

Tomography STEM facilitates the following steps in the data acquisition procedure:

- Screening of multiple specimens to select the specimens and features with the highest potential for high quality data acquisition.
- High throughput, high resolution data collection.

1.2 Audience

This manual is aimed at users of Thermo Scientific Transmission Electron Microscopes. In particular, you should be able to successfully identify issues with the microscope. Morover, you should be able perform the necessary corrective actions to resolve the identified issues, provided that these actions are available. For higher level corrective actions, the assistance of a Thermo Fisher Scientific service engineer may be required.

For online training materials to help you improve your Cryo-EM skills, visit the EM-learning.com website.

1.3 System and software compatibility

The Thermo Scientific Tomography STEM software is available for Thermo Scientific TEM systems and FEI TEM systems that run up-to-date microscope software. For detailed system and software version compatibility information, see the Tomography STEM Release Notes.

Note Not all features and functions in this manual are available on all systems and all supported microscope software versions.

2 Getting Started

Start

Verify that the TEM server is running before starting Tomography STEM software.

Tomography STEM software can be started from the Windows Start menu (**All programs** > **Tomography STEM**).

2.1 Prepare for a Tomography STEM session

Before starting an Tomography STEM session, make sure the following prerequisites are met and preparations are completed.

1. Verify that an **Alignments File** and a **FEG Register** are loaded that match with the High Tension and Extractor settings of the system.
2. Verify that the system is in **STEM mode**.
3. (Optional) Verify that the **EDX detectors** are cooled and at a stable temperature.

2.2 Recommendations for loading the specimen on a holder for a Side Entry system

For most Tomography experiments, a flat specimen with a diameter of 3 mm is used. This specimen is often mounted on a copper grid. In such flat samples, the tilt angle at which high quality Tomography data can be acquired is limited by the orientation of the copper grid and the holder geometry. At higher tilt angles, the holder tip and the grid bars may cast a shadow.

To minimize or prevent shadowing:

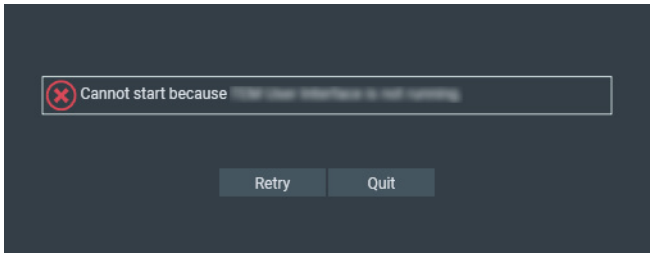
- Load the specimen onto the holder with its grid bars at a 45 degree angle relative to the tilt axis. This increases the angle at which the shadow of the grid bars reaches the center of the GridSquare, so the usable tilt angle range is enlarged.
- Use a rod shaped specimen with a Fischione 2040/2045 or similar holder.

2.3 Start the Tomography STEM software

1. Verify that the system is in **STEM mode**.
2. Start **Tomography STEM**
   Shortcuts can be found on the desktop and in the Windows Start menu.

   Tomography starts the user interface for the current optics mode.

3. A splash screen appears while Tomography STEM runs the startup checks.
   If one or more checks fail, the pop-up displays the related messages.

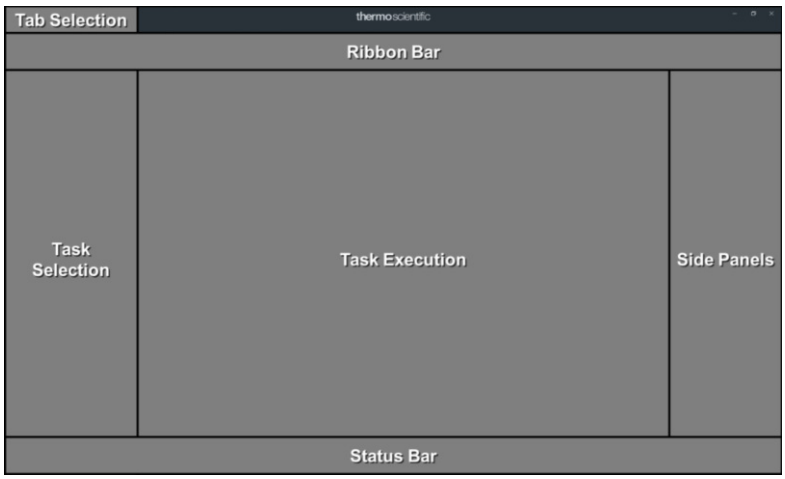


- Solve the reported issue(s).
- Select **Retry**

3 The User Interface

3.1 User interface panels

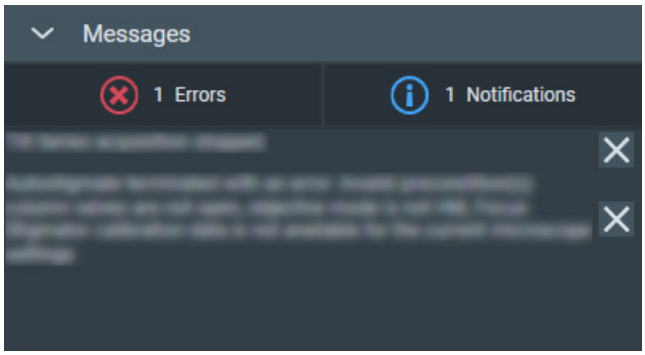
The Tomography STEM user interface guides the user through all actions that are needed to prepare and execute an Automated Acquisition run.



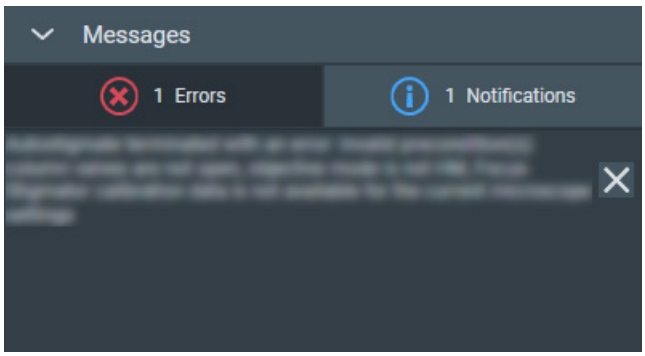
- **Tab Selection**
  The tabs are typically worked through from left to right. Each tab provides a set of tasks.
- **Task Selection**
  Tasks are typically executed from top to bottom. The set of available tasks depends on the selected tab.
- **Task Execution**
  The content of the Task Execution pane depends on the active task. It can display an input dialog, one or multiple acquired images, or progress information for an ongoing function.
- **Ribbon Bar**
  The Ribbon Bar offers a set of controls that are necessary or helpful for completing the active task.
- **Side Panels**
  The Side Panels pane contains a set of collapsible panels. The set of available side panels depends on the active task and/or the selected image in the Task Execution pane.
- **Status Bar**
  The Status Bar displays the CryoFlow login and status. The Status Bar is only visible when CryoFlow is available.

3.2 Messages side panel

The Messages panel shows a list of *Error* and *Notification* messages in chronological order.



By default, all Errors and Notifications are displayed.
Select **Notifications** to hide the Notification messages.

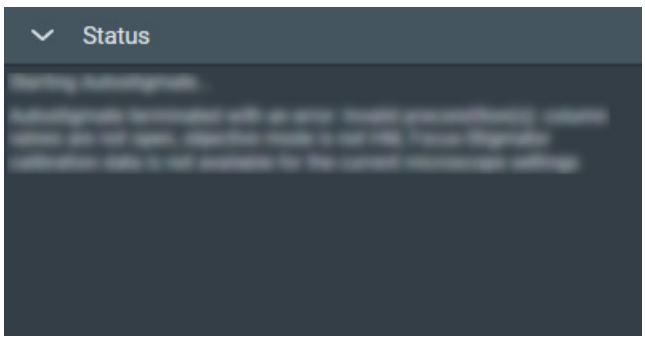


The same applies to the Error messages.

To clear messages that are no longer relevant:

- Select the **cross** at the right-side of each individual message.
- Right-click on **any message** and select **Delete All Messages**

3.3 Status side panel



The Status panel displays various types of messages in chronological order, such as:

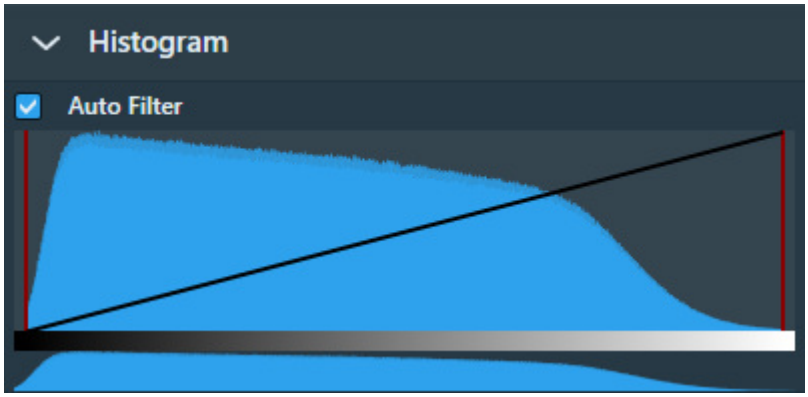
- Errors and Notifications.
- Progress messages for ongoing automated procedures.
- Intermediate and final results of automated procedures.
- Instructions and recommendations to the user.

3.4 Histogram side panel

If two or more images are present in the Task Execution panel, then the Histogram applies only to the selected image. The selected image is recognizable by the highlighted image title and frame.

3.4.1 Histogram side panel

The Histogram side panel shows a histogram of image-pixel intensities for the selected image in the Image Display.



The Histogram side panel offers the following functionalities:

Auto Filter:

- Ticked:
  Tomography STEM automatically calculates the optimal contrast and brightness settings and applies these values when a new image is acquired or selected.
- Cleared:
  Manual adjustments of the contrast, brightness or gamma values are also applied to the next acquisition.

When ticked, it is possible to adjust contrast, brightness and gamma for the current image. To reset contrast, brightness and gamma to their default values, clear *Auto Filter* and tick it again.

Black Level:

Drag the **red line at the left-side** of the main histogram to adjust the black level. Pixels with an intensity below the black level value are displayed with zero intensity (black).

White Level:

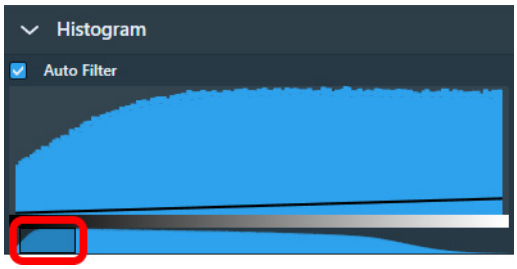
Drag the **red line at the right-side** of the main histogram to adjust the white level. Pixels with an intensity above the white level value are displayed with maximum intensity (white).

Gamma:

Drag the **diagonal black line** up or down to adjust the Gamma curve.

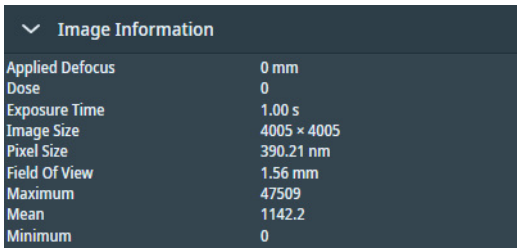
If the Black Level and/or White Level are adjusted, then the Gamma curve is scaled proportionally in the range between the Black Level and White Level values.

Zoom:
In the lower histogram, drag a **range** to zoom in on a section of the spectrum.
Click outside the zoom range in the lower histogram to reset the zoom level.



3.5 Image Information side panel

The Image Information panel displays a small basic set of the image meta data.



Example for a camera image.
For STEM images, different parameters can be displayed.

3.6 Image and plot display area

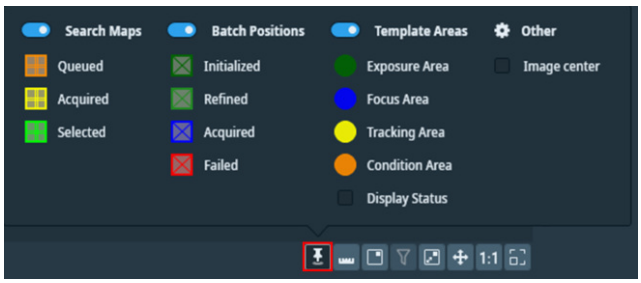
The availability of functionalities for the Image Display depends on the active task, the applied Acquisition and Optics Preset and the selected image.

Zoom slider

Drag the slider with the mouse to change to the zoom level.

Image Markers menu

Specify which image markers are displayed on an image.



Measurement tool

Enables you to measure real distances on an acquired image by drawing rulers.

Show/hide tiles locations

Shows or hides the outlines of the tiles in the image.

Show/hide tilt axis

Shows or hides the Tilt Axis in the image.

Show/hide template areas

Shows or hides the Template Areas in the image.

Color Enhancement

Applies a color mapping to the intensity values in the image. This makes it easier to recognize intensity gradients and areas with similar intensity.

Show/hide the inset image (FFT)

By default, the Inset window displays the FFT of the image.

Filter the FFT image

The FFT filter optimizes the contrast and brightness of the FFT, so that for example Thon Rings are shown clearer. The FFT filter does not change the acquired image data for which the FFT is displayed.

Note This filter is not adjustable and has not been optimized for performance and low resource usage.
Activate the FFT filter only when necessary. Deactivate the FFT filter when there is no direct need to display the FFT for an image.

Swap the inset and main image

Swap the images in the Inset and the Main windows. If the Inset window is hidden, then the Main window will toggle between the FFT and the main image.

Zoom to fit

Adjust the zoom level, so the entire image fits in the image display frame.
Zoom to fit is also available in the right-click context menu of the image display.

Zoom to 100%

Adjusts the zoom level to 100%, so the image is displayed in actual size.
Zoom 1:1 is also available in the right-click context menu of the image display.

Show/hide the panning window

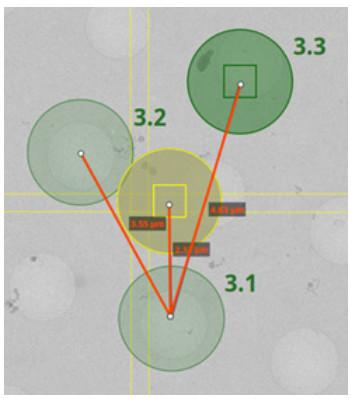
Show or hide the panning inset window.

3.6.1 Zoom in/out

1. Place the **mouse cursor** at the **region of interest** in the image display.
2. Scroll up or down with the **mouse wheel**.
   The image will zoom in or out around the cursor location.

3.6.2 The Measurement tool

The measurement tool enables you to measure real distances on an acquired image by drawing rulers.



Basic use: To draw a single ruler:

1. Click on the Measurement tool icon .
2. Click on a point of interest on the image.

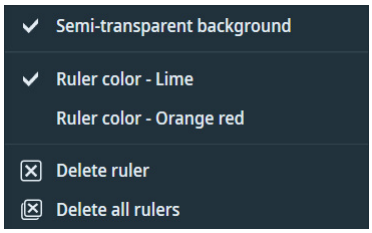
3. Move the mouse to a second point of interest on the image and click to place the second end of the ruler.

Multiple rulers: To draw more rulers, repeat the steps above.

Measurement: The length of a ruler is displayed next to it. This measurement is the real distance on the sample, e.g. measured in μm.

Edit: Click and drag any of the two endpoints of a ruler to change its position. Click and drag the body of the ruler to change its position.

Delete: Right-click a ruler to display the following context menu, then click Delete ruler.



The right-click menu also allows you to:

- Delete all rulers on this image.
- Choose between two colors for all rulers on all images.

Persistence: The rulers drawn on an image remain there until you either:

1. Quit Tomo.

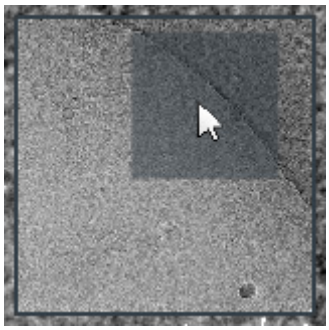
2. Perform a new acquisition, a new search image, etc.

3.6.3 Navigate and pan in a zoomed image

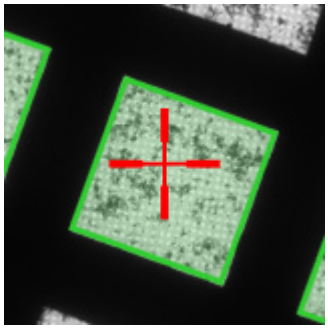
To navigate and pan in an image, either drag the image with the mouse, or use the panning inset.

1. Select the **panning inset**

2. Drag the **dark gray square** across the **panning inset**



3.6.4 The red crosshair

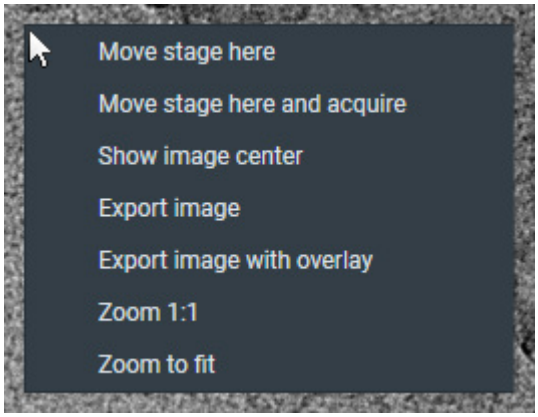


The red crosshair is commonly referred to as the *stage position*. Although the red crosshair moves when the stage moves, it does not mark the stage position itself. The red crosshair marks the *center of the field of view* if a new image were acquired.

3.6.5 Export an image to file

Images that are displayed in the Task Execution panel can be exported to file.

1. Right-click in the image to open the context menu.



The options in the context menu depend on the active task and image type.

2. Select either:
   - **Export image**
     Create a file of the image. The image is saved with the original resolution.
   - **Export image with overlay**
     Create a file of the image with scales, markers and other visual aids. The resolution of the image file is the same as as displayed in Tomography STEM. This may be less detailed than the original image.

4 Preparation Tab

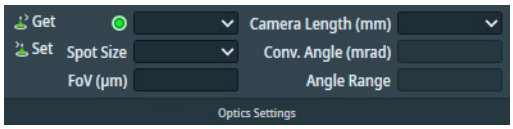
The Preparations tab provides a set of tasks and functionalities to set up the microscope and the Tomography STEM application for successful automated acquisition.

4.1 Acquisition and Optics Settings task

For each step in the preparation and automated acquisition process, a Preset must be prepared that fulfills a specific set of requirements. Each Preset consists of:

- Optics Settings.
- Detector Settings.
- Acquisition Settings.

4.1.1 Description of the Optics Settings



Get

Imports the Optics Settings parameter values from the microscope.

Set

Applies the Optics Settings parameter values to the microscope.

Probe mode

- *NanoProbe* is typically used for high magnification images with a narrow focus depth.
- *MicroProbe*: is typically used for medium and high magnification images with a wider focus depth.

  MicroProbe STEM must be configured in the microscope software, and must be calibrated before it can be used. You will be prompted to calibrate it at the startup of Tomography STEM if needed.

  This option is not visible in systems with no MicroProbe equipped.
- *LM* is typically used for the acquisition of images with a large field of view and large focus-depth.

Spot Size

The Spot Size determines the beam current. A higher Spot Size value corresponds to a lower beam current, and therefore a lower Dose Rate.

FoV (Field of View)

The size of the imaged area.

Camera Length

At larger camera lengths, the amount of elastically scattered electrons that contributes to the recorded signal increases. A drawback of large camera lengths is the occurrence of diffraction contrast in the acquired image.

The Camera Length value is coupled to the Probe mode. If the Camera Length value is adjusted, then the new value applies to all Presets that use the same Probe mode.

Convergence Angle and **Angle Range**

Only available on microscopes with three condenser lenses.

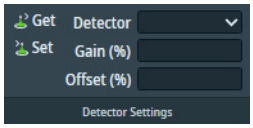
The Convergence Angle and Angle Range are read-only values. These values are adjusted depending on the other Optics Settings.

The Angle Range has two states:

- *Normal*:
  The C1 lens is used to demagnify the spot, while C2 and C3 adjust the beam so that a spot is formed at the image plane.
- *Large*:
  The C1 and C2 lenses are used to demagnify of the spot, which results in a stronger demagnification. *Large* mode is typically used in MicroProbe mode, where the convergence angle is very small (< 2 mrad) and the extra demagnification is needed to create a probe that is as small as possible.

Changes in Convergence Angle and Angle Range induce a change in the excitation of the C3 lens for eucentric focus and focus scale, and will also shift the scan frame. Make sure that a FEG register is loaded in which all Direct Alignments are completed for the given Convergence Angle and the Field of View is identical to the Exposure Preset in NanoProbe.

4.1.2 Description of the Detector Settings



Get

Imports the Detector Settings parameter values from the microscope.

Set

Applies the Detector Settings parameter values to the microscope.

Detector

The primary STEM detector. This detector is used for:

- All image acquisitions during preparation of the Data Acquisition run.
- Execution of the auto-functions during the Data Acquisition run.
- Image acquisition at each tilt step during the Data Acquisition run.

The primary detector selection is coupled to the Probe mode. If a different detector is selected, then the new selection applies to all Presets that use the same Probe mode.

If additional STEM detectors are available on the system, then these can be used for simultaneous image acquisition at each tilt step during the Data Acquisition run. Additional detectors will be selected later.

Gain and **Offset**

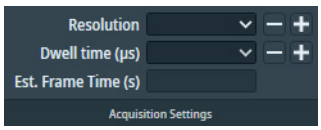
With well-chosen Gain and Offset values, the signal counts fit well with the dynamic range of the detector. How to determine the Gain and Offset values is described in Define the Exposure Preset on page 20.

- Offset shifts the signal count, so that the minimum signal count aligns with the lower boundary of the dynamic range.
- Gain adjusts the amplitude of the signal, so that the maximum signal count aligns with the upper boundary of the dynamic range.

The Gain and Offset values are coupled to the Probe mode. If the Gain and/or Offset values are adjusted, then the new values apply to all Presets that use the same Probe mode.

If additional STEM detectors will be used during the Data Acquisition run, then their Gain and Offset values will be determined later.

4.1.3 Description of the Acquisition Settings



Resolution

The pixel dimensions of the image that will be acquired.

Dwell Time

The acquisition time per individual pixel.

Estimated Frame Time

The esitmated time it takes to acquire an image. This is a read-only value.

$\texttt{EstFrameTime} \approx \texttt{PixelCount} \times \texttt{DwellTime}$

The actual frame time is typically a little bit longer than $\texttt{PixelCount} \times \texttt{DwellTime}$ due to processing and control overhead. The estimated value is improved after an image is acquired.

4.1.4 The recommended order to define the Acquisition and Optics Presets

The most efficient sequence to define the Presets is slightly different from their order in the Presets list:

1. Exposure
2. Presets that use the same *Probe Mode* as the Exposure Preset:
    - Tracking
    - Focus
    - Search / Template
3. Eucentric Height
4. Overview / Positioning
5. Atlas

To get the best quality images for 3D reconstruction, the Exposure Preset must be optimized without sacrifices to the other Presets. To determine good starting values for Optics Settings, Detection Settings and Acquisition Settings, it can be helpful to use the Velox software and the handpanels. The settings that are found with Velox and the handpanels can be easily imported into the Tomography software.

Although the optics system is highly reproducible, it is always better to avoid changes that are not strictly necessary. To achieve maximum stability, use the Exposure Preset as the basis for all other Presets that are used during the Data Acquisition run.

The following settings are not directly coupled to the selected Preset, but to the Probe mode that is used by the Preset. This means that an update of the value or selection applies to all Presets that use the same Probe mode.

- Optics Settings:
    - Spot Size
    - Camera Length
- Detector Settings:
    - Detector
    - Gain
    - Offset

The remaining Optics Settings and the Acquisition Settings are specific for the selected Preset.

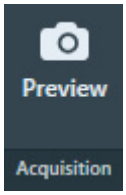
4.1.5 Define the Exposure Preset

The Exposure Preset is used to acquire the images that will be used for 3D reconstruction of the imaged feature. The parameter values of the Exposure Preset are dictated by the requirements for a successful 3D reconstruction of the specimen, and depend on the type and dimensions of specimen, and on the selected detector. There are some generic guidelines:

Settings	Typical Settings and Recommendations
Optics	• Probe Mode: for most experiments, NanoProbe is the most suitable mode. For experiments that require a larger focus-depth, MicroProbe or even LM mode can also be used. • Spot Size: higher values result in a smaller spot with a lower beam current. • Field of View: appropriate for the feature size and the required resolution.
Detector	—
Acquisition	• Resolution: high values for more accurate 3D reconstructions. • Dwell time: appropriate for the maximum dose rate that the specimen can endure. *Note: if there is a noticeable amount of drift, then a high resolution in combination with a long dwell time can result in a distorted image.*

Use the procedure below to set the Exposure Preset.

1. Use the **handpanels** and the **live view functions in Velox** to:
   a. Move the **specimen** to an area that can be sacrificed for tuning of the Preset values.
   b. Select the **STEM detector** that will be used for all Presets.
      Velox automatically starts a live view for the selected detector.
   c. Create an image that meets the requirements for the selected Preset.
      To assess the image quality it can be helpful to inspect the FFT of the live image.
   d. Acquire a **still STEM image** and assess the image quality.
2. Select **Presets**: **Exposure**
3. Select the same **Detector Settings** > **Detector** as used in Velox
4. Synchronize all relevant settings from the microscope and Velox to Tomography:
   a. Select **Optics Settings** > **Get**
   b. Select **Detector Settings** > **Get**
   c. In **Acquisition Settings**, enter the values as they are determined with Velox.
5. Select **Acquisition** > **Preview**



6. Verify that the acquired image meets the requirements above.
   If not, make any necessary adjustments and select **Preview** again.

7. (Optional) If the Tilt Series must be acquired with multiple STEM detectors, then optimize the Gain and Offset values for the additional STEM detector(s).
   a. In **Detector Settings** > **Detector**, select the **additional STEM detector**
   b. Select **Preview**
   c. If necessary, adjust the **Gain** and **Offset** to optimize signal counts for the dynamic range of the selected detector.

Note

Do *not* adjust the Optics Settings.
Do *not* adjust the Acquisition Settings.

   d. Select **Preview** again to verify that the image uses the full dynamic range of the detector.
   e. Select the **Detector** again that will also be used for the other Presets.

Note

Make sure to select the additional STEM detector(s) at a later phase of the preparation workflow in *Tomography* > *Data Acquisition* > *Additional Detectors*.

4.1.6 Define the Tracking Preset

The Tracking Preset is used to center the feature of interest after a tilt angle step.

The Tracking Preset typically uses the same Probe mode as the Exposure Preset, so some settings are common. The Tracking Preset also uses the same *High SA* filter settings.

Settings	Typical Settings and Recommendations
Optics	Same Probe mode as the Exposure Preset. At high magnifications, the feature of interest may shift too far away from the center of the image for robust and accurate centering. If this happens, then increase the *FoV* value.
Detector	Same as the Exposure Preset.
Acquisition	Same as the Exposure Preset.

Use the procedure below to set the Tracking Preset.

1. Move the **specimen** to an area that can be sacrificed for tuning of the Preset values.
2. Select **Preset Selection** > **Presets**: **Tracking**
3. Select the same **Optics Settings** > **Probe Mode** as in the Exposure Preset.
4. In **Acquisition Settings**, select the same settings as used in the Exposure Preset.
5. Select **Acquisition** > **Preview**
6. Verify that the acquired image meets the requirements above.
7. If necessary:
   - Increase **Optics Settings** > **FoV** to make sure that the feature of interest remains visible near the center of the image after a tilt step.
   - Adjust other Optics, Detector and/or Acquisition Settings, but be aware that some settings are shared with all other Presets that use the same Probe Mode,
     (see: The recommended order to define the Acquisition and Optics Presets on page 19).

4.1.7 Define the Focus Preset

The Focus Preset is used for:

- Calibration of the Autofocus function.
- Execution of the Autofocus step during the automated run.
- Execution of the manual focus action in case the Autofocus function fails to find the optimal focus.

The Focus Preset typically uses the same Probe mode as the Exposure Preset, so some settings are common. The Focus Preset also uses the same *High SA* filter settings.

Settings	Typical Settings and Recommendations
Optics	Same Probe mode as the Exposure Preset.
Detector	Same as the Exposure Preset.
Acquisition	Same as the Exposure Preset, but Dwell Time must be > 0.4 µs for proper performance of the Dynamic Focus function

Use the procedure below to set the Focus Preset.

1. Move the **specimen** to an area that can be sacrificed for tuning of the Preset values.
2. Select **Preset Selection** > **Presets**: **Focus**
3. Select the same **Optics Settings** > **Probe Mode** as in the Exposure Preset.
4. In **Acquisition Settings**:
   - Specify a **Dwell Time** > **0.4 µs**.
     Shorter Dwell Time values are too fast for proper performance of the Dynamic Focus function.
   - All other Acquisition Settings use the same values as in the Exposure Preset.
5. Select **Acquisition** > **Preview**
6. In **Auto Functions** > **Auto-Functions (STEM)**:
   a. Select **Autofocus linear**
      (*See:* Run the 'Autofocus linear' auto-function on page 47).
   b. Select **Presets**: **Focus**
   c. Select **Start**
   d. Wait until the *Autofocus linear* function is completed.
   e. Verify that the *Autofocus linear* autofunction is successful.

7. If the *Autofocus linear* autofunction fails, then:
   a. Increase the **Auto Function Settings** > **Coarse focus step**, then select **Start** again.

   If the autofunction still fails, then:
   b. In the **TEM User Interface** > **Calibrations** control panel, select **Calibrations**, then select **STEM Focus**.
   c. Select **Start**
   d. Follow the **instructions** in the Calibrations control panel to complete the STEM Focus calibration.
   e. Perform the **Tomography** > **Auto Functions** > **Autofocus linear** autofunction again.

   If the autofunction still fails, then:
   - Increase the **Optics Settings** > **FoV** and try again.
   - Adjust other Optics, Detector and/or Acquisition Settings, but be aware that some settings are shared with all other Presets that use the same Probe Mode,
     (see: The recommended order to define the Acquisition and Optics Presets on page 19).

4.1.8 Define the Search / Template Preset

The Search / Template Preset is used:
- To acquire the Search Map.
- To acquire the Search image, in which:
  - The feature of interest is identified and centered
  - The Template Areas are assigned to their locations.

The Search / Template Preset must fulfill the following requirements:
- The contrast must be sufficient to reliably identify features of interest.
- The field of view must be at least 3 µm.
  On a Quantifoil specimen, the field of view is typically three Foil Holes wide.
- On Life Science specimens, the Dose Rate should not exceed 0.1 $e^-/Å^2s$. The easiest way to achieve this is to use a lower resolution and smaller dwell time.

Settings	Typical Settings and Recommendations
Optics	• Probe Mode: same as the Exposure Preset • FoV: typically > 3 µm or three Foil Holes.
Detector	Same as Exposure Preset.

Acquisition	• For so-called 'radiation hard' specimens: same as the Exposure Preset.
	• For dose sensitive specimens, limit the exposure per pixel area:
	  • Resolution: lower than the Exposure Preset.
	  • Dwell time: smaller than the Exposure Preset.

Use the procedure below to set the Search / Template Preset.

1. Move the **specimen** to an area that can be sacrificed for tuning of the Preset values.
2. Select **Preset Selection** > **Presets**: **Search / Template**
3. Select the same **Optics Settings** > **Probe Mode** as in the Exposure Preset.
4. Specify the **Optics Settings** > **FoV** according to the requirements above.
5. Specify the **Acquisition Settings**
   - For so-called 'radiation hard' specimens, use the values as in the Exposure Preset.
   - For dose sensitive specimens, limit the exposure per pixel area:
     - Resolution: lower than the Exposure Preset.
     - Dwell time: smaller than the Exposure Preset.
6. Select **Acquisition** > **Preview**
7. Verify that the acquired image meets the requirements above.
8. If necessary, adjust the Optics, Detector and Acquisition Settings, but be aware that some settings are shared with all other Presets that use the same Probe Mode,
   (see: The recommended order to define the Acquisition and Optics Presets on page 19).

4.1.9 Define the Eucentric Height Preset

The Eucentric Height Preset is used to perform the Auto-eucentric Height function during the automated run. The Eucentric Height Preset must result in an image with sufficient contrast and brightness to clearly identify a contrast-rich feature on the specimen.

Settings	Typical Settings and Recommendations
Optics	Probe Mode: MicroProbe or LM. If available, MicroProbe is preferred. The limited focus-depth of the NanoProbe mode is less suitable for the Eucentric Height auto-function due to changes in the Z-position of the stage.
Detector	—
Acquisition	Resolution: 512x512 (recommended, not required). Higher resolution images are re-binned for increased processing speed.

Use the procedure below to define the Eucentric Height Preset.

1. Move the **specimen** to an area that can be sacrificed for tuning of the Preset values.
2. Select **Preset Selection** > **Presets**: **Eucentric Height**
3. Select **Optics Settings** > **Probe Mode**: **MicroProbe** (preferred, if available) or **LM**
4. If no Detector Settings have been specified for the selected Probe Mode yet, then specify the **Detector Settings**
5. In **Acquisition Settings**:
   - Select **Resolution**: **512x512**
   - All other settings can have the same values as in the Exposure Preset.
6. Select **Acquisition** > **Preview**

7. In **Auto Functions** > **Auto-Functions (STEM)**:
   a. Select **Auto-eucentric height**
      (*see:* Run the 'Auto-eucentric height' auto-function on page 48)
   b. Select **Presets**: **Eucentric Height**
   c. Select **Start**
   d. Wait until the *Auto-eucentric height* function is completed.
   e. Verify that the *Auto-eucentric height* autofunction is successful.
8. If necessary, adjust the Optics, Detector and Acquisition Settings, but be aware that some settings are shared with all other Presets that use the same Probe Mode,
   (see: The recommended order to define the Acquisition and Optics Presets on page 19).

4.1.10 Define the Overview / Positioning Preset

The Overview / Positioning Preset is used during target selection to view an area of interest where features of interest are located.

This Preset is not used during the automated Data Acquisition run, so there are no strict requirements for the Overview / Positioning Preset.

Settings	Typical Settings and Recommendations
Optics	• Probe Mode: LM • FoV: for slab-like specimens, typically large enough to cover an entire Grid Square.
Detector	—
Acquisition	—

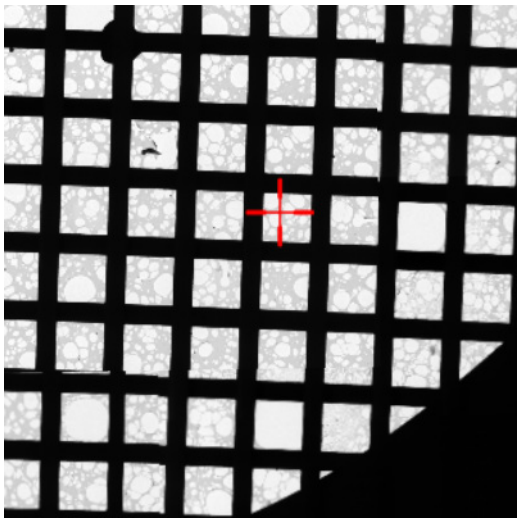
Use the procedure below to set the Overview / Positioning Preset.

1. Select **Preset Selection** > **Presets**: **Overview / Positioning**
2. Select **Probe Mode**: **LM**
3. Select **Optics Settings** > **Set**
4. Use the **handpanels** and the **live view functions in Velox** to:
   a. Move the **specimen** to an area that can be sacrificed for tuning of the Preset values.
   b. Create an image that meets the requirements.
5. Select **Optics Settings** > **Get**
   Be aware that some Optics Settings also apply to other Presets that use Probe Mode: LM.
6. If no Detector Settings have been specified for the selected Probe Mode yet,
   then select **Detector Settings** > **Get**
   Be aware that the Detector Settings also apply to other Presets that use Probe Mode: LM.
7. In **Acquisition Settings**, copy the **Resolution** and **Dwell time** from Velox.

8. Select **Acquisition** > **Preview**
9. Verify that the acquired image meets the requirements above.
   If not, make any necessary adjustments and select **Preview** again.
   Be aware that some settings are shared with all other Presets that use the same Probe Mode,
   (see: The recommended order to define the Acquisition and Optics Presets on page 19).

4.1.11 Define the Atlas Preset

An Atlas is an overview of the specimen that is assembled from a series of individual images, so-called *Tiles*. At the edges of the Tiles, small offsets can be visible. This is acceptable.



The Atlas is typically used to identify areas of interest, and to quickly navigate to such areas. If this step of the workflow is routinely skipped, then it is not necessary to define the Atlas Preset.

This Preset is not used during the automated Data Acquisition run, so there are no strict requirements for the Atlas Preset. For the identification and navigation purposes, the Atlas Preset should meet the following requirements:

- The field of view typically covers a specimen area that is large enough, so that an Atlas of 4x4 tiles covers the entire specimen.
- The grid bars of the carbon foil are clearly recognizable at the edges of each image.
- For Cryo Tomography experiments, the contrast and brightness must be good enough to assess the ice thickness.

Settings	Typical Settings and Recommendations
Optics	• Probe Mode: LM • FoV: approximately a quarter of the specimen width.
Detector	• —
Acquisition	—

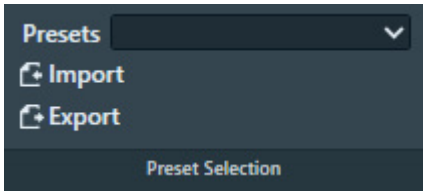
Use the procedure below to define the Atlas Preset.

1. Select **Preset Selection** > **Presets**: **Atlas**
2. Select **Probe Mode**: **LM**
3. Select **Optics Settings** > **Set**
4. Use the **hand panels** and the **live view functions in Velox** to:
   a. Move the **specimen** to an area that can be sacrificed for tuning of the Preset values.
   b. Create an image that meets the requirements.

5. Select **Optics Settings** > **Get**
   Be aware that some Optics Settings also apply to other Presets that use Probe Mode: LM
6. If no Detector Settings have been specified for the selected Probe Mode yet,
   then select **Detector Settings** > **Get**
   Be aware that the Detector Settings also apply to other Presets that use Probe Mode: LM.
7. In **Acquisition Settings**, copy the **Resolution** and **Dwell time** from Velox.
8. Select **Acquisition** > **Preview**
9. Verify that the acquired image meets the requirements above.
   If not, make any necessary adjustments and select **Preview** again.
   Be aware that some settings are shared with all other Presets that use the same Probe Mode,
   (see: The recommended order to define the Acquisition and Optics Presets on page 19).

4.1.12 Import and export the Acquisition and Optics Presets

With the Import and Export functions it is possible to archive the values for all current Presets, and to load them again at a later time. This way, setting up a new Automated Acquisition run for a regularly used specimen type can be done much easier and much faster.



Export

Writes the current parameter values for all Presets to an XML file. The Presets file contains the following values for all Presets:

- The Optics Settings.
- The Camera Settings.
  Depending on the selected camera the Advanced Camera Settings and/or Exposure Settings are also included.

Import

Overwrite all current parameter values for all Presets with the values from the selected Presets file.

- There is no *Undo* function. It may be wise to export the current Presets to a file before importing a different Presets file.
- Presets files that are created with previous software versions are supported with limitations. When Tomography STEM can not import or convert a legacy Presets file it will display an error message.

Note A Presets file contains the parameter values for *all* Presets. It is not possible to export or import the settings for a single Preset.

4.2 Calibrate Image Shifts task

Even on a well-aligned and calibrated system, a centered feature may shift away from the image center when a new image is acquired at a different magnification. For optimum performance of the Data Acquisition run, any remaining shifts must compensated.

The Image Shift Calibration acquires images with each Preset. In each image, Tomography STEM requests to mark the exact location of an easily recognizable feature. Tomography STEM uses the distance between the marked locations to compensate for image center offsets between the Presets.

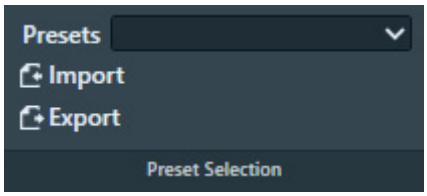
All shifts are relative to the Exposure Preset, so reconstruction is always based on zero-shift images.

4.2.1 Prepare for Image Shift Calibration

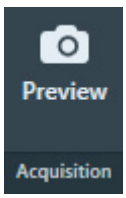
The Image Shift Calibration acquires images for each Preset. In each image, the exact location of an easily recognizable feature must be pinpointed. The shifts between the pinpointed locations are used to compensate for image center offsets at various magnifications.

To prepare for the Image Shift Calibration, follow the procedure below:

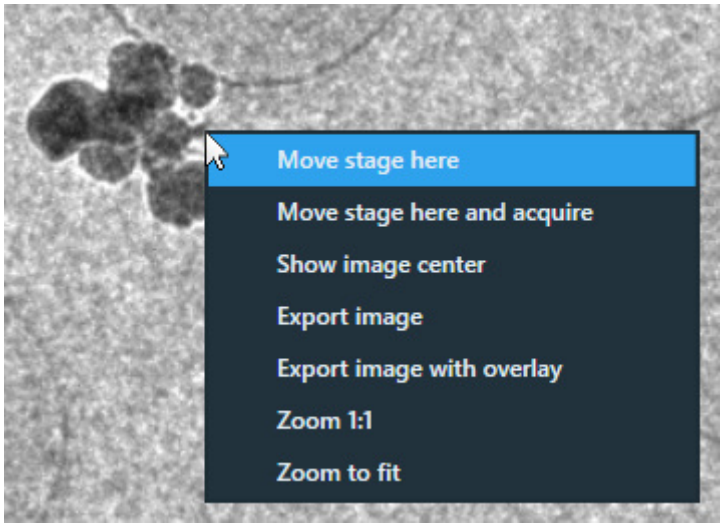
1. In **Auto Functions**:
   a. Select **Auto-eucentric height** > **Presets**: **Eucentric Height**, then select **Start** and wait until the autofunction is completed.
   b. Select **Autofocus linear** > **Presets**: **Focus**, then select **Start** and wait until the autofunction is completed.
2. Select **Preparation** tab > **Acquisition and Optics** task.
3. Select **Preset Selection** > **Presets**: **Exposure**



4. Select **Optics Settings** > **Set**
5. Use the **hand panels** and the **live view function in Velox** to find an **easily recognizable feature**, and center it in the image.
   Select an asymmetric feature that is easily recognizable at low magnifications. If you are not sure about the recognizability of the feature details at low magnifications, switch to low magnifications for a quick check.
6. Select **Preset Selection** > **Presets**: **Overview / Positioning**
7. Select **Acquisition** > **Preview**



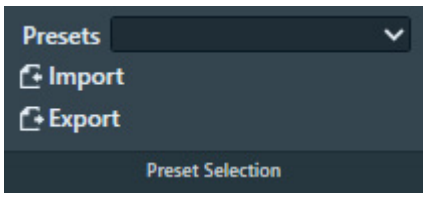
8. In the acquired image, right-click on the feature and select **Move stage here**
   If necessary, zoom in to make it easier to pinpoint the feature.



9. Select **Preset Selection** > **Presets**: **Eucentric Height**
10. Select **Acquisition** > **Preview**
11. In the acquired image, check if the selected feature is easily recognizable.
    It doesn't have to be centered, but on a well-aligned system it should be well within the field of view.
    When not in view:
    a. Use the **hand panels** and the **live view function in Velox** to find and center the recognizable feature.
    b. Select **Preview** again.
12. In the acquired image, right-click on the feature and select **Move stage here**
13. Select **Preset Selection** > **Presets**: **Exposure**
14. Select **Acquisition** > **Preview**
15. In the acquired image, check if the selected feature is visible near the image center.

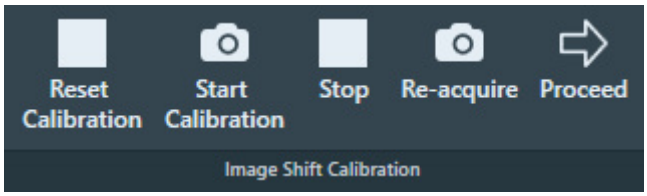
4.2.2 Perform the Image Shift Calibration

1. In the **Preparation** tab > **Acquisition and Optics** task:
   a. Select **Preset Selection** > **Presets**: **Exposure**

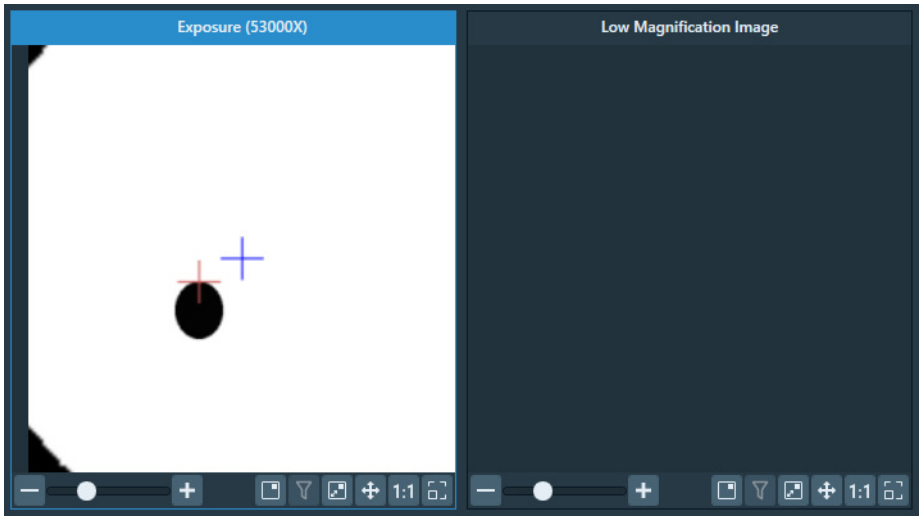


   b. Select **Optics Settings** > **Set**
2. Roughly center a **recognizable feature**.
3. Select **Preparation** > **Calibrate Image Shifts** task.

4. Select the **Image Shift Calibration** > **Start Calibration**



The calibration procedure acquires the first image and displays it on the left side of the Task Execution panel.



5. Accurately center the recognizable feature in the center of the image.
   a. In the **left-side image**, double-click on the **recognizable feature**.
      The red crosshair moves to the selected position. The red crosshair can be relocated as often as necessary.
      Optionally zoom in for better accuracy.
   b. Select **Re-acquire**
      Tomography STEM uses a *backlash corrected stage move* to center the marked feature and then acquires a new image.
   c. If the feature is not properly centered, select **Re-acquire** again.
      On a well-aligned and calibrated system this should solve the offset.
      Note that the CompuStage is not infinitely accurate. A very small offset may still exist at the highest magnification. This is acceptable.
6. After the feature is properly centered, the shifts between the Presets can be determined:
   a. Select **Proceed**
      Tomography STEM acquires a new image using the next Preset, and displays it on the right-side of the Task Execution panel.
   b. In the **new right-side image**, double-click on the **distinctive location** of the **recognizable feature**.
      The red crosshair moves to the selected position.
      - The red crosshair can be relocated as often as necessary.
      - Zoom in for better accuracy.
   c. Repeat step *a* and step *b* until the image shifts between all Presets have been calibrated.
      For every shift:

- The left-side image serves as a reference.
- Use the new right-side image to mark the distinctive location on the recognizable feature.

d. After the last shift is calibrated, the Status panel reports the successful completion of the calibration.

4.2.3 When to reset and renew the Image Shift Calibration

The Image Shifts Calibration needs to be renewed:

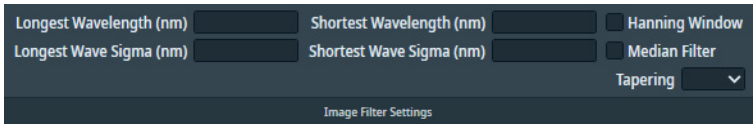
- When the Optics Settings of a Preset are updated.
- When a different detector is selected. The center of the field of view may shift.
- When the Alignment file that was used to define the Presets is updated, or when a different Alignment file is loaded.
- When the FEG Register that was used to define the Presets is updated, or when a different FEG Register is loaded.
- When a feature does not stay centered after selecting a different Preset.

4.3 The Image Filters Settings Task

The image shift calculation that is used to center the feature uses the cross-correlation of the Fourier transforms of two images.

A Fourier transform is based on the assumption that the image can be extended periodically in all directions. In reality however, the image is finite and has edges. This results in artifacts in the Fourier transform, such as horizontal and vertical lines.

Besides the Fourier transform artifacts, there can be other factors that can have a negative impact on the accuracy of the image shift calculation. To overcome these factors, the following Image Filters are available.



- The *Band Pass* filter.
  This filter is defined by:
  - *Longest Wavelength (nm)* and *Shortest Wavelength (nm)*
  - *Longest Wave Sigma (nm)* and *Shortest Wave Sigma (nm)*
- The *Hanning Window*
- The *Median Filter*
- The *Tapering* filter

4.3.1 Description of the image filters

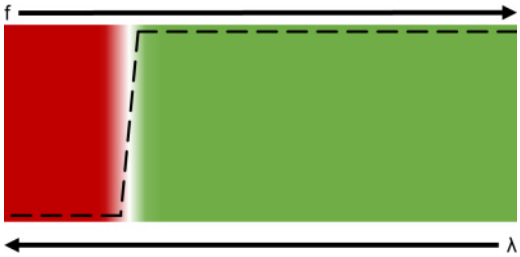
4.3.1.1 The Band Pass filter

The Band Pass filter is a combination of a High Pass filter and a Low Pass filter.

Although the terms High Pass and Low Pass apply to the passed-through *frequency* ranges, the filter parameters are specified as *wavelengths [nm]*. These wavelengths are directly related to feature sizes in nanometers, so that they remain valid when a Preset is changed.

High Pass filter

In the frequency domain, the High Pass filter suppresses frequencies below the cut-off frequency.



In the wavelength domain, the High Pass filter suppresses features that are larger than the cut-off wavelength.

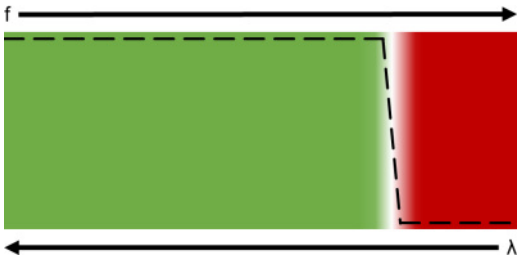
In the Tomography STEM software, the High Pass filter is specified by:

- **Longest Wavelength**
  The filter suppresses features that are larger than the specified value.
- **Longest Wave Sigma**
  The abruptness (Gaussian width) of the filter's cut-off. A low value corresponds to a sharp cut-off.

  In the illustrations above, the Sigma value corresponds to the steepness of the cut-off, where 0.0 would be a vertical cut-off.

Low Pass filter

In the frequency domain, the Low Pass filter suppresses frequencies above the cut-off frequency.



In the wavelength domain, the Low Pass filter suppresses features that are smaller than the cut-off wavelength.

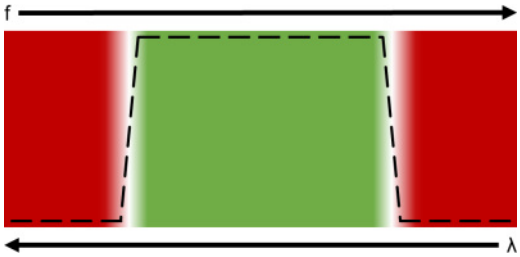
In the Tomography STEM software, the Low Pass filter is specified by:

- **Shortest Wavelength**
  The filter suppresses features that are smaller than the specified value.
- **Shortest Wave Sigma**
  The abruptness (Gaussian width) of the filter's cut-off. A low value corresponds to a sharp cut-off.

  In the illustrations above, the Sigma value corresponds to the steepness of the cut-off, where 0.0 would be a vertical cut-off.

Band Pass Filter

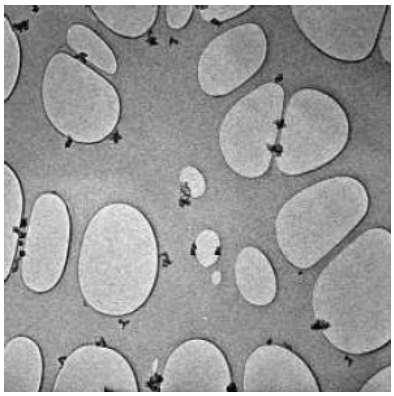
The Band Pass filter multiplies the amplification factors of the High Pass and the Low Pass filters.



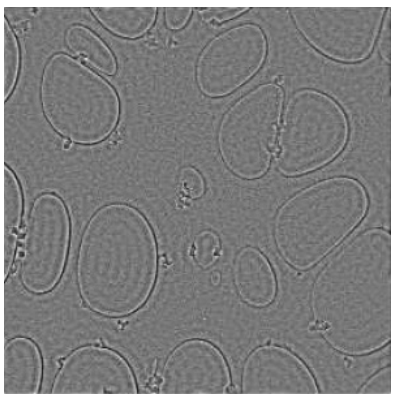
The result is a filter that cuts off the highest and the lowest frequencies, or in the wavelength domain: suppresses the smallest and the largest features.

Example for the application of the Band Pass filter:

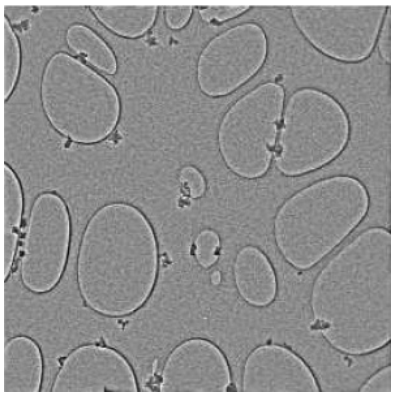
Use the *Shortest Wavelength* and *Shortest Wave Sigma* of the Low Pass filter to suppress noise, features and patterns that are smaller than the markers (gold particles).



Use the *Longest Wavelength* and *Longest Wave Sigma* of the High Pass filter to suppress features and patterns that are larger than the foil holes.



The result is a Band Pass filter that shows the foil hole outlines and the markers.



4.3.1.2 The Hanning Window filter

The first and last pixels of a row or column typically have significantly different counts values. The FFT function processes an image as if it repeats endlessly in horizontal and vertical directions. In the FFT image, the counts difference between pixels on opposing edges of the image will show up as an artefact with a frequency that matches the dimensions of the image and an intensity that matches the counts difference.

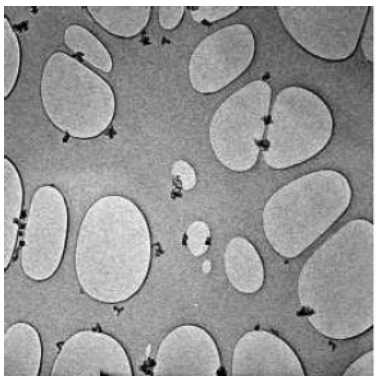
The Hanning Window filter reduces these artefacts by reducing the counts difference between pixels on opposing edges of the image. In Tomography STEM, the Hanning Window filter adjusts the counts value for each pixel as follows:

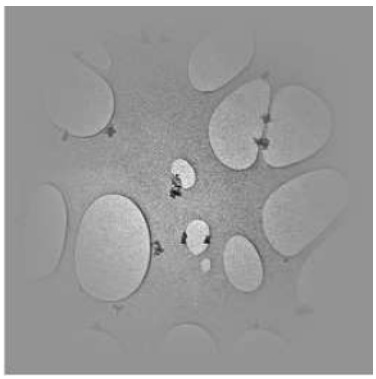
$C_{Hanning} = C_{avg} + H \times (C_{acq} - C_{avg})$

where:

- $C_{Hanning}$: counts value of the pixel after applying the Hanning factor H.
- C_{avg}: average counts value of all pixels in the image.
- H: the Hanning factor, a *raised cosine* from 1 in the image center to 0 at the image edge.
- C_{acq}: counts value of the pixel before applying the Hanning factor H.

The result is an image in which the contrast is progressively reduced as the distance to the center of the image increases.

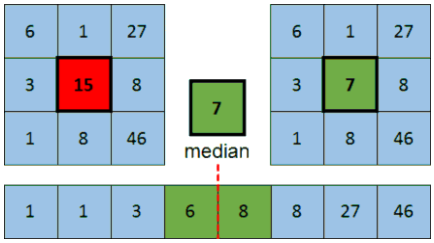




A drawback of the Hanning Window is that contrast-rich features that are not near the center of the image become useless for finding cross-correlation peaks.

4.3.1.3 The Median filter

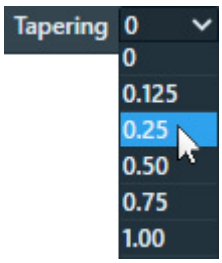
The Median filter reduces noise by replacing the acquired counts value for each individual pixel by the median value of its direct neighbors.



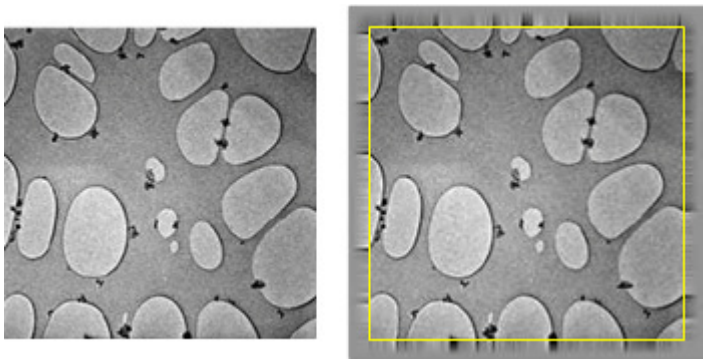
4.3.1.4 The Tapering filter

The FFT function processes an image as if it repeats endlessly in horizontal and vertical directions. The first and last pixels of a row or column typically have significantly different counts values. In the FFT image, the counts difference between pixels on opposing edges of the image will show up as an artefact with a frequency that matches the dimensions of the image and an intensity that matches with the counts difference. The Tapering filter reduces these artefacts, so they do not play a noticeable role anymore.

The Tapering filter first adds a margin around the acquired image. The width of the margin is specified as a fraction of the image size.



The Tapering filter then applies a gradient from the edge of the acquired image toward the outer edge of the added margin. The gradient goes from the counts value at the edge of the acquired image towards the average counts value of the entire image.



Left: acquired image.
Right: acquired image with tapered margin.

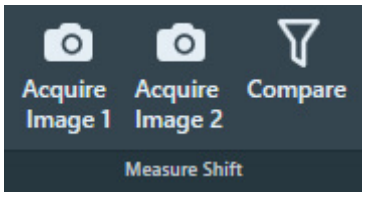
4.3.2 Define the Image Filter Presets

To set the Filter Presets, the steps below must be performed for each magnification range in **Filter Settings Selection** > **Settings**:

For the procedure below, use the following combinations of Acquisition and Optics Preset and Image Filter Preset:

Image Filter Preset	Acquisition and Optics Preset
High LM range	Overview / Positioning
Low HM range	Search
High HM range	Tracking

1. Set the specimen to eucentric height.
2. In the **Acquisition and Optics Settings** task:
   a. Select the appropriate **Preset** for the Image Filter Setting that will be specified.
   b. Select **Optics Settings** > **Set**
   c. Select **Detector Settings** > **Set**
3. Select the **Image Filters Settings** task.
4. Select the desired **Preset Selection** > **Presets**
5. Use the **handpanels** and the **live view functions in Velox** to:
   a. Move the **specimen** to an area that can be sacrificed for tuning the Image Filter Settings.
   b. Create an **accurately focused image** with a contrast-rich feature in the center.
6. Select **Measure Shift** > **Acquire Image 1**



7. Tilt or move the **specimen** a bit, so that the centered feature shifts a small, but noticeable distance from the image center.
8. Select **Acquire Image 2**
9. Select **Compare**

10. Select and/or specify the filter parameters, so that the cross-correlation image shows a single clear bright peak that is not at Zero Shift.
    a. Tune the **Band Pass filter**.
       As a rule of thumb, start with the following initial values:
       - Longest Wavelength: $^1/_4$ of the scale bar in the image.
       - Shortest Wavelength: $^1/_{40}$ of the scale bar in the image.
       - Longest Wave Sigma and Shortest Wave Sigma: 0.0

       If the scale bar is 2 μm, then start with a Longest Wavelength of 500 nm, and a Shortest Wavelength of 50 nm.

    b. If the cross correlation image shows undesirable peaks or other artifacts, then select the applicable filters to suppress these peaks.

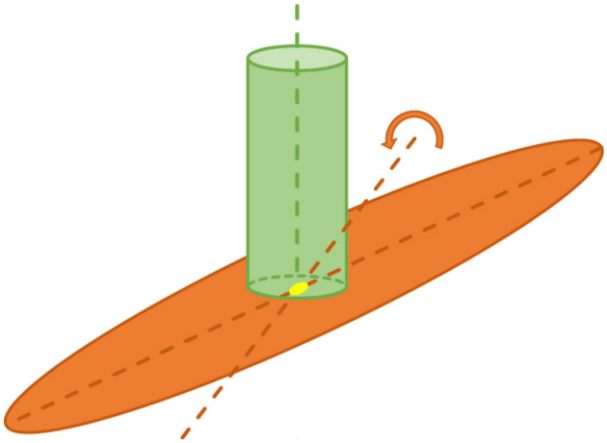
4.3.3 Suggestions for the application of image filters

- **Low dose imaging of a specimen with markers**
  Specify the **Band Pass filter** to suppress noise and enhance the markers.
  Remove the shorter wavelengths to smoothen the image and the peak at zero shift, while enhancing the signal from the markers.
- **Low dose imaging of a specimen without markers**
  Apply one or more of the following filtering strategies:
  - Specify the **Band Pass filter** to suppress noise and enhance the signal counts of the features of interest.
  - Add the **Median** filter to decrease single noise peaks, but retain edges.
- **Multiple less suitable features near the feature of interest**
  - Specify the **Band Pass filter** to enhance features of a specific size range.
    If the less suitable features are similar in size as the most suitable feature than it can be difficult to find a robust combination of parameter values.
  - Apply a **Hanning Window** to decrease the contrast of features that are not in the image center.
- **Edge artifacts**
  If the image is dark on one edge and bright on the opposite edge, then a bright line will appear through the center of the cross-correlation image. This is consequence of the Fourier transform. The Fourier transform function assumes that the image can be repeated indefinitely in all directions, so it joins the bright edge to the opposing dark edge and falsely detects a contrast-rich feature with a size that is approximately the same as the acquired image. This artifact can severely decrease the quality of the shift measurement.
  To avoid the bright line artifact:
  - Apply a **Hanning Window**, possibly in combination with a **Tapering** filter to decrease contrast between opposing edges of the image.
  - Specify the **Band Pass filter** to enhance features of a specific size range.

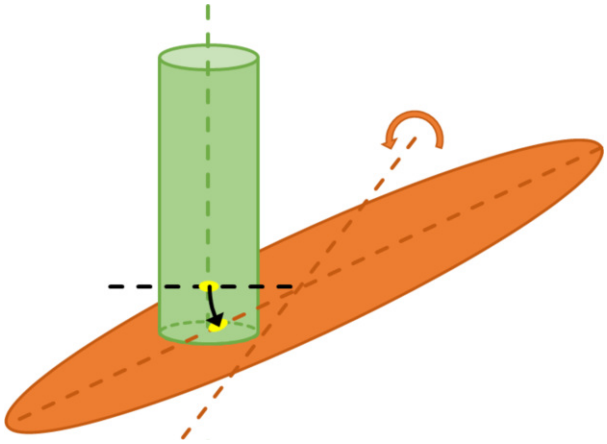
4.4 The Optimized Position task

In an ideal microscope, the optical axis intersects exactly with the tilt axis. In a real microscope however, there can be a small offset between the optical axis of the column and the tilt axis of the stage. This offset has the following undesirable effects:

- The feature of interest goes out of focus.
- At large tilt angles, the feature of interest shifts in Y-direction.
- The result of the Auto Eucentric Height function becomes inaccurate.



Optical axis and tilt axis intersect.
The feature of interest stays in focus and centered.



Optical axis and stage axis have an offset.
The feature of interest goes out of focus
and shifts in Y-direction.

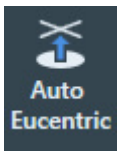
In principle, every optical mode and magnification could have its own offset. On a well-aligned microscope, the offset between the optical axis and the tilt axis is the same for all modes and magnifications. The Optimized Position calibration measures this offset, so that Tomography STEM can compensate for it. After a successful Optimized Position calibration, the center of the beam intersects with the tilt axis, and their intersection is centered in the field of view.

The Optimized Position value is specific for the microscope and its alignment. The Optimized Position calibration must be renewed when the CompuStage is replaced or re-adjusted, or after the optical alignment of the microscope is thoroughly revised.

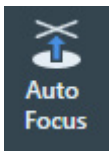
4.4.1 Prepare for the Optimized Position calibration

The Optimized Position calibration automatically selects the Focus Preset. It is recommended to use the same Optics, Detector and Acquisition settings during preparation of the Optimized Position calibration.

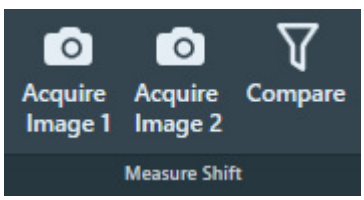
1. Load the **Combined Test Specimen (Agar S142)**
   This specimen is a holey carbon foil with gold particles and graphitized carbon. This specimen is delivered with the microscope.
2. In **Preparation** > **Acquisition and Optics Settings**:
   a. Select **Preset Selection** > **Presets**: **Focus**
   b. Select **Optics Settings** > **Set**
   c. Select **Detector Settings** > **Set**
3. Use the **hand panels** and the **live view functions in Velox** to move the **specimen** to an area of thin carbon film, so that:
   - Multiple features, such as markers, are visible in the field of view.
   - A suitable feature is centered in the image.
4. Verify and, if necessary, adjust the **Autofocus Linear** settings. The default settings are:
   - **Coarse focus step**: **3.00**
   - **Fine Focus Step**: **0.60**
   - **Number of steps**: **10**
   - **Fine search only**: **No**
   - **Apply filters**: **No**
5. In the **Preparation** > **Optimized Position**:
   a. Select **Auto Eucentric**

   b. Select **Auto Focus**

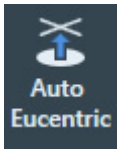
6. Verify and, if necessary, adjust the *High HM* image filter:
   a. In **Preparation** > **Image Filters Settings**,
   select **Preset Selection** > **Presets**: **High HM**
   b. Select **Measure Shift** > **Acquire Image 1**

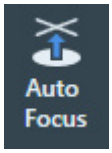
c. Tilt the **specimen** a few degrees, so that the centered feature shifts a small, but noticeable distance from the image center.
d. Select **Acquire Image 2**
e. Select **Compare**
f. Verify that the **Cross-Correlation image** shows a well-defined peak, away from the zero-shift location.
If not, see The Image Filters Settings Task on page 33 for instructions how to tune the Image Filters.

4.4.2 Perform the Optimized Position calibration

1. Select **Preparation** > **Optimized Position**
2. Select **Auto Eucentric**



3. Select **Auto Focus**

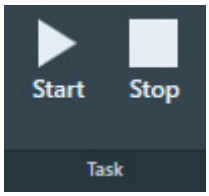


4. If the *CompuStage* or the *FEG* has been serviced, then reset the Optimized Position value:

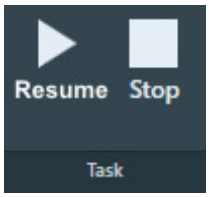
a. Specify **New Value**: **0.00**
b. Select **Apply**

Else, the *Current value* is typically a good starting point for the Optimized Position calibration.

5. Select **Task** > **Start**



6. Monitor the progress and intermediate results in the **Status** side panel.
   The procedure may request to perform manual actions. If so, perform the requested action and select **Resume**



   The Optimized Position calibration procedure starts with the *Current Value*. The *Current Value* is a read-only value.
   - If the result is within 0.05 µm of the *Current Value*, then Tomography STEM updates the *New Value*.
   - If the result is *not* within 0.05 µm of the *Current Value*, then Tomography STEM repeats the calibration once.
     - If the second result is within 0.05 µm of the first result, then Tomography STEM updates the *New Value*.
     - If the second result is *not* within 0.05 µm of the first result, then the *New Value* remains equal to the *Current Value.*
7. Verify that **Optimized Position Calibrations** > **New Value** is not equal to **Current Value**
   This indicates that the calibration is completed successfully. If the calibration is not successful, then New Value is equal to Current Value.
8. If the calibration completed successfully, then select **Apply** to accept the *New Value*.
9. With the **TEM User Interface** and the **Handpanels**:
   a. Verify the **Direct Alignments** control panel > **Tomo Rotation Center** alignment(s).
      If necessary, adjust the Direct Alignment.
   b. If necessary, correct **astigmatism**
10. Update the Direct Alignments that are stored in the FEG Register:
    a. In the **FEG Registers** control panel, select the **currently loaded FEG Register**
    b. Select **Update** and confirm.

4.4.3 Considerations after a successful Optimized Position calibration

The Optimized Position is a system property. It does not depend on the used specimen, the holder or the software application.

- The Optimized Position calibration is specific for Tomography in STEM mode. The result is not shared with Tomography for TEM / EFTEM mode, or EPU-D.
- The Optimized Position calibration should only be renewed:
  - After the stage is replaced or re-adjusted.
  - After the optical alignments are thoroughly revised.
- To make proper use of the calibrated Optimized Position value, use the *Direct Alignments* > *Tomo* versions of the alignments while Tomography STEM is running. The regular, *non-Tomo* Direct Alignment versions will reset the calibrated Image Beam Shift value.
  If a regular Direct Alignment version is performed, then select an Acquisition and Optics Preset and select *Set* to re-apply the optics settings, including the Optimized Position shift.

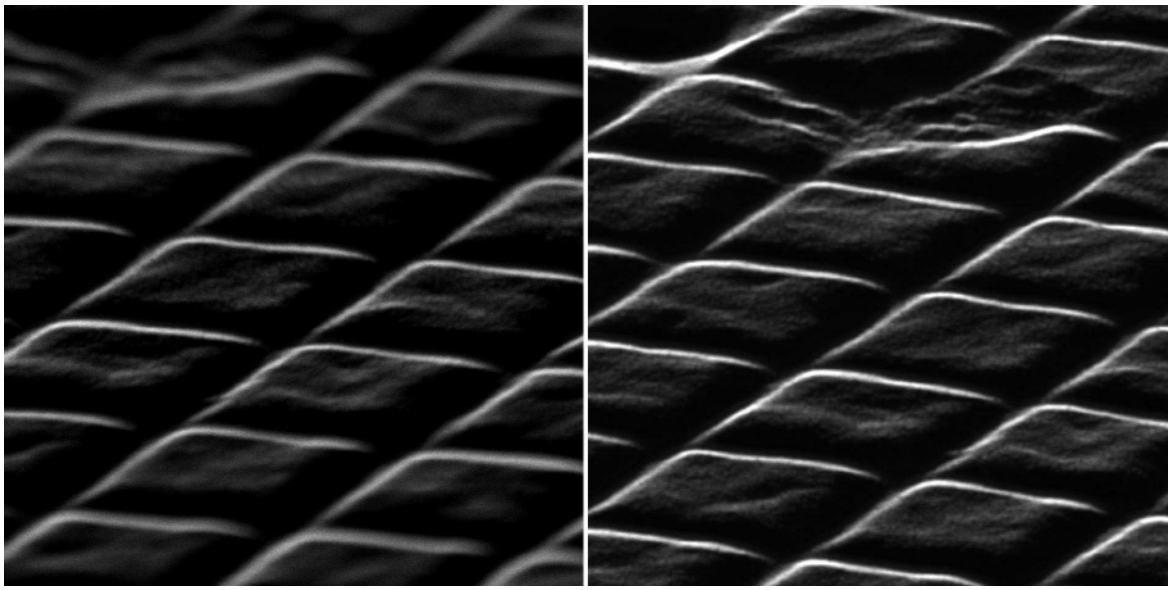
Tomography STEM sets the Optimized Position when the software starts. When the Tomography STEM software is stopped, the Optimized Position is revoked.

Note While Tomography STEM is running, always use the *Tomo* versions of the Direct Alignments.

The regular *TEM User Interface > Direct Alignments* reset the image shift to 0, which cancels the Optimized Position image shift that Tomography STEM has set.

4.5 The Dynamic Focus task

On a tilted specimen, the areas that are furthest from the tilt axis have a different Z-height than central region around the tilt axis. The *Dynamic Focus* function adjusts the focus during the acquisition, so that the areas that are farther away from the tilt axis stay in focus.



Left: without Dynamic Focus.

Right: with Dynamic Focus.

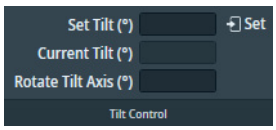
To do so:

- The scan rotation is aligned with the tilt axis so that the scan lines are parallel to tilt axis.
- The focus is adjusted for each scan line, depending on the tilt angle and the distance from the tilt axis.

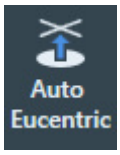
Dynamic focus is used throughout the calibrations and (optionally) also during the acquisition of Tilt Series with a slab-like specimen.

4.5.1 Perform the Dynamic Focus task

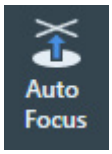
1. Specify **Set Tilt**: **0.00** and select **Set**



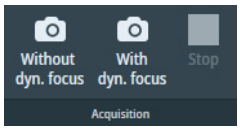
2. Select **Auto Eucentric**



3. Select **Auto Focus**



4. Select **Without dyn. focus**



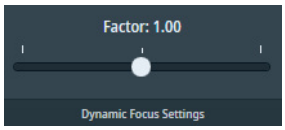
5. Verify that the **entire image** is accurately focused.
6. Identify a few **recognizable features** near the edges of the image that are furthest away from the tilt axis. These features mark the area that is imaged at 0 degrees.

   Make a **screenshot** for reference.
7. In **Set Tilt** specify the **highest tilt angle** that is supported by the system, and select **Set**
8. Verify that the **area close to the tilt axis** is still in **focus**.

   If necessary, manually adjust focus
9. Select **With dyn. focus**
10. Check if the **area that was imaged at 0°** is focused. Use the *screenshot* as a reference.

    It is acceptable if the regions outside the *0° image area* not accurately focused and/or have a small deformation due to stretching or shrinking. These areas do not contribute to the 3D reconstruction.
11. If the central region around the tilt axis is not accurately focused,

    then:
    a. Adjust the **Dynamic Focus Settings** > **Factor**



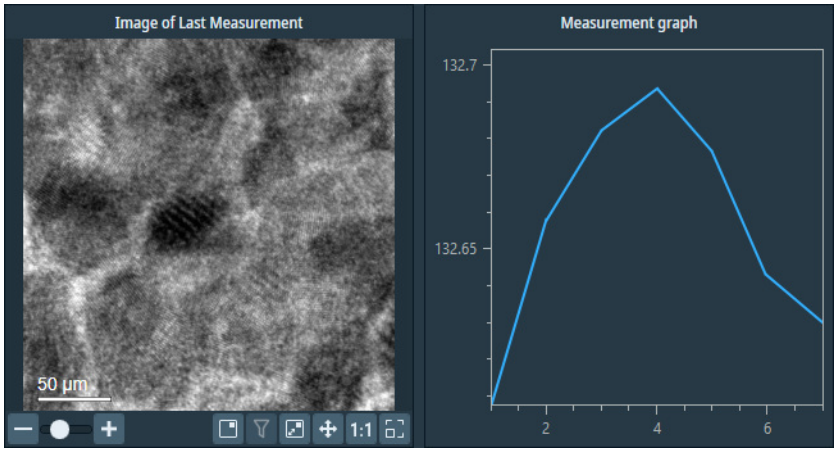
    b. Select **With dyn. focus** again.
    c. Verify that the **central region of image** around the tilt axis is accurately focused.

5 Auto Functions Tab

The Auto Functions tab provides various (semi-)automated tasks. The Auto Functions use the Acquisition and Optics Presets.

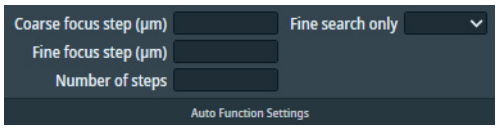
5.1 Run the 'Autofocus linear' auto-function

The *Autofocus linear* function searches for the sharpest image by varying the focus value.



The Autofocus function typically starts with a rough search to find the approximate focus. After the rough search, a fine search around the approximate focus starts.

5.1.1 Description of the 'Autofocus linear' settings



Coarse focus step

This is the focus step size for the rough search. If the contrast on the image decreases during the first 4 steps, then the procedure stops.

Fine focus step

This is the focus step size for the fine search. Please make sure that the search range covers at least one step of the rough search.

Number of steps

This is the total number of focus steps, rough and fine search steps combined.

Fine search only

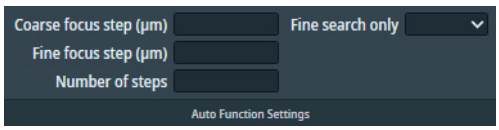
If the current focus value is already close to the optimum, then the rough search can be skipped.

5.1.2 Perform the 'Autofocus linear' auto function

1. Use the **hand panels** and the **live view functions in Velox** to:

a. Move the **specimen** to an area with a few recognizable features, for example an edge of a Foil Hole.
b. With the **Focus** knob, adjust the focus value, so that the image is at or near focus. It is *not* necessary to *accurately* focus the image.

2. Tomography STEM selects the relevant preset automatically. Otherwise, in **Auto Functions** > **Autofocus linear**, select **Presets**: **Focus**.
3. Execute the *Autofocus linear* function for the first time:
   a. In the **Auto Function Settings**, specify the following initial values:



   - **Coarse focus step**: **3.00**
   - **Fine focus step**: **0.60**
   - **Number of steps**: **10**
   - **Fine search only**: **No**

   b. Select **Start**
   c. If the procedure fails, then use the **hand panels** and the **live view functions in Velox** to manually improve focus, then select **Start** again.
   d. Verify that:
      - The **Measurement graph** shows a clear maximum.
      - The **image** is accurately focused.

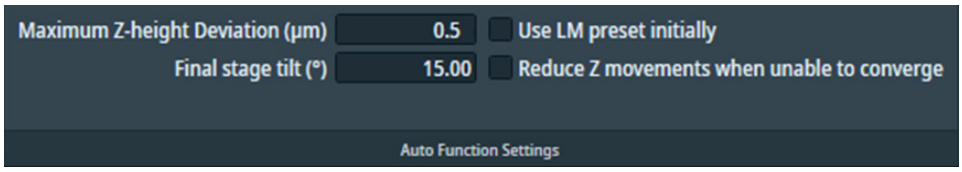
5.2 Run the 'Auto-eucentric height' auto-function

The *Auto-eucentric height* auto-function uses stage tilt to set the specimen to eucentric height. To run the *Auto-eucentric height* auto-function, follow the procedure below:

1. Select **Auto Functions** > **Auto-eucentric height**
2. Tomography STEM selects the appropriate preset automatically. Otherwise, in **Preset Selection** > select **Presets**: **Eucentric Height**.

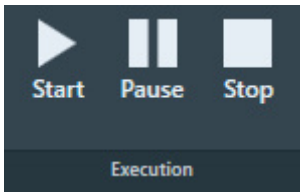
   Technically, auto-functions can be executed with any Preset. For this auto-function, the *Eucentric Height* Preset is the most suitable option.

3. In the **Auto Function Settings** ribbon:



   a. Specify the **Maximum Z Deviation**
      The default value is suitable for the majority of all experiments and specimens.
   b. Specify the **Final stage tilt**
      This is the maximum tilt angle at which the CompuStage Alpha tilt axis wobbles. The default value is 15 degrees. Larger and smaller values can be entered, depending on the start conditions.
   c. Select **Adjust focus**: **Yes** to use Dynamic Focus
      If the Dynamic Focus factor is not calibrated, then either:
      - Select *No*
      - Perform the *Preparations* > *Dynamic Focus* task.
        For instructions, see: The Dynamic Focus task on page 45.

4. Select **Execution** > **Start**



6 Atlas Tab

An Atlas is an overview of the specimen. To create an Atlas, Tomography STEM acquires a set of large area images at multiple stage positions, and stitches these images together.

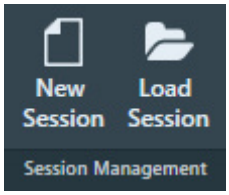
An Atlas can be used to navigate to features of interest during the preparation of an Automated Acquisition run. For Tomography STEM experiments in which only one or a few features at known locations will be processed, the Atlas has little added value and can be omitted.

6.1 Session Setup task

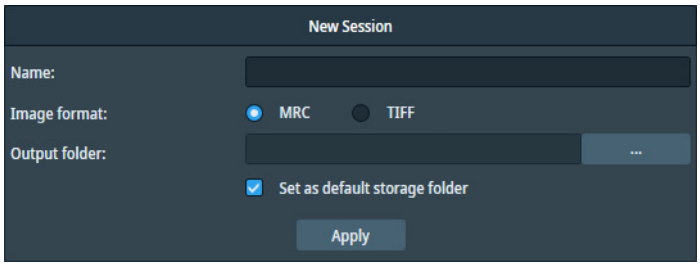
6.1.1 Create a new Atlas Session

To setup a new Atlas session, follow the procedure below:

1. Select the **Atlas** tab > **Session Setup** task.
2. Select **New Session**



3. Enter a **Name** for the session.



4. Select the **Image format** that will be used to store the acquired images.
   - **MRC**: Electron microscopy image format. The MRC format includes an extensive set of metadata about the microscope and the microscope settings.
     See The MRC2014 Image Format on page 112.
   - **TIFF**: Raster image format: TIFF file format.

5. At *Output folder*, select **[...]** and navigate to the target folder.
   In the specified folder, Tomography STEM creates a sub-folder with the *Name* of the Atlas session.

Note Do *not* rename or move the Output folder.

In this folder, Tomography STEM stores the following files:

- The Atlas session file `ScreeningSession.dm`
- For each specimen that is processed in the session: the images and metadata for the Atlas and for all Tiles.
  - On a system with an Autoloader, the Screening task can process multiple specimens.
  - On a system without an Autoloader, the Atlas Acquisition task processes only the specimen that is loaded on the stage.

6. (Optional) Tick **Set as default storage folder**.
   If *Set as default storage folder* is ticked, then the specified folder is used as the default *Output folder* for subsequent Atlas sessions.
7. Select **Apply**

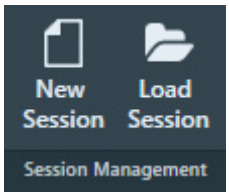
6.1.2 Load an existing Atlas Session

Note If a specimen has already been (partly) processed in an Automated Acquisition run, then do not load an existing Screening Session file for a new Tomography STEM session with that specimen.

The Screening Session file does not contain data about which Grid Squares have been processed already in a preceding Automated Acquisition run. Processed areas may be too damaged to yield new high quality data. Revisiting these areas is therefore not a productive use of system time.

To load an existing Atlas Screening Session, follow the steps below:

1. In the **Atlas** > **Session Setup** task, select **Session Management** > **Load Session**



2. Navigate to the Output folder of the desired Screening Session and select the `ScreeningSession.dm` file.

Note Do not load an `Atlas.dm` file.

6.2 Screening task

The Screening functionality is used to acquire an Atlas. On a system with an Autoloader the Atlas acquisition can be done for multiple specimens from a selection of Slot Positions.

6.2.1 The Screening task for systems with an Autoloader

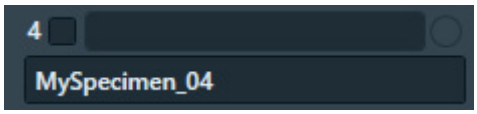
6.2.1.1 Acquire Atlases for multiple specimens

To acquire Atlases for specimens from multiple Slot Positions, follow the steps below:

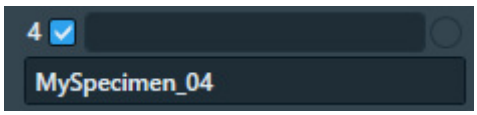
1. Select the **Atlas** > **Screening** task.
   The Task Selection panel displays all Slot Positions in the Autoloader.
   - Empty slot:

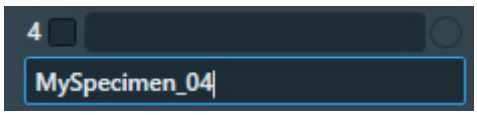
   - Occupied slot:

2. For each occupied slot:
   a. (Optional) Tick the **Slot Position** to schedule it for Atlas acquisition.

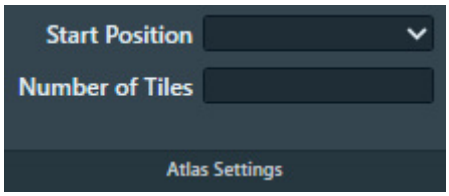
      

      It is not possible to select an empty Slot Position.
   b. (Optional) Edit the default **description**

      At the creation of a new Screening session, Tomography STEM copies the slot descriptions that are present the *TEM User Interface* > *Autoloader* control panel. After the Screening session is created, Tomography STEM and the TEM User Interface do not synchronize their slot descriptions.

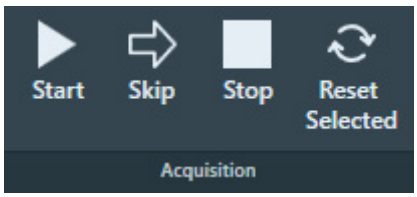
3. In **Atlas Settings**:



   a. If the currently loaded specimen is included in the set of selected Slot Positions, then select the **Start position**. The selected Start Position only applies to the Slot Position of the currently loaded specimen. For all other Slot Positions, Atlas acquisition starts at *Close to center*. :
      - **Close to center**
        Atlas acquisition starts close to the center of the specimen.
      - **Close to current**
        Atlas acquisition starts close to the current stage position.

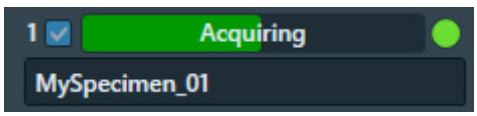
   b. (Optional) Specify the **Number of tiles** to restrict the area that will be covered in the new Atlas. The specified *Number of tiles* applies to all Slot Positions. It is not possible to specify a different value per Slot Position.

4. Select **Acquisition** > **Start** to begin the screening procedure.

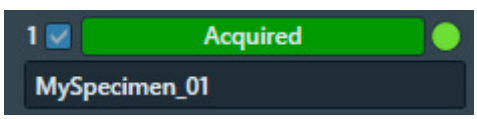


   Tomography STEM prepares the system for Atlas acquisition.
   - If the Objective Aperture Mechanism is enabled and inserted, then Tomography STEM retracts the aperture before acquisition starts. After the acquisition is completed, the aperture is inserted again.
   - If the Autoloader TMP is not running, then Tomography STEM will start it to shorten the exchange time between Slot Positions. After the Screening acquisition is completed, Tomography STEM returns the Autoloader TMP to its previous status.

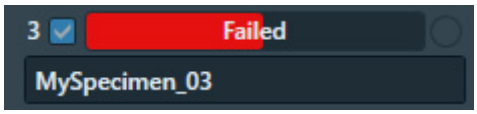
5. Wait until all selected Slot Positions are processed.
   For each included Slot Position, Tomography STEM loads the specimen and acquires an Atlas.
   The progress bar displays the progress or the result of the Atlas acquisition.
   - Acquiring



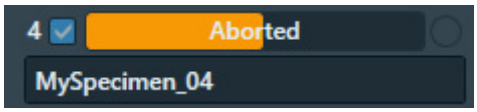
     The **green LED** shows that the specimen from this Slot Position is currently loaded on the CompuStage.
   - Acquired



- Failed



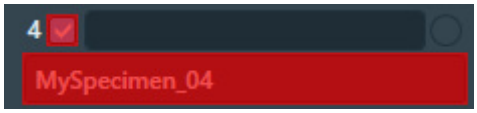
- Aborted



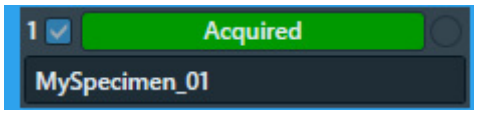
While the screening procedure is running, it is possible to:

- Schedule or unschedule Slot Positions for which Atlas acquisition has not started yet.
- Select any Slot Position to view the Atlas.

  To select a Slot Position, click anywhere in the Slot Position, except for the checkbox and the description.



The Slot Position is highlighted and the Atlas for the specimen appears in the Image Display.



Viewing the Atlas for a specimen does not affect an ongoing acquisition for a different specimen.

6.2.1.2 Acquire a single Atlas from an unknown specimen on the stage

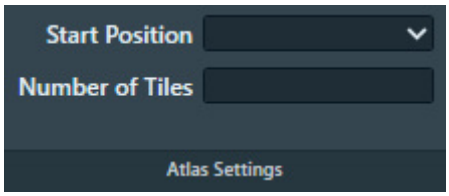
Sometimes the occupation status of the stage is not known. It is unknown if a specimen is currently present on the stage, or it is unknown from which Slot Position the specimen on the stage was loaded. This situation can occur when, for example, the cassette is undocked or when the Autoloader is not initialized.

Under these circumstances, the Single Atlas functionality allows the acquisition of an Atlas from the unknown specimen. If no specimen is present, then the Atlas will be blank.

The Single Atlas functionality is available in the Screening task as an additional (virtual) Slot Position, below the regular Slot Positions. Unlike regular Slot Positions, the Single Atlas slot has its own Acquire function. The Acquire function is only available when the occupation status of the stage is unknown. An Atlas that is acquired with the Single Atlas functionality behaves the same and is treated the same as an Atlas that is acquired for a regular Slot Position in a regular Screening session.

To acquire an Atlas from an unknown specimen on the stage, follow the steps below:

1. (Optional) Verify that a specimen is present on the stage:
   a. In **Preparation** > **Acquisition and Optics Settings**,
      select **Preset Selection** > **Presets**: **Atlas** or another Preset with a large illuminated area to prevent that the specimen is exposed to a converged beam.
   b. Select **Optics Settings** > **Set**
   c. With the TEM User Interface and/or Hand Panels,
      insert the **FluScreen** and open the **Column Valves**.
2. In **Atlas** > **Screening** > **Atlas Settings**:



   a. Select the **Start position**:
      - **Close to center**
        Atlas acquisition starts close to the center of the specimen.
      - **Close to current**
        Atlas acquisition starts close to the current stage position.
   b. (Optional) Specify the **Number of tiles** to restrict the area that will be covered in the new Atlas.
   c. At the bottom of the Autoloader Slot Positions, select **Single Atlas** > **Acquire**

      If the Objective Aperture Mechanism is enabled and inserted, then Tomography STEM retracts the aperture before the acquisition starts. After the acquisition is completed, the aperture is inserted again.
   d. Wait until the Single Atlas acquisition is completed.

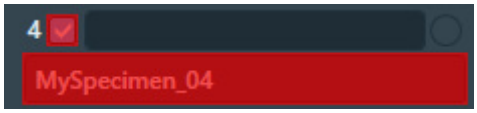
6.2.1.3 Reset the Slot Position status

After a Slot Position is processed by the Screening task, it is possible to reset the status back to the initial value. This means that the following information is erased or returned to its initial value:

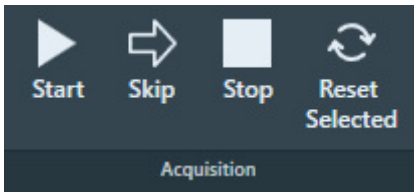
- Slot Position status
- Atlas
- Selected Grid Square categories

To reset the status of a Slot Position:

1. Select the **Slot Position**
   To select a Slot Position, click anywhere except for the checkbox or the description.



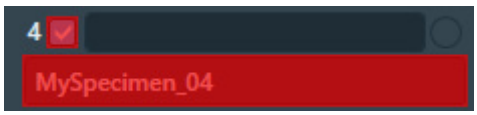
2. Select **Reset Selected**



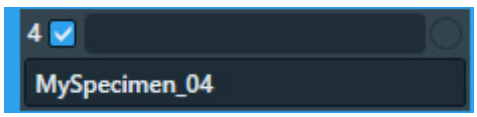
The status, Atlas, name and selection Grid Square categories are erased, or are returned to their initial values.

6.2.1.4 Load a specimen on the stage

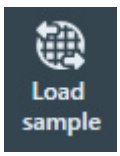
1. Select **Atlas** > **Screening** to display all Slot Positions in the Autoloader.
2. Select the **Slot Position** at which the desired specimen is located.
   To select a Slot Position, click anywhere except for the checkbox or the description.



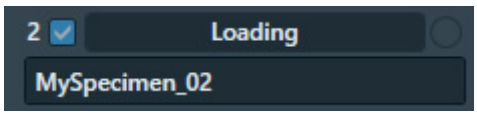
The Slot Position is highlighted and the Atlas for the specimen appears in the Image Display.



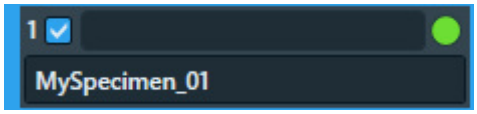
3. Select **Load sample**



The status of the selected Slot Position changes to *Loading*



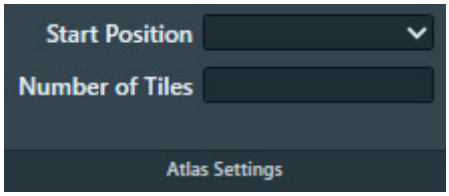
4. Wait until the loading procedure is completed and the **LED** is **green**



6.2.2 Acquire an Atlas for a single specimen on a system with removable holders (side entry)

To acquire an Atlas on a system with a removable holder (a so-called *side entry* system), follow the instructions below:

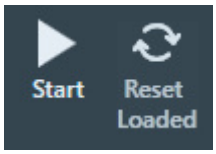
1. Select **Atlas** > **Screening**
2. In the **Atlas settings** section:



   a. Select the **Start position**:
      - **Close to center**
        Atlas acquisition starts close to the center of the specimen.
      - **Close to current**
        Atlas acquisition starts close to the current stage position.

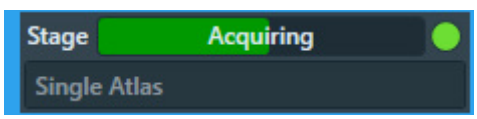
   b. (Optional) Specify the **Number of tiles** to restrict the area that will be covered in the new Atlas.

3. Select **Acquisition** > **Start**



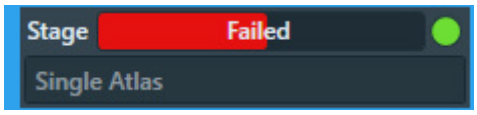
   Tomography STEM prepares the system for Atlas acquisition.
   - If the C2 aperture mechanism is enabled, then Tomography STEM first selects the largest C2 aperture. After the acquisition is completed Tomography STEM returns the aperture mechanism to its initial position.
   - If the Objective aperture mechanism is enabled and inserted, then Tomography STEM first selects the retracted position. After the acquisition is completed Tomography STEM returns the aperture mechanism to its initial position.

4. Wait until Tomography STEM has acquired all images for the Atlas.
   The progress bar displays the progress or the result of the Atlas acquisition.
   - Acquiring

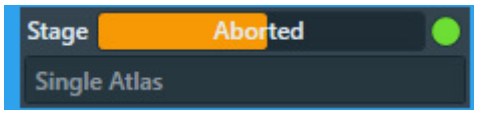


   - Acquired

- Failed



- Aborted



If the image quality is not good enough, or if stitching the tiles does not result in a good Atlas:

a. Select **Stop**
   This can be done at any time during the Atlas acquisition and may take a few seconds.
b. Select **Acquisition and Optics Settings** > **Presets**: **Atlas** and adjust the settings.
c. (For side entry loader) Select **Reset Loaded** to unload the current specimen and prepare for re-acquiring the Atlas.
d. Select **Atlas** > **Screening** > **Start** again to acquire a new Atlas.
   All tiles that were acquired before adjusting the Atlas Preset are discarded.

Note If the Optics Settings parameters of the Atlas Preset are changed, and the system has a Cryobox, then the Atlas Optics Alignment might have to be performed again.

7 Tomography Tab

The Tomography tab provides tasks and functionalities to setup and execute an Automated Acquisition run. The sequence of actions in the Tomography tab depends on the selected Experiment Settings.

Before starting the tasks in the Tomography tab:

- The tasks in the Preparations tab must be completed.
- The Auto Functions calibrations and alignments must be completed.
- For the Batch option, it is recommended that an Atlas of the specimen is acquired for easy navigation, but this is not a prerequisite.

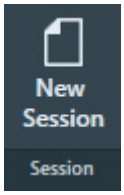
7.1 Session Setup task

7.1.1 Create a new Tomography Session

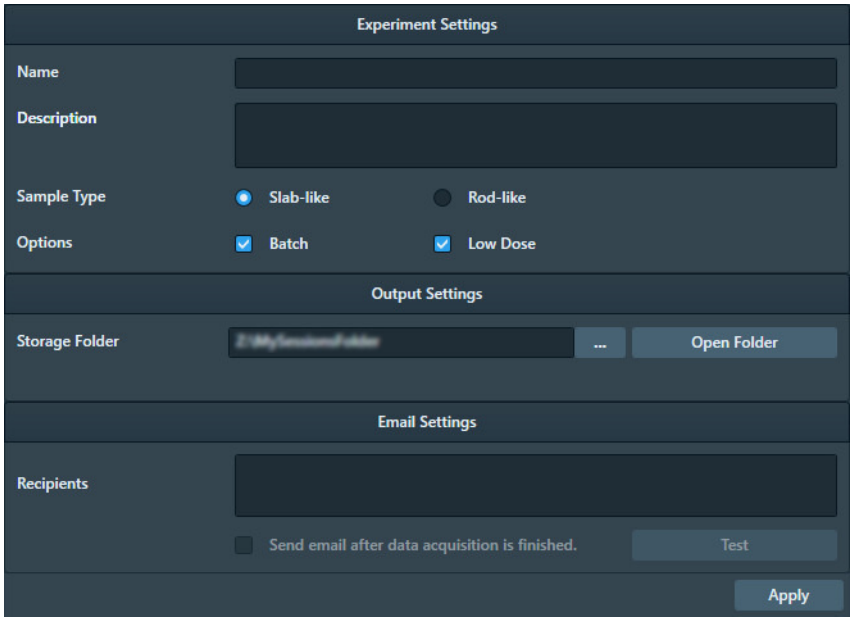
To setup a new Data Acquisition session, follow the procedure below:

Note Create a new Tomography Session during Atlas Screening.

1. Select the **Tomography** > **Session Setup** task.
2. Select **Session** > **New Session**



The **New Session** form appears.



3. In the **Experiment Settings** section:
   a. Specify a **Name** for the new Tomography session.
   b. Optionally enter a **Description** for the new Tomography session.
   c. Select the **Sample Type**:
      - **Slab-like**
        The specimen is carried by a flat foil. The cross-section of the specimen in the direction of the electron beam increases as the tilt angle increases.
      - **Rod-like**
        The specimen is needle shaped. The cross-section of the specimen in the direction of the electron beam is fairly constant at every tilt angle.
   d. (Optional) Tick **Batch**
      Depending on the selection, Tomography STEM activates either:
      - The *Batch Positions* task to acquire data from multiple features of interest.
      - The *Sample Navigation* task to acquire data from a single feature of interest.
   e. (Optional) Tick **Low Dose**
      In a Low Dose experiment Tomography STEM does not execute the Autofocus and Tracking actions at the same location as Exposure Area where the feature of interest is located. Instead, these action take place at dedicated areas near the feature.
      Use the Low Dose option:
      - To prevent over-exposure of dose sensitive features.
      - To prevent charging of the feature of interest.
      - To prevent failure of the Focus and Tracking functions when the feature of interest has very low contrast.

      If Low Dose is cleared, then the Autofocus and Tracking actions take place at the same location as the Exposure Area.
4. In the **Output Settings** section, specify the **Storage Folder** path,
   or select **[...]** to navigate to the desired file system location.
5. In the **Email Settings** section:
   a. Specify the **Recipients**
      Enter the email addresses to which a notification is sent when the Automated Acquisition run is completed or otherwise stopped. When entering multiple addresses they need to be separated by commas, semi-colons or spaces.
   b. Tick or clear **Send email...**

c. If desired, select **Test** to confirm if the entered email addresses are valid and if the email services are configured correctly.

The notification emails are sent via the email service components that are installed on the Microscope PC and on the Support PC. If the test email is not delivered to at least one of the specified addresses, contact Thermo Fisher.

The email settings can not be updated after a run is started.

6. Select **Apply**

If an Atlas is available for the currently loaded specimen, then that Atlas is used for the new session.

Depending on the selected options, Tomography STEM makes the applicable tasks available.

It is possible to change the selected options after the session is created. Depending on the progress and status of the session, some actions may have to be performed again.

7.1.2 Load an existing Tomography Session

You can load a Tomography session file (`.dm`) which contains the following data:

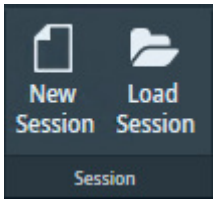
- Experiment Settings
- Output Settings
- Email Settings.

In addition, loading a Tomography session automatically loads the data saved in the corresponding Storage Folder. This allows you to review acquired data and utilize Tomography features, such as batch annotations.

Note To ensure proper functionality, manually load the corresponding Atlas session before loading the Tomography session. This ensures that the Atlas session is correctly displayed within the Tomography session, if applicable.

To load an existing Tomography session, follow the steps:

1. Go to the **Tomography > Session Setup task**.
2. Select **Session Management > Load Session**.



Navigate to the Output folder of the desired Tomography session and select the session's `.dm` file.

7.2 The Search Maps task

To identify each individual feature, Tomography uses *Search Maps*. A Search Map is matrix of Search images that is assembled like an Atlas.

In the Search Maps task, multiple Search Maps on the specimen can be added and acquired automatically. During the acquisition of multiple Search Maps, Tomography ensures that the specimen is at Eucentric Height before acquiring each individual Search Map.

For easy navigation and accurate target selection, Tomography provides a process flow that uses a stepped sequence of views on the specimen at increasing magnifications. This flow helps accurately identify multiple features of interest and helps prepare a Tilt Series for each individual feature.

Tomography also provides a way to select an Atlas that does not have to be currently loaded on the stage, allowing you to define Search Maps for multiple samples without needing to load each sample individually. However, acquiring images (including Overview images) and navigating on them with the stage is not allowed until the corresponding sample is loaded.

To speed up defining Batch Positions (see The Batch Positions task on page 80), multiple Search Maps can be acquired separately without user interaction.

To acquire multiple Search Maps for areas of interest, perform the following actions:

1. (Optional) Acquire an Overview of the area of interest.

   The Overview has a wide field of view. On a slab-like specimen, this is typically a single GridSquare. In the Overview view it is possible to:

   - Acquire a Search Map immediately.
   - Add a Search Map to the list of Search Maps to be acquired.
2. Once an Alas or Overview image is available, add a Search Map of the area of interest to the list of Search Maps to be acquired.

   A Search Map has a smaller field of view at a higher accuracy than the Overview image. The tiles of a Search Map are marked in the Atlas and in the Overview. The currently active Search Map has green tile outlines, all acquired Search Maps have yellow tile outlines, and all Search Maps yet to be acquired have orange tile outlines.
3. (Optional) Edit, refine, re-order, and delete the defined Search Maps as needed.

7.2.1 Add a Search Map

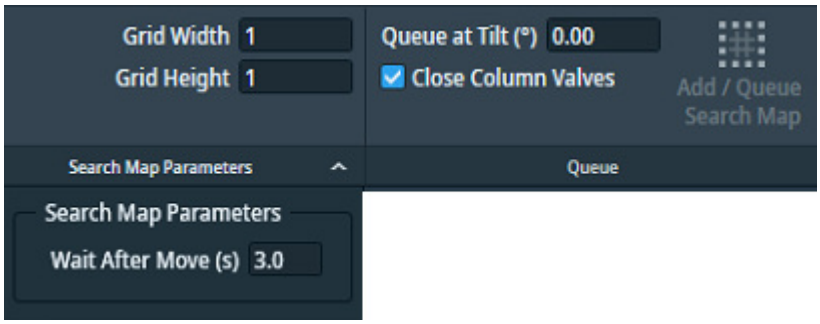
Search Maps can be added on the Atlas image, or on an Overview image. For information on how to acquire an overview image, refer to Acquire an Overview image on page 72, Acquire an Overview image on page 81.

It is also possible to acquire a single Search Map directly using the Acquire Search Map button. However, using this button does not run the Auto-eucentric function automatically. See Acquire a Search Map.

Note Add Search Maps to the list of Search Maps to be acquired already during Atlas Screening, while the acquisition of queued Search Maps is in progress, or while Batch Position acquisition is in progress.

To define and add a Search Map:

1. In the **Search Map Parameters**:

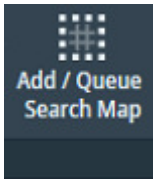


   a. Specify the **Grid Width** (X direction) and **Grid Height** (Y direction).

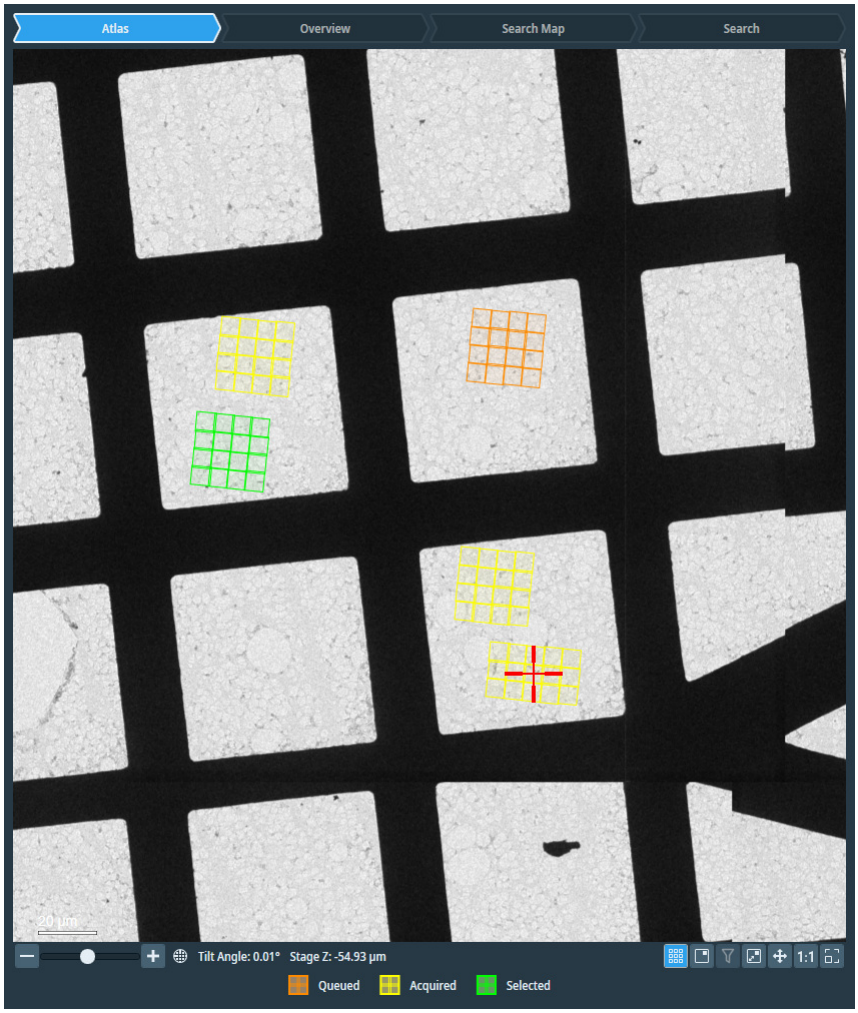
b. Specify **Wait After Move (s)**: the time between the stage move and the acquisition of a search map image.

c. Specify **Queue at Tilt (˚)**: the Alpha tilt used for Search Map acquisition.

2. In the Atlas image or in the Overview image, right-click on the center of the **area of interest** and select **Add Search Map here**.
3. (Optional) Alternatively, in the Atlas image or the Overview image, right-click on the center of the **area of interest** and select **Move Stage here**.
4. Click **Add / Queue Search Map**.



The outlines of the Search Map are visible in the Atlas, and are displayed in orange for Search Maps that are not yet acquired. If an Overview is available that contains the Search Map area, then the outlines of the Search Map tiles are also visible in that Overview.

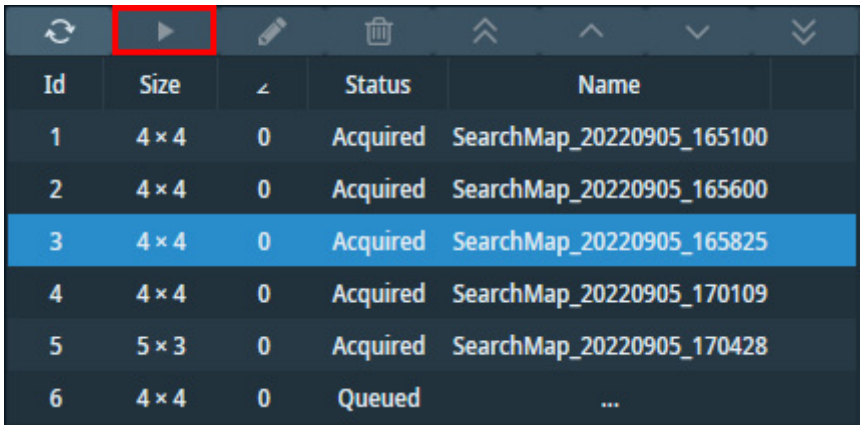


7.2.2 Manage Search Maps

7.2.2.1 Acquire a single Search Map

To acquire a single Search Map from the Search Maps List:

1. In the Image Display or in the Search Maps List, select the Search Map that must be acquired.
2. Select the Acquire button above the Search Maps List column header.



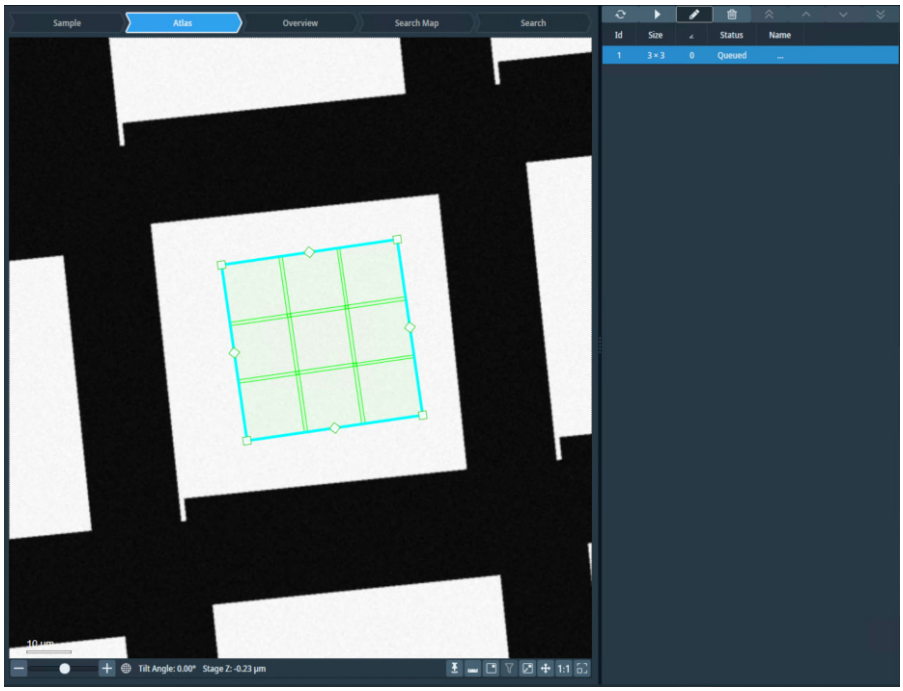
Id	Size	∠	Status	Name
1	4 × 4	0	Acquired	SearchMap_20220905_165100
2	4 × 4	0	Acquired	SearchMap_20220905_165600
3	4 × 4	0	Acquired	SearchMap_20220905_165825
4	4 × 4	0	Acquired	SearchMap_20220905_170109
5	5 × 3	0	Acquired	SearchMap_20220905_170428
6	4 × 4	0	Queued	...

7.2.2.2 Edit a single Search Map

To change a Search Map:

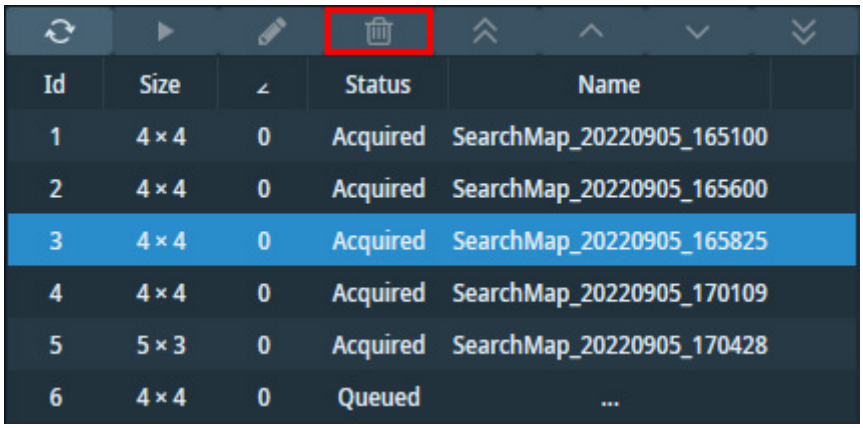
- Select the Search Map (either in the list of Search Maps, or by clicking on the Search Map annotation).
- Click, hold and move the center of a Search Map to pan the position of Search Map.
- Click, hold and move the squares on edges of the Search Map to change size of Search Map.
- To enter/exit the edit mode of a search map, either:
  - Double click on a search map annotation or
  - Click the edit button in the search maps list

Note You cannot edit the Acquired Search Maps.



7.2.2.3 Delete a single Search Map

1. In the Image Display or in the Search Maps List, select the Search Map that must be deleted.
2. Either:
   - Right-click and select Delete
   - Select the Delete button above the Search Maps List column header.



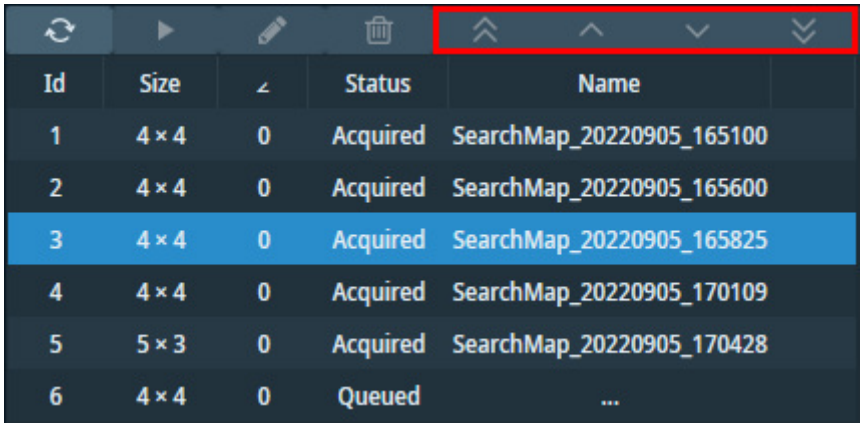
Note There is no Undo function. It is not possible to retrieve deleted Search Maps.

7.2.2.4 Change the processing order of the Search Maps

During the Automated Acquisition run, the Search Maps in the list are processed in top-to-bottom order. By default, this is the order in which the Search Maps have been added to the list.

To change the processing order, either:

- Select a Search Map, then select a re-ordering action from the toolbar above the Search Maps List.
- Right-click on a Search Map and select a re-ordering action from the context menu.



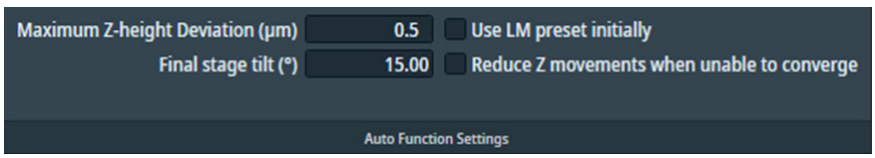
Id	Size	∠	Status	Name
1	4 × 4	0	Acquired	SearchMap_20220905_165100
2	4 × 4	0	Acquired	SearchMap_20220905_165600
3	4 × 4	0	Acquired	SearchMap_20220905_165825
4	4 × 4	0	Acquired	SearchMap_20220905_170109
5	5 × 3	0	Acquired	SearchMap_20220905_170428
6	4 × 4	0	Queued	...

Note Note: The Acquired Search Maps will always be displayed at the top of the list.

7.2.3 Acquire all Search Maps in the list

During the automatic acquisition of all Search Maps, the Auto-eucentric function runs at the center of each area of interest before acquiring the Search Map. After the Search Map is acquired, an Overview image is acquired at the center of the Search Map and saved. This image can then be used to gain information about the surroundings of the area of interest.

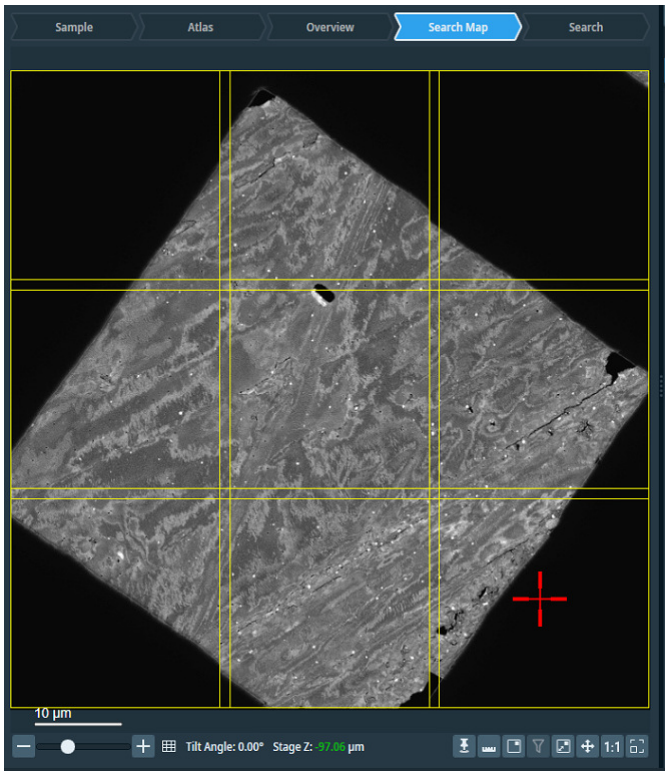
1. In **Auto Functions > Auto-eucentric by stage tilt > Auto Function Settings**:
   a. Specify Maximum Z-height Deviation (µm) to specify the maximum acceptable Z deviation from Eucentric Height.
   b. Select **Reduce Z movements when unable to converge**.



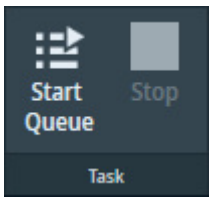
Before the first Search Map is acquired, Auto-eucentric is run with the *Use LM preset initially* enabled. Every subsequent Auto-eucentric during automatic Search Map acquisition is run with the *Use LM preset initially* disabled. The X, Y shift that is measured during the first Auto-eucentric is applied to all subsequent Search Maps. If the Search Map Queue Acquisition is stopped and restarted, Auto-eucentric starts again with the *Use LM preset initially enabled.*

Note The selection in Auto-eucentric height auto-function settings > Final Stage Tilt, Use LM preset initially does not have an effect on the Auto-eucentric performed during Search Map Queue Acquisition.

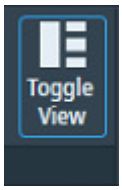
Search Maps acquired from the queue have a highlighted Z height, indicating that they have been acquired at Eucentric height.



2. Select **Start Queue** to acquire the Search Maps in the list.



A Search Map is marked as *In Progress* already while its Auto-eucentric is running. The progress of Search Map acquisition is shown when clicking on the **Toggle View** button.



7.2.4 Define the Lamella orientation

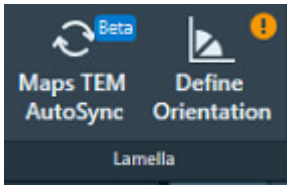
When loading specimens containing lamellae, it is recommended to align a lamella with the tilt axis. If the specimen is not oriented correctly with respect to the tilt axis, features of interest at Exposure Areas that are not located on the tilt axis might not be tracked and acquired accurately. Tomography STEM allows you to correct for small misalignment in the orientation of a lamella with respect to the tilt axis in order to acquire exposure areas relatively far from the tilt axis more accurately.

Defining orientation of lamella is available only when the Lamella option is selected in session setup. The orientation of a lamella needs to be specified manually once per specimen.

Follow these steps to define the orientation of a lamella:

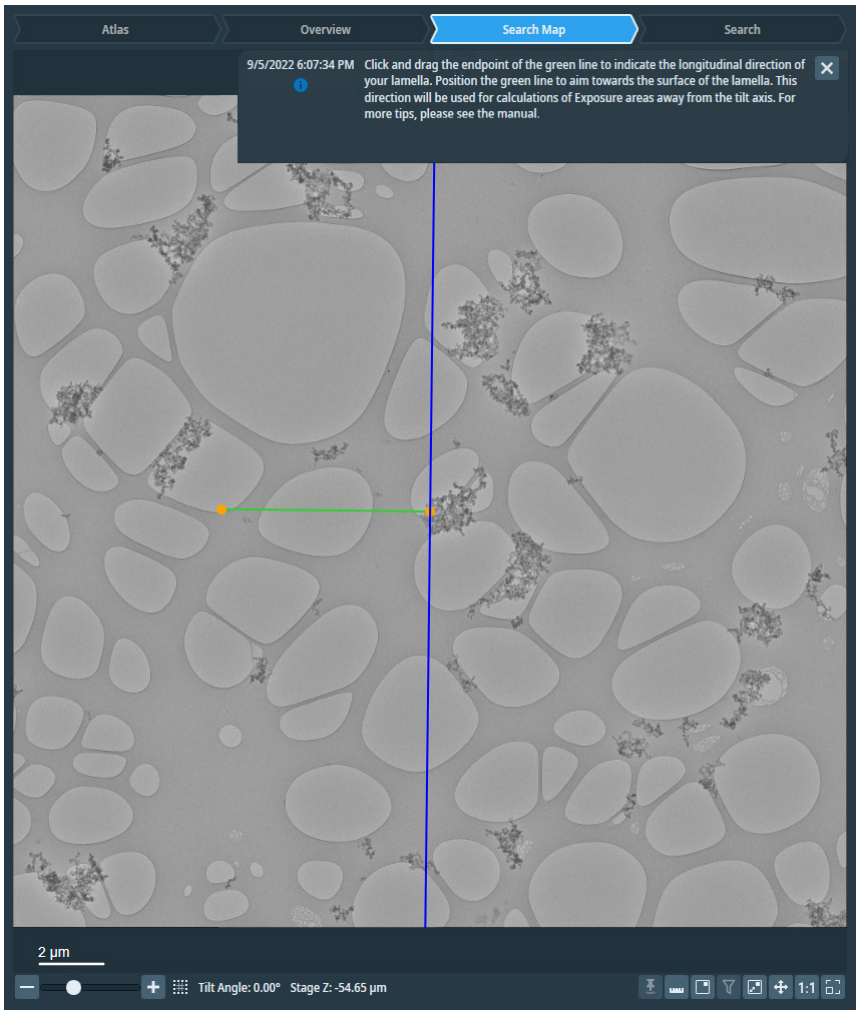
1. Open a previously-acquired Search Map.

2. You will see an exclamation mark on the **Define Orientation** button, when the lamella orientation is not defined for the sample.



3. Select **Define Orientation**.

A green line with two orange endpoints appears in the image display. One end is fixed to the center of the tilt axis while the other end is free to drag around.

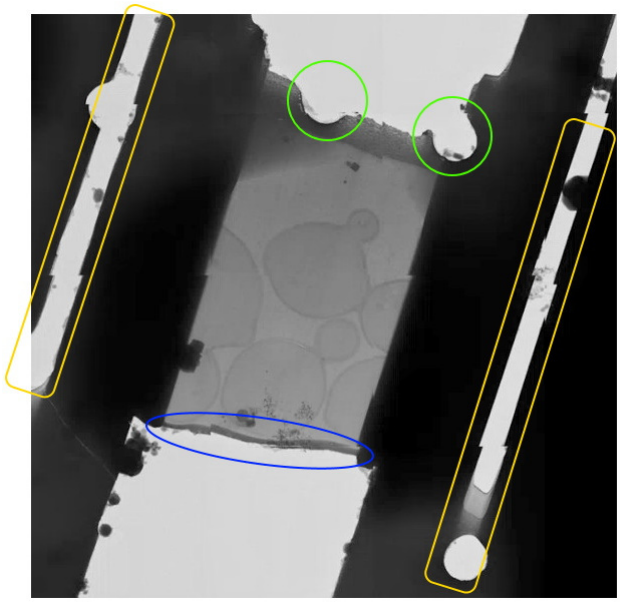


Click and drag the free end to adjust the alignment and the direction of the line according to the following.

a. Alignment: The green line needs to be longitudinal to the lamella, i.e., parallel to the orientation of the milling.

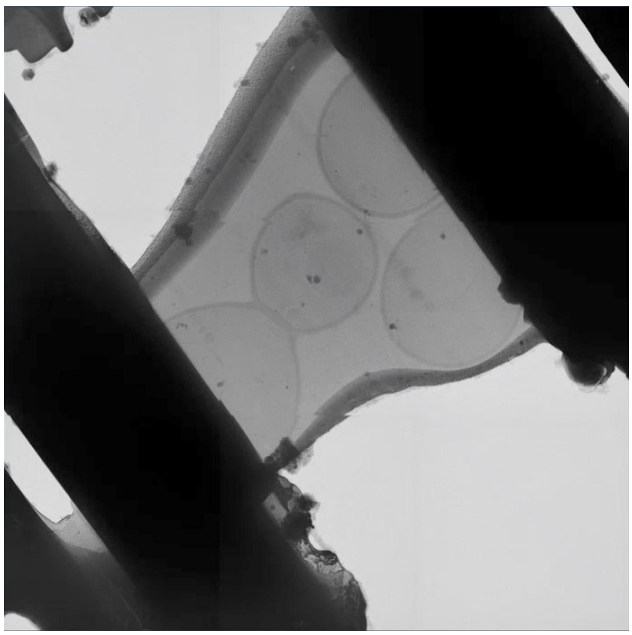
b. Direction: The green line needs to be aimed towards the surface or the higher part of the lamella. That is the the point from which the milling started, i.e., the source point of the ion beam.

To help you identify the orientation of any particular lamella, we present the following illustrated example.

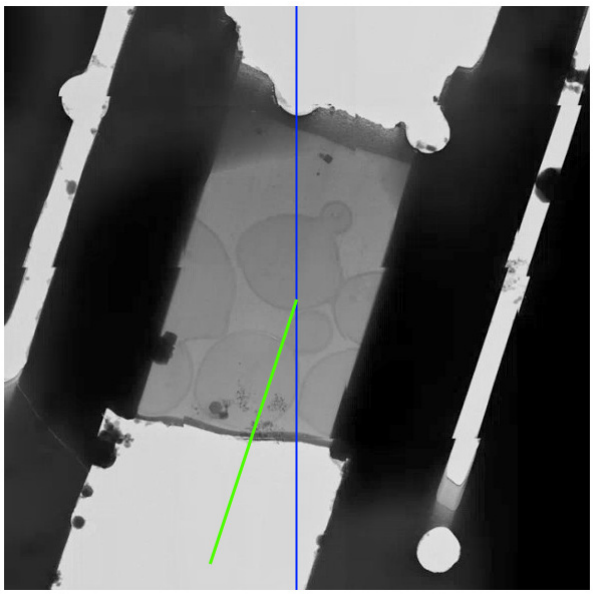


- (Marked yellow) The tension-relief cuts indicate the longitudinal axis of the lamella. Therefore, we look for the higher and lower parts of the lamella on the two ends of that longitudinal axis. In addition, you should notice which of the two sides across the length of a tension-relief cut has an abrupt start indicating the position where the cut started. The starting positions of the two tension-relief cuts are usually on the side where the milling started, i.e., on the higher side of the lamella.
- (Marked blue) The sputter-deposited platinum indicates the source of the ion beam.
- (Marked green) The round holes are foil holes which indicate the lower side of the lamella.

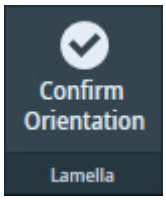
Tip regarding similar-looking sides: In some instances, the lower side of the lamella has no visible holes, and looks similar to the higher side. Observe the image below where the southeast side is the higher side of the lamella. While the lower side of the lamella (northwest in the image) has no visible holes, we can see a double layer indicating that this is not where the milling started; so we exclude this side from being the higher side. Another clue from this image is the tension-relief cut appearing on the left side; the starting position of this cut is towards the southeast where the milling started from.



Once you have determined the correct orientation, adjust the green line to the correct alignment and direction as shown below.



4. Select **Confirm Orientation**.



7.3 The Sample Navigation task

In the Sample Navigation task, a single position on the specimen is prepared for the acquisition of Tilt Series during the Data Acquisition run.

To prepare a Tilt Series for a feature of interest, perform the following actions.

1. Acquire an *Overview* of the area of interest.
   The Overview has a wide field of view. On a slab-like specimen, this is typically a single Grid Square. In the Overview view it is possible to:
   - Acquire a *Search Map* for easier and more accurate navigation and target selection.
   - Acquire a *Search* image at the location of a single feature of interest.
2. Acquire a *Search Map* of the area of interest; or use a previously-acquired Search Map.
   A Search Map has a smaller field of view at a higher accuracy than the Overview image. It is a matrix of Search images that is assembled like an Atlas. The tiles of a Search Map are marked in the Atlas and in the Overview. The currently active Search Map has green tile outlines, all other Search Maps have yellow tile outlines.
3. Acquire a *Search* image with the feature of interest in the center.
   The Search view is used to define the locations of the Template Areas and the Tilt Series settings.
   For single positions, this step is mandatory.
   For batch positions, this step is not mandatory.
4. Define the Template Area locations and the Tilt Series settings.

The sections below provide detailed information and instructions for the actions above.

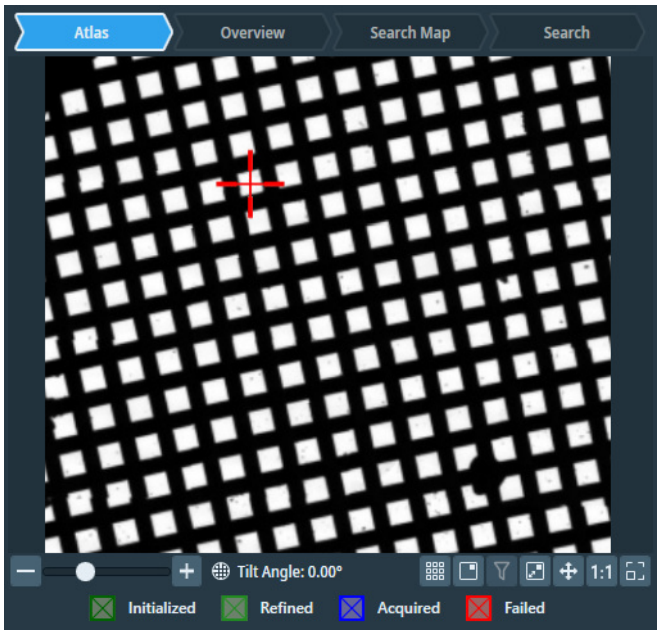
7.3.1 Acquire an Overview image

An Overview image can be acquired in the context of an Atlas of the specimen. How to do this is described in the steps below.
It is also possible to acquire an Overview image without an Atlas of the specimen. How to do this is also described below.

7.3.1.1 Acquire an Overview with using an Atlas

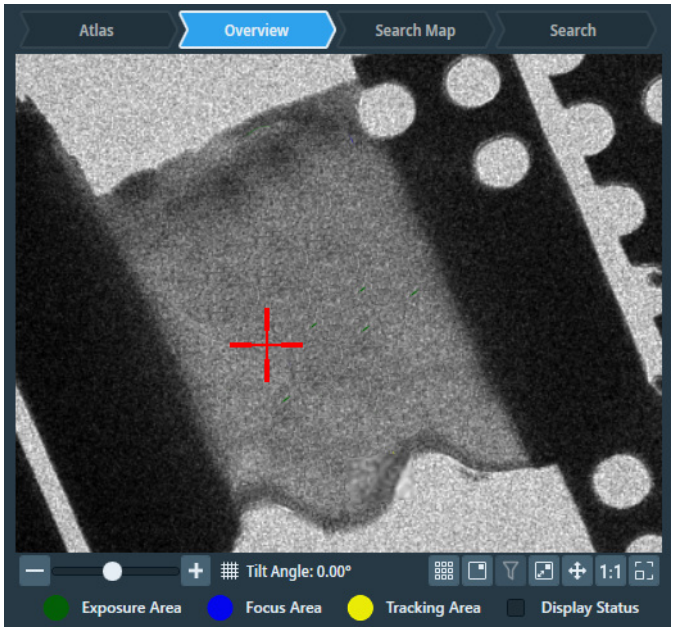
To acquire an Overview image *with* the use of an Atlas:

1. Select the **Atlas** view.

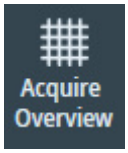


- If no Atlas is available yet, then go to *Atlas* > *Screening* task for the acquisition of a new Atlas. For instructions how to acquire an Atlas for the currently loaded specimen, see: Atlas Tab on page 50.
- If an Atlas is available for the currently loaded specimen, then Tomography displays the Atlas.

2. Right-click in **the center of the area of interest**,
   then select **Move Stage Here and Acquire Overview Image**
   Tomography STEM moves the specimen to the selected position, and then acquires and displays an Overview image.



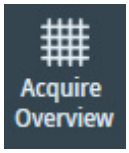
3. If the Overview image does not show the entire area of interest, then:
   a. In the current Overview image, right-click on the **center of the area of interest** and select **Move stage here**
   b. Select **Acquire Overview**



7.3.1.2 Acquire an Overview without using an Atlas

To acquire an Overview image *without* using an Atlas:

1. In **Velox**, start a *live image view*.
2. Use the **handpanels** to move the **area of interest** to the center of the live image.
3. Select **Acquire Overview**

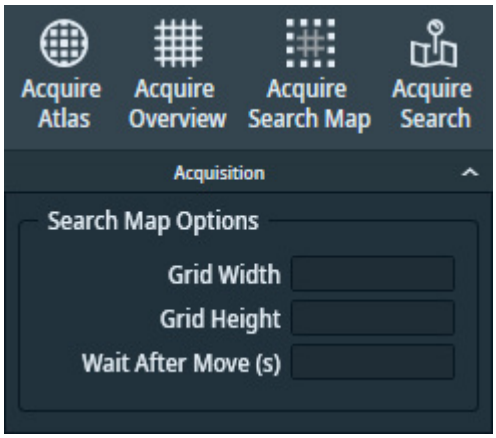


4. If the Overview image does not show the entire area of interest, then:
   a. In the current Overview image, right-click on the **center of the area of interest** and select **Move stage here**
   b. Select **Acquire Overview** again.

7.3.2 Acquire a Search Map

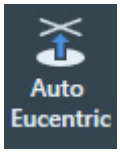
To acquire a Search Map:

1. In the **Acquisition** > **Search Map Options**:

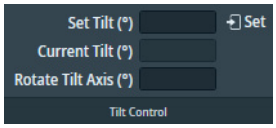


   a. Specify the **Grid Width** (X direction) and **Grid Height** (Y direction).
   b. Specify **Wait After Move (s)**: the time between the stage move and the acquisition of a search map image.
   c. Specify **Queue at Tilt (˚)**: the Alpha tilt used for Search Map acquisition.

2. If this is the first Search Map that will be acquired in this field of view, then select **Auto Eucentric**

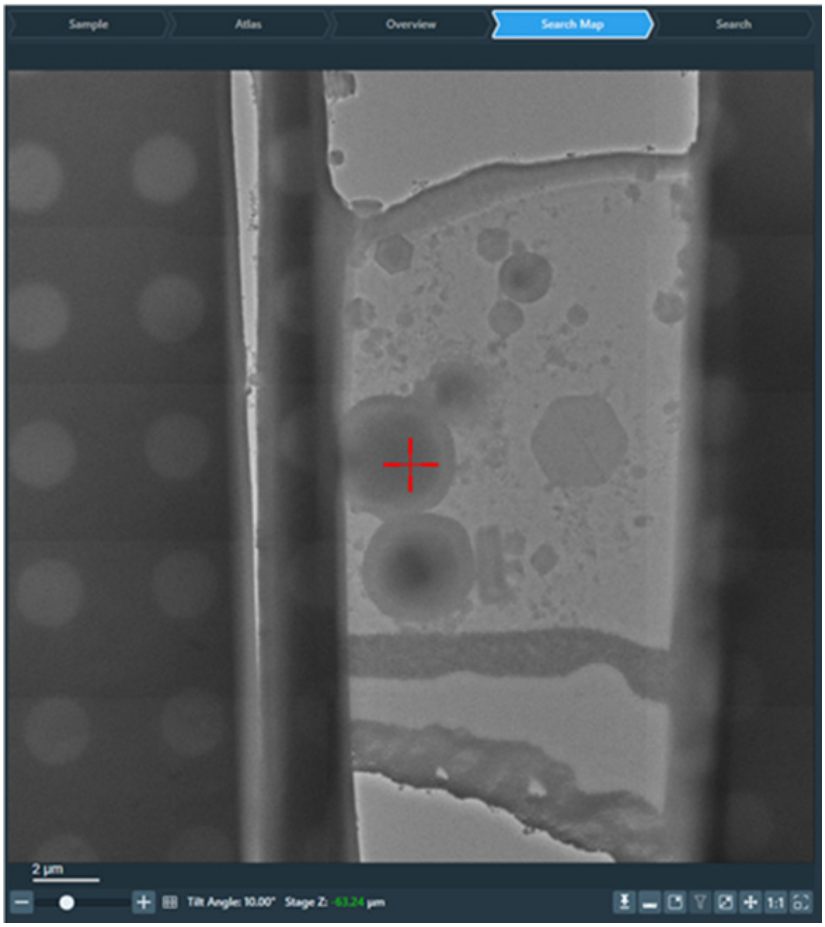


3. (Optional) Specify the *Tilt Control* settings:

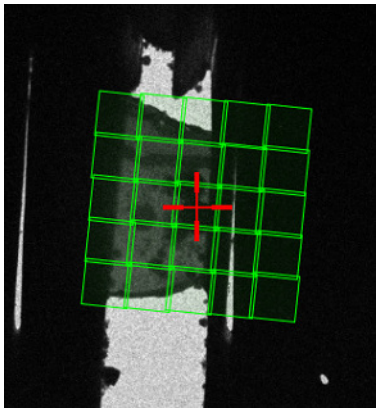


- Specify **Set Tilt**, then select **Set** to move the stage to a different tilt angle.
  On narrow screens, the Tilt Control ribbon must be expanded to make the settings visible.
- The **Rotate Tilt Axis** value is a system property. It specifies the offset of the tilt axis in the Search Map image relative to the physical tilt axis of the stage. The Rotate Tilt Axis value can be calculated with 3D reconstruction software, based on the acquired data from preceding Tilt Series.

4. In the Overview image, right-click on the center of the **area of interest** and either:
   - Select **Move stage here**, then select **Acquisition** > **Acquire Search Map**
   - Select **Move stage here and acquire Search Map**
     Tomography STEM moves the specimen to the selected position, and then acquires and displays a Search Map.

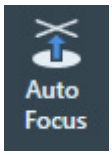


The outlines of the Search Map tiles are visible in the Atlas. If an Overview is available that contains the Search Map area, then the outlines of the Search Map tiles are also visible in that Overview.

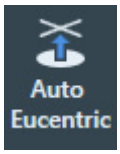


7.3.3 Acquire a Search image

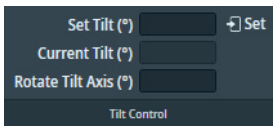
1. In the Overview image or Search Map view, right-click on the **feature of interest** and select **Move Stage Here and Acquire Search Image**
2. (Optional) Select **Auto Focus**



3. If this is the first feature of interest in the current area of interest, then select **Auto Eucentric**

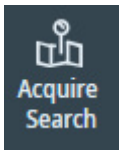


4. Accurately center the feature of interest:
   a. (Optional) Specify the *Tilt Control* settings:



   - Specify **Set Tilt**, then select **Set** to move the stage to a different tilt angle.
     On narrow screens, the Tilt Control ribbon must be expanded to make the settings visible.
   - The **Rotate Tilt Axis** value is a system property. It specifies the offset of the tilt axis in the Search image relative to the physical tilt axis of the stage. The Rotate Tilt Axis value can be calculated with 3D reconstruction software, based on the acquired data from preceding Tilt Series.

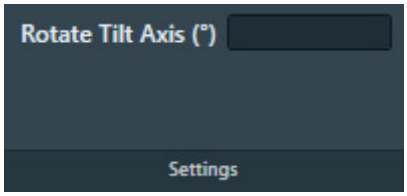
b. Select **Acquire Search**



c. If the feature of interest is not accurately centered in the Search image, then right-click on the **feature of interest** and select **Move Stage Here and Acquire Search Image**

7.3.4 Define the Tilt Series position

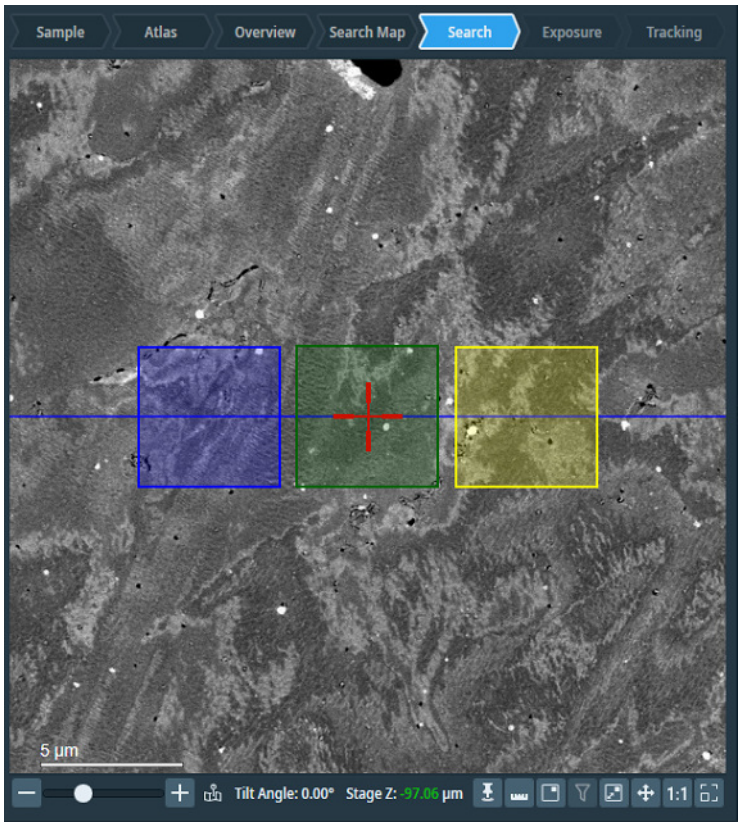
1. (Optional) Specify **Rotate Tilt Axis**



The Rotate Tilt Axis value specifies the offset of the tilt axis in the Search image relative to the physical tilt axis of the stage. The Rotate Tilt Axis value can be calculated with 3D reconstruction software, based on the acquired data from preceding Tilt Series.

2. If *Session Setup > Low Dose* is ticked, then:
   a. (Optional) Right-click in the Search image and select **Lock Focus and Tracking Area**
      When locked, the Focus Area and the Tracking Area stay on top of each other. In most experiments, these areas can be on the exact same position to save time without sacrificing accuracy.

b. Drag the **Template Areas** to their desired locations on the tilt axis.



The size of each Template Area is determined by the FoV value in its related *Acquisition and Optics Preset*.

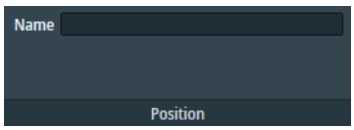
c. Drag the **Focus Area** and the **Tracking Area** to their desired locations on the tilt axis.
It is not possible to assign a location away from the tilt axis.

Note

Do not change *Rotate Tilt Axis* for the purpose of dragging Template Areas to positions away from the current tilt axis.

The purpose of the Rotate Tilt Axis parameter is to compensate for a difference between the tilt axis orientation in Tomography STEM and the physical tilt axis of the stage. If the tilt axis in Tomography STEM is not aligned with the physical tilt axis, then the Z-height of the Focus Area will vary with the tilt angle, which may result in unfocused images of the Exposure Area.

3. Enter the **Name** for the Tilt Series position.



7.3.5 Guidelines for the locations of the Template Areas

The Tracking and Focus Areas can usually be located at the same position, preferably on an area of amorphous carbon foil. For an optimal location of the Template Areas, keep the following guidelines in mind:

- The Tracking and Focus Areas must not overlap with the Exposure Area.
  The accumulated dose of multiple illuminations can cause local damage to the feature of interest.

- It is common practice to put the Focus Area and Tracking Area at the same position. This saves time and has a negligible impact on the result of the focus, tracking and conditioning functions. Use the *Lock Focus and Tracking Area* option to assign the exact same position for the Focus Area and Tracking Area.
- In a Low Dose Tomography session, the Tracking function and Focus function are performed on an area near the feature of interest, so that the feature itself is not exposed. If the feature of interest moves more than half of the field of view after a tilt step, then move the Tracking Area and Focus Area a bit further away from the Exposure Area, so that the feature of interest is not exposed.
- There must be no large structures nearby any of the Template Areas that could cast a shadow on the Template Area when the specimen is tilted.

  For example, avoid positions near:

  - Grid bars.
  - Ice crystals.
  - The edge of a FIB lamella.
- The Z-height of the specimen at the *Focus Area* must be as close as possible to the Z-height of the feature of interest. This is typically the case close to the Exposure Area.

  Avoid positions near damaged or unstable carbon foil.
- The field of view of the *Tracking Area* must contain features with sufficient contrast, so that the Cross-Correlation image shows a well-defined peak.

  See The Image Filters Settings Task on page 33 for suggestions how to tune the filter settings to achieve a well-defined peak in the Cross-Correlation image.

7.4 The Batch Positions task

In the Batch Positions task, multiple positions on the specimen can be prepared for a Data Acquisition run. During the automated Data Acquisition run, Tomography STEM will acquire a Tilt Series at each individual Batch Position.

For easy navigation and accurate target selection, Tomography offers a process flow that uses a stepped sequence of views on the specimen at increasing magnifications. This flow helps to accurately identify multiple features of interest, and to prepare a Tilt Series for each individual feature. In Batch-mode, a prepared Tilt Series for a feature of interest is called a *Batch Position*.

Note You can view the Atlas you have selected and loaded, but you cannot change this selection here. If you need to change the selected Atlas, you must do so in the Search Maps task.

To prepare a Tilt Series for a feature of interest, perform the following actions.

1. Acquire an *Overview* of the area of interest.
   The Overview has a wide field of view. On a slab-like specimen, this is typically a single Grid Square. In the Overview view it is possible to:
   - Acquire a *Search Map* for easier and more accurate navigation and target selection.
   - Acquire a *Search* image at the location of a single feature of interest.
2. Acquire a *Search Map* of the area of interest; or use a previously-acquired Search Map.
   A Search Map has a smaller field of view at a higher accuracy than the Overview image. It is a matrix of Search images that is assembled like an Atlas. The tiles of a Search Map are marked in the Atlas and in the Overview. The currently active Search Map has green tile outlines, all other Search Maps have yellow tile outlines.
3. Acquire a *Search* image with the feature of interest in the center.
   The Search view is used to define the locations of the Template Areas and the Tilt Series settings.
   For single positions, this step is mandatory.
   For batch positions, this step is not mandatory.
4. Define the Template Area locations and the Tilt Series settings.
5. Add the new Batch Position to the Batch Positions list.
6. (Optional) Edit, refine, re-order and/or delete the defined Batch Positions.

The sections below provide detailed information and instructions for the actions above.

7.4.1 Add a Batch Position

To add a Batch Position, the following workflows are possible:

- **Acquire an Overview image**
  > Acquire a *Search Map* image
  > Acquire a *Search* image
  > Define and add a *Batch Position*.
- **Acquire an Overview image**
  > Acquire a *Search* image
  > Define and add a *Batch Position*.
- **Recommended workflow**:
  Using Atlas or Overview image, define search Maps (see Manage Search Maps on page 63)
  > Acquire *Search Maps in a Queue (See* Acquire a single Search Map on page 64)
  > Define and add a *Batch Position on Search Maps.*

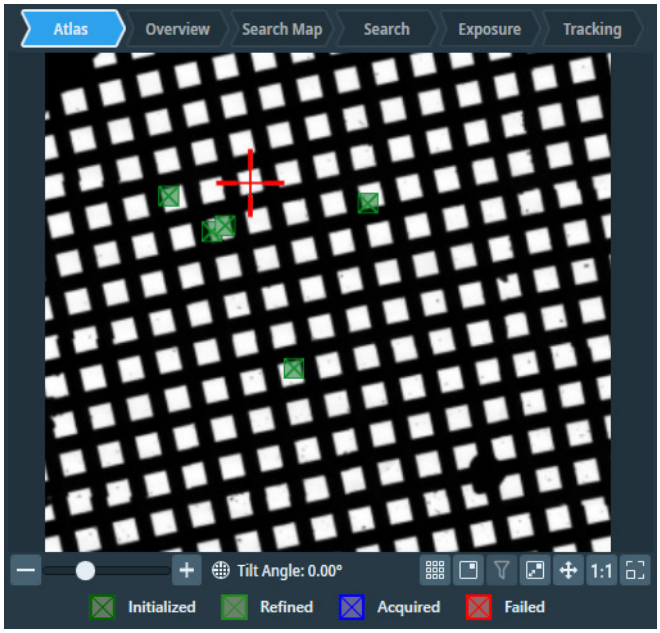
7.4.1.1 Acquire an Overview image

An Overview image can be acquired in the context of an Atlas of the specimen. How to do this is described in the steps below.
It is also possible to acquire an Overview image without an Atlas of the specimen. How to do this is also described below.

7.4.1.1.1 Acquire an Overview with using an Atlas

To acquire an Overview image *with* the use of an Atlas:

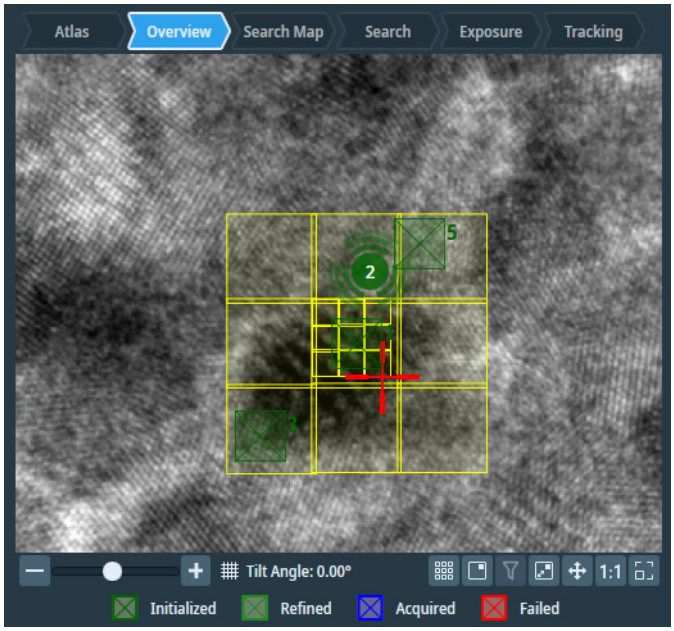
1. Select the **Atlas** view.



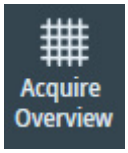
- If no Atlas is available yet, then go to *Atlas* > *Screening* task for the acquisition of a new Atlas. For instructions how to acquire an Atlas for the currently loaded specimen, see: Atlas Tab on page 50.

If an Atlas is available for the currently loaded specimen, then Tomography displays the Atlas.

2. Right-click in **the center of the area of interest**,
   then select **Move Stage Here and Acquire Overview Image**
   Tomography STEM moves the specimen to the selected position, and then acquires and displays an Overview image.



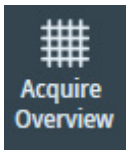
3. If the Overview image does not show the entire area of interest, then:
   a. In the current Overview image, right-click on the **center of the area of interest** and select **Move stage here**
   b. Select **Acquire Overview**



7.4.1.1.2 Acquire an Overview without using an Atlas

To acquire an Overview image *without* using an Atlas:

1. In **Velox**, start a *live image view*.
2. Use the **handpanels** to move the **area of interest** to the center of the live image.
3. Select **Acquire Overview**



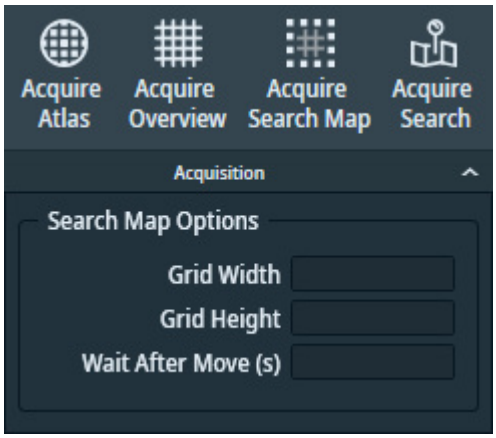
4. If the Overview image does not show the entire area of interest, then:
   a. In the current Overview image, right-click on the **center of the area of interest** and select **Move stage here**
   b. Select **Acquire Overview** again.

7.4.1.2 Acquire a Search Map and/or a Search image

7.4.1.2.1 Acquire a Search Map

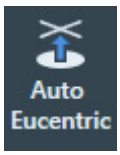
To acquire a Search Map:

1. In the **Acquisition** > **Search Map Options**:

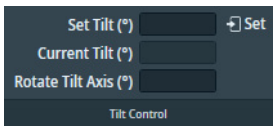


   a. Specify the **Grid Width** (X direction) and **Grid Height** (Y direction).
   b. Specify **Wait After Move (s)**: the time between the stage move and the acquisition of a search map image.
   c. Specify **Queue at Tilt (˚)**: the Alpha tilt used for Search Map acquisition.

2. If this is the first Search Map that will be acquired in this field of view, then select **Auto Eucentric**

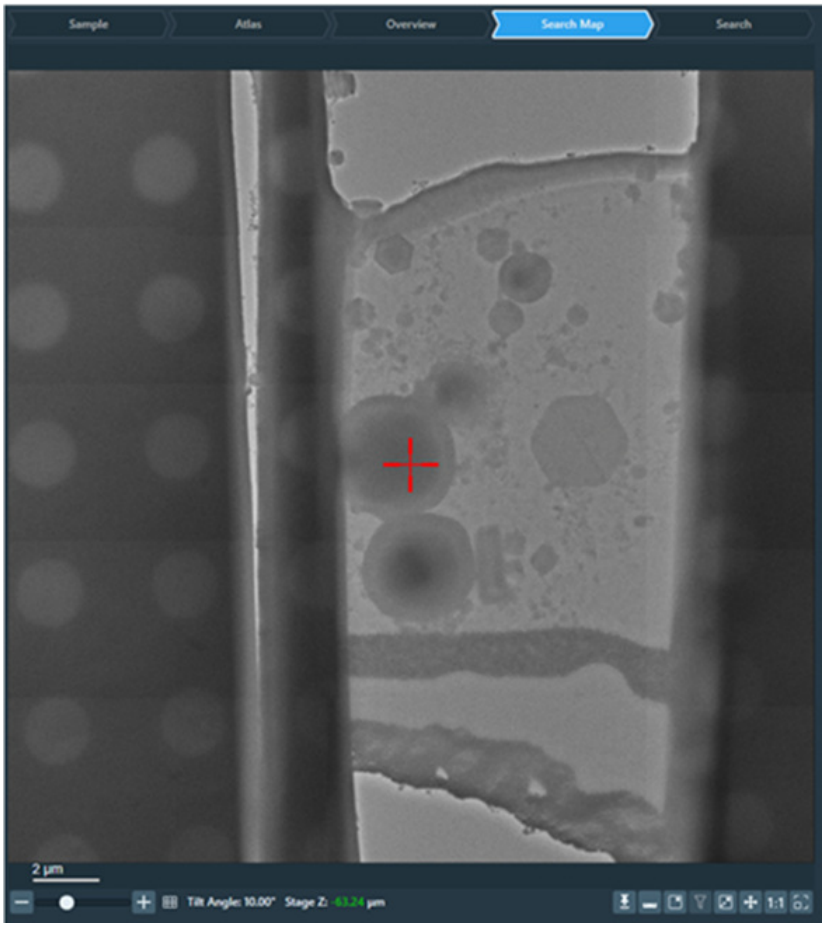


3. (Optional) Specify the *Tilt Control* settings:

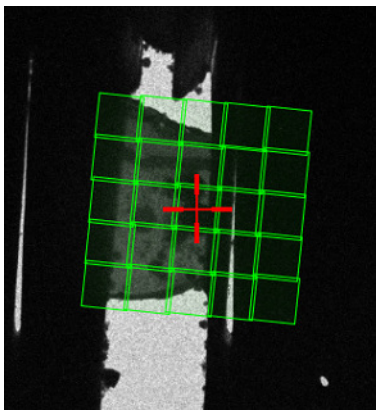


   - Specify **Set Tilt**, then select **Set** to move the stage to a different tilt angle.
     On narrow screens, the Tilt Control ribbon must be expanded to make the settings visible.
   - The **Rotate Tilt Axis** value is a system property. It specifies the offset of the tilt axis in the Search Map image relative to the physical tilt axis of the stage. The Rotate Tilt Axis value can be calculated with 3D reconstruction software, based on the acquired data from preceding Tilt Series.

4. In the Overview image, right-click on the center of the **area of interest** and either:
   - Select **Move stage here**, then select **Acquisition** > **Acquire Search Map**
   - Select **Move stage here and acquire Search Map**
     Tomography STEM moves the specimen to the selected position, and then acquires and displays a Search Map.

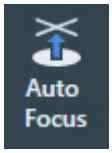


The outlines of the Search Map tiles are visible in the Atlas. If an Overview is available that contains the Search Map area, then the outlines of the Search Map tiles are also visible in that Overview.

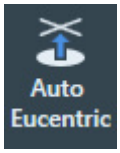


7.4.1.2.2 Acquire a Search image

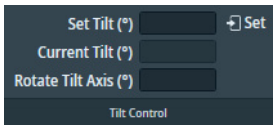
1. In the Overview image or Search Map view, right-click on the **feature of interest** and select **Move Stage Here and Acquire Search Image**
2. (Optional) Select **Auto Focus**



3. If this is the first feature of interest in the current area of interest, then select **Auto Eucentric**

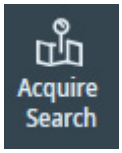


4. Accurately center the feature of interest:
   a. (Optional) Specify the *Tilt Control* settings:



   - Specify **Set Tilt**, then select **Set** to move the stage to a different tilt angle.
     On narrow screens, the Tilt Control ribbon must be expanded to make the settings visible.
   - The **Rotate Tilt Axis** value is a system property. It specifies the offset of the tilt axis in the Search image relative to the physical tilt axis of the stage. The Rotate Tilt Axis value can be calculated with 3D reconstruction software, based on the acquired data from preceding Tilt Series.

   b. Select **Acquire Search**

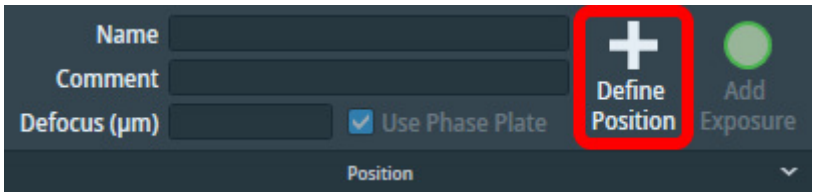


   c. If the feature of interest is not accurately centered in the Search image, then right-click on the **feature of interest** and select **Move Stage Here and Acquire Search Image**

7.4.1.3 Define and add the Batch Position

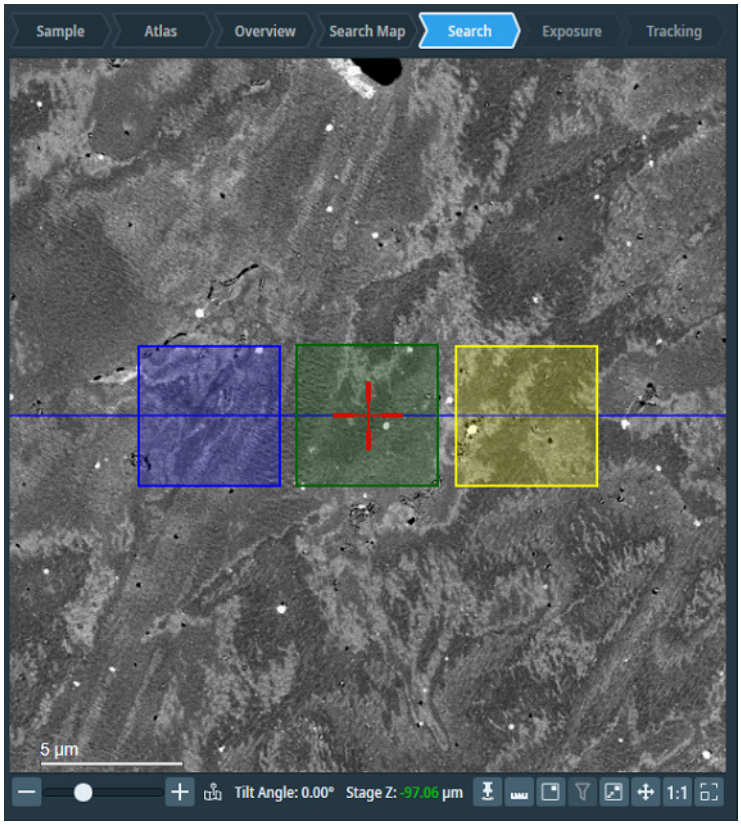
A new Batch Position can be defined in a *Search Map* image or in a *Search* image.

1. Determine the location of the new Batch Position:
   - In a *Search* image, select **Define Position**



   - In a *Search Map* image, right-click at the **exact location in the Search Map** where the new Batch Position must be created, and either:
     - Select **Define new position** from the context menu.
     - Select **Move stage here** from the context menu, then select **Position** > **Define Position** in the ribbon bar.

2. Define the locations for the Template Areas:
   a. (Optional) Right-click in the Search image and select **Lock Focus and Tracking Area**
      When locked, the Focus Area and the Tracking Area stay on top of each other. In most experiments, these areas can be on the exact same position to save time without sacrificing accuracy.
   b. Drag the **Template Areas** to their desired locations on the tilt axis.



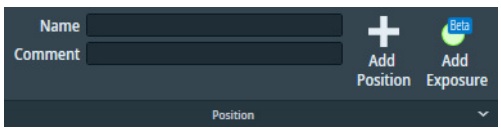
      The default template is copied from the most recently defined Batch Position.
      For guidelines, see: Guidelines for the locations of the Template Areas.
      The size of each Template Area is determined by the FoV value in its related *Acquisition and Optics Preset*.
   c. Drag the **Focus Area** and the **Tracking Area** to their desired locations on the tilt axis.
      It is not possible to assign a location away from the tilt axis.

Note

Do not change *Rotate Tilt Axis* for the purpose of dragging Template Areas to positions away from the current tilt axis.

The purpose of the Rotate Tilt Axis parameter is to compensate for a difference between the tilt axis orientation in Tomography STEM and the physical tilt axis of the stage. If the tilt axis in Tomography STEM is not aligned with the physical tilt axis, then the Z-height of the Focus Area will vary with the tilt angle, which may result in unfocused images of the Exposure Area.

3. Specify the **Name** and (optional) **Comment** for the Batch Position.

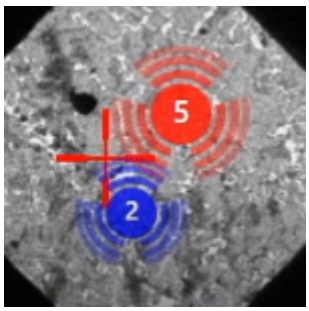
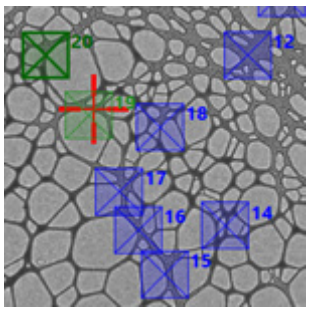
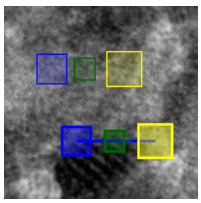


4. (Optional) Select **Add Exposure (Beta)** to add an additional Exposure Area to the template.
   This is Beta functionality. For feedback and questions, please contact tomo5@thermofisher.com
   - The location of the additional Exposure Area is restricted the Tilt Axis.
   - Because additional Exposure Areas are part of the Batch Position template, the next new Batch Position will also have more than one Exposure Area.

   It is also possible to right-click in the image and then select **Add new exposure area**
5. (Optional) To remove an additional Exposure Area.
   right-click on the Exposure Area and select **Remove Exposure Area**
   It is not possible to remove the primary Exposure Area.
6. Select **Add Position**
   Tomography STEM adds the new Batch Position to the Batch Positions List.
   The Start Angle depends on how the Batch Position is created:
   - In a Search Map: the Start Angle is the Tilt Angle that is displayed at the bottom of the Search Map image.
   - In a Search image: the Start Angle is the current A-tilt position.

7.4.2 Manage the Batch Positions

7.4.2.1 Description of the position markers in the Image Display

Marker type	Description
	*Only used in the Batch Positions task*. Location of multiple Batch Positions. The color of the marker indicates the status of the Batch Positions. Hover on the Group marker with the mouse to display a tooltip with the Batch Position IDs and statuses of the grouped Batch Positions.
	Location and status of a Tilt Series. In Batch-mode, the label shows the Batch Position ID.
	Location of the Template Areas.

The displayed marker type can change depending on the view and the zoom level. The meaning of the color of the marker is described in the legend at the bottom of the Image Display.

Depending on the availability of acquired images, the highlighted Batch Position in the Image Display and in the Batch Positions list is the same:

- When a marker is selected in the Image Display, then the corresponding row in the Batch Positions list is also highlighted.
- When a row is selected in the Batch Positions list, then the Image Display highlights the corresponding marker. If the location of a Batch Position is outside the currently displayed field of view, then the Image Display switches to a same-type view in which the selected Batch Position is visible. The Image Display will not switch to a different type of view.

For example: in the Image Display, the currently selected Batch Position is highlighted in a Search Map view. In the Batch Positions list, a different Batch Position is selected that is not visible in the currently displayed Search Map. If a different Search Map is available that contains the selected Batch Position, then that Search Map will be displayed. If such a Search Map is not available, then the Image Display will not follow the selection in the Batch Positions list.

7.4.2.2 Edit a single Batch Position

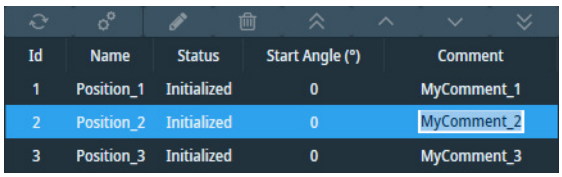
Of all Batch Position properties, only *Comment* can be edited after the Batch Position is added to the Batch Positions list. All other properties are fixed, or will be updated automatically by Tomography STEM.

To edit the Comment of a Batch Position, either:

- Double-click on the **Batch Position** > **Comment** and edit the text, then select **Enter**
- Follow the steps below:

1. In the Image Display or in the Batch Positions List,
   select the **Batch Position** that must be edited.
2. Select **Edit**

The Comment is selected and editable.



Id	Name	Status	Start Angle (°)	Comment
1	Position_1	Initialized	0	MyComment_1
2	Position_2	Initialized	0	MyComment_2
3	Position_3	Initialized	0	MyComment_3

3. Edit the **Comment** and select **Enter.**
4. (Optional) Select **Edit** to update the template.

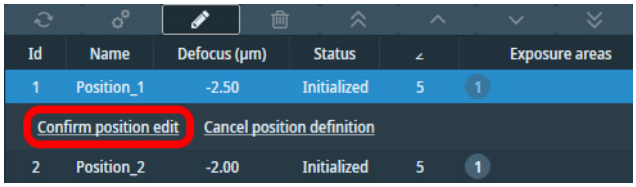
The following updates and adjustments are now available:

a. Add or remove an Exposure Area.
   - It is possible to move the main/primary Exposure Area only on the Search Maps, not on Search images.

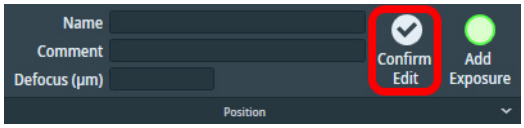
b. Adjust the locations of the template areas.

To save the changes, either:

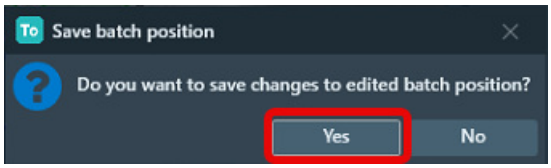
c. Select **Confirm position edit.**



d. Select **Confirm Edit.**



e. Click away from the current image (i.e. open an Overview image) and select **Yes**.



To cancel the template adjustments and revert to the stored template area positions, either:

f. Select **Cancel position definition.**

g. Select **Edit** again.

h. Click away from the current image (i.e. open an Overview image) and select **No.**

7.4.2.3 Refine a single Batch Position

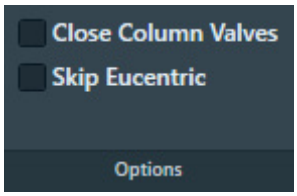
The Refine function:

- Adjusts the Batch Position to compensate for any mechanical play or hysteresis on the X, Y and Alpha axis of the stage.
- Acquires focused reference images for increased centering accuracy during the Data Acquisition run.

If the Refine action is not performed before the Data Acquisition run is started, then Tomography STEM refines the Batch Positions during the Data Acquisition run.

To refine a single Batch Position:

1. Select the *Batch Position* that will be refined.
   Either:
   - If the desired Batch Position is visible in the Image Display,
     then select the desired **Image Display** > **Batch Position**
     Tomography STEM automatically highlights the selected Batch Position in the Batch Positions list.
   - In the **Batch Positions** List, select the desired **Batch Position**
     In the Image Display, the marker for the selected Batch Position is highlighted. If necessary, Tomography STEM first switches to a different Overview or Search image to display the Batch Position.
2. If *Auto Function* > *Auto Eucentric* has already been executed for this Grid Square,
   then tick **Skip Eucentric**

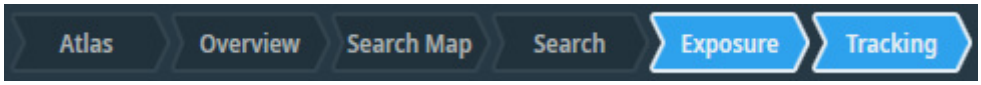
   

   By default, the Refine Batch Positions procedure executes the *Auto-eucentric height* function for each Batch Position. Within the same Grid Square, the Z-height of the features of interest does not change significantly. To speed up refinement, re-adjusting the eucentric height can be skipped.
3. Either:
   - Right-click and select **Refine**
   - Select the **Refine** button above the Batch Positions List.

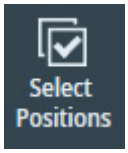
   After the Refine action has completed successfully, the status of the Batch Position changes from *Initialized* to *Refined*.

4. (Optional) Above the Image Display area, select **Exposure** and/or select **Tracking** to inspect the acquired Exposure and Tracking images for the refined Batch Position.

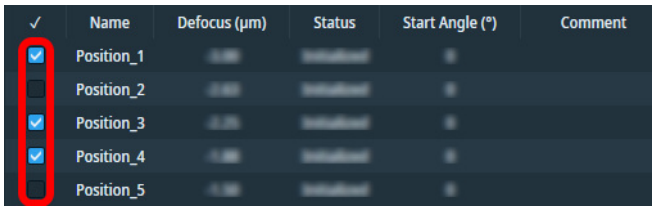


7.4.2.4 Refine multiple Batch Positions

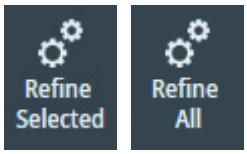
1. (Optional) Select a subset of Batch Positions:
   a. Select **Select Positions**



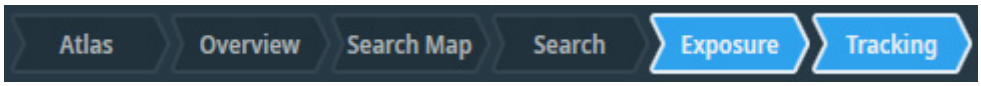
   b. In the Batch Positions list, tick the desired **Batch Positions**



2. Select **Refine Selected** / **Refine All**



3. (Optional) Inspect the Exposure and Tracking images for each refined Batch Position.
   a. In the **Batch Positions** list, select the desired **Batch Position**
   Tomography loads the image view for the selected Batch Position.
   b. Above the Image Display area, select **Exposure** and/or select **Tracking** to inspect the acquired Exposure and Tracking images for the refined Batch Position.



7.4.2.5 Delete a single Batch Position

It is not possible to delete a Batch Position for which a Tilt Series has been acquired.

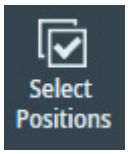
To delete a single Batch Position:

1. In the Image Display or in the Batch Positions List, select the **Batch Position** that must be deleted.
2. Either:
   - Right-click and select **Delete**
   - Select the **Delete** button above the Batch Positions List column header.

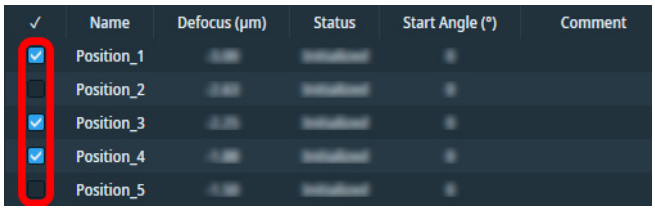
Note There is no Undo function. It is not possible to retrieve deleted Batch Positions.

7.4.2.6 Delete multiple Batch Positions

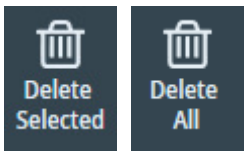
1. (Optional) Select a subset of Batch Positions:
   a. Select **Select Positions**



   b. In the Batch Positions list, tick the desired **Batch Positions**



2. Select **Delete Selected** / **Delete All**



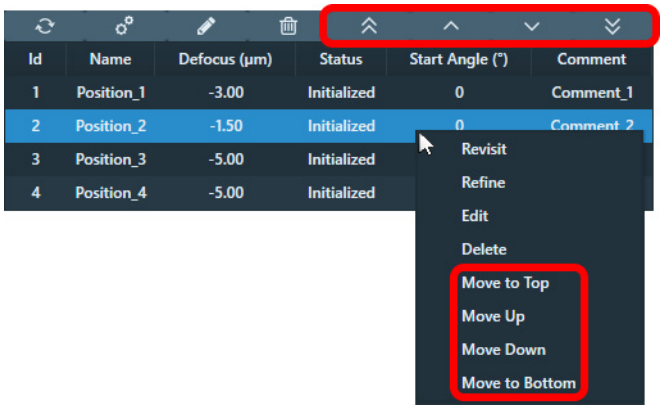
Note There is no Undo function. It is not possible to retrieve deleted Batch Positions.

7.4.2.7 Change the processing order of the Batch Positions

During the Automated Acquisition run, the Batch Positions in the list are processed in top-to-bottom order. By default, this is the order in which the Batch Positons have been added to the list. If a Template Area overlaps with the Exposure Area of a nearby Batch Position that is lower on the list, then it is advised to adjust the processing order so that the Exposure Area is not illuminated before data is acquired from it.

To change the processing order, either:

- Select a **Batch Position**, then select a **re-ordering action** from the toolbar above the Batch Positions List.
- Right-click on a Batch Position and select a **re-ordering action** from the context menu.



7.4.3 Define and Manage Batch Positions During Data Acquisition

Once the acquisition of Batch Positions is started in Data Acquisition task, additional Batch Positions can be defined and edited. These actions are possible during Data Acquisition:

1. Defining Batch Positions.
2. Editing positions of template areas which are not Acquired or currently in progress.
3. Changing Comment of Batch Positions.
4. Deleting Batch Positions which are not Acquired or currently in progress.
5. Using the Select Positions button.
6. Using the Delete All button.

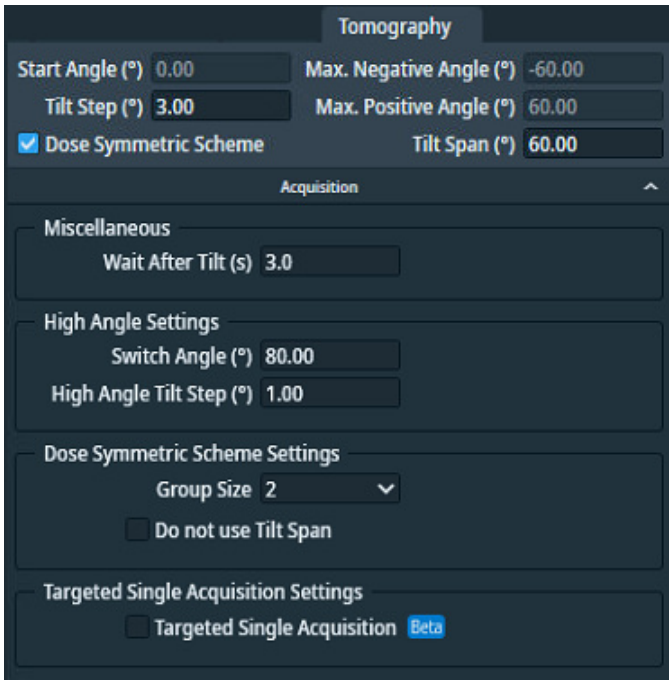
Changing the acquisition order of batch positions is not permitted.

Newly defined Batch Positions are automatically added to the list and acquired according to the defined order. Selecting Batch Positions using the Select Positions button does not effect which Batch Positions will be acquired, if Data Acquisition is already in progress.

7.5 The Data Acquisition task

7.5.1 Description of the Acquisition Settings

The Acquisition settings define the tilt scheme that is executed during a Tilt Series acquisition. The basic settings are available in the main ribbon bar. Select the ribbon bar title to display the advanced settings.



Start Angle

The tilt axis position at which the first image of the Tilt Series will be acquired.

Tilt Step

The tilt axis rotation between consecutive Tilt Series images.

Dose Symmetric Scheme

A Dose Symmetric Scheme is a sequence of angles that alternate between the positive and negative side in increasing tilt angles. This way, the accumulated dose at low Tilt Angles is minimized.

Targeted Single Acquisition

When activated, automatic acquisition is executed only on the specified angle. This type of acquisition is useful on samples with sparse distribution of particles. This type of acquisition is available only in Batch mode.

This feature is in Beta. For feedback and questions, please contact tomo5@thermofisher.com.

Max. Negative Angle, Max. Positive Angle

The outer most tilt axis positions at which a Tilt Series image will be acquired.

On a system with TEM Server 7.10 or later, the *Max. Negative Angle* and *Max. Positive Angle* are validated against the actual maximum tilt angle of the stage.

Tilt Span

Only for Dose Symmetric Scheme

The maximum deviation from the Start Angle. Tomography automatically calculates the Max. Negative Angle and Max. Positive Angle, based on the Start Angle and the Tilt Span.

Group Size

Only for Dose Symmetric Scheme

Select the number of tilts desired in a single sweep by setting the Group Size to 2, 3, or 4.

File Name

Filename of the MRC image to which the acquired Tilt Series images are recorded. This MRC file is saved in the **Session Setup** > **Storage Folder**

Wait After Tilt

After each Tilt Step, Tomography waits the specified amount of time to let any mechanical tension or drift stabilize before executing the Template or acquiring an image.

Do not use Tilt Span

Only for Dose Symmetric Scheme

Disables *Tilt Span* and the automatic calculation of the Max. Negative Angle and Max. Positive Angle. For a Dose Symmetric Scheme, the Max. Negative Angle and Max. Positive Angle must be symmetric relative to the *Start Angle*.

Switch Angle and **High Angle Tilt Step**

When the tilt axis rotation exceeds the Switch Angle, then the rotation increment for the next Tilt Series image changes from the Tilt Step value to the High Angle Tilt Step.

- For experiments with a *slab-like specimen*, the Switch Angle value is typically set at around 40º.
- For experiments with a *rod-like* specimen, the Switch Angle can be specified equal to largest value of *Max. Positive Angle* and *Max. Negative Angle*.

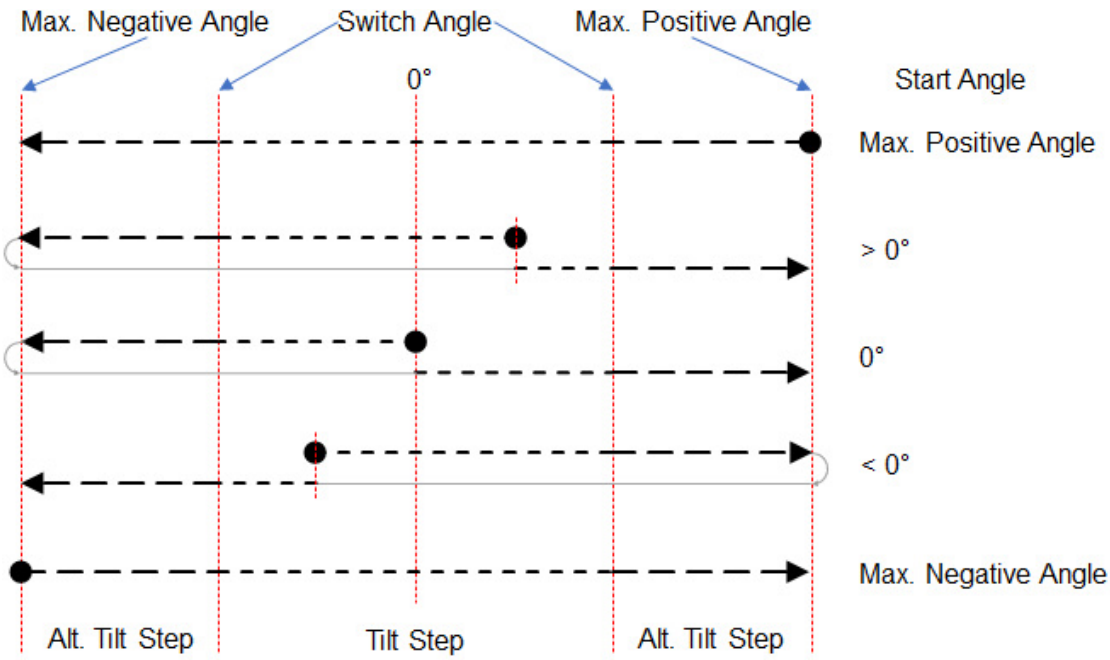
7.5.1.1 Tilt sequences when Dose Symmetric Scheme is not selected

If Dose Symmetric Scheme is cleared, then a Tilt Series is typically acquired in two branches:

- The first branch always passes through 0° tilt.

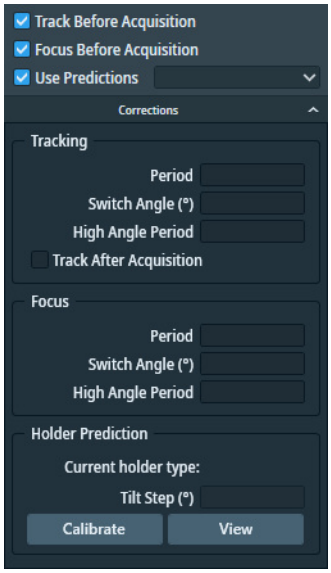
  After the first branch is acquired, the specimen returns to the Start Angle to acquire the second branch.

  If the Start Angle is 0°, then the first branch starts in the negative direction.
- If the Start Angle is equal to the Max. Positive Angle or Max. Negative Angle, then there is no second branch. All tilt angles are covered by the first branch. This is also referred to as a *Continuous Scheme*.


Max. Negative Angle
Switch Angle
Max. Positive Angle
0°
Start Angle
Max. Positive Angle
> 0°
0°
< 0°
Max. Negative Angle
Alt. Tilt Step
Tilt Step
Alt. Tilt Step

7.5.2 Description of the Corrections Settings

The basic correction settings are available in the main ribbon bar. Select the ribbon bar title to display the detailed settings.



Track Before Acquisition

The Tracking Before Acquisition function executes the Tracking function before a Tilt Series image is acquired. The Tracking > Period value determines how often the Tracking function is executed.

In a Low Dose experiment the Tracking function is performed on the Tracking Area.

Focus Before Acquisition

The Focus Before Acquisition function executes the Autofocus function before a Tilt Series image is acquired. The *Focus > Period* value determines how often the Autofocus function is executed.

In a Low Dose experiment the AutoFocus function is performed on the Focus Area.

Use Predictions

If the *Holder Prediction Calibration* has been performed, then Tomography STEM applies a calibrated XY and/or Focus correction before executing the Tracking and/or Autofocus functions.

- The XY correction is performed by applying an image shift.
- The Focus correction is applied by adjusting the Objective lens value.

Predictions improves the accuracy and reliability of the Focus and Tracking action, and does not add a time penalty.

Period

The number of tilt steps between consecutive Autofocus or Tracking function executions.

Switch Angle

The tilt angle (positive and negative) at which the Autofocus or Tracking execution interval toggles to from *Period* to *High Angle Period* or vice versa.

High Angle Period

The number of tilt steps between consecutive Autofocus or Tracking function executions, when the tilt axis position is beyond the *Switch Angle*.

Track After Acquisition

After an image acquisition, Tomography STEM measures the tracking error in the acquired image and calculates a corresponding correction for the next image acquisition in the Tilt Series.

The Tracking After Acquisition function is enabled by default. It does not require an additional image acquisition and does not take noticeable extra time. In Low Dose experiments, Tracking After Acquisition is a necessity. The specimens are typically not flat, so the movement of the Tracking Area may not accurately represent the movement of the feature of interest.

Holder Prediction:

- **Current Holder Type**
  The type of specimen holder that is currently inserted in the stage.
  Systems with an Autoloader have a fixed holder.
- **Tilt Step**
  The interval at which images are acquired during the Holder Prediction calibration.
- **Calibrate**
  Start the calibration procedure. See Perform the Holder Calibration on page 101 for instructions.
- **View**
  View the most recent calibration result for the current tilt scheme. It is also possible to manually select a calibration result file from a different tilt scheme.

7.5.2.1 Working principles of the Track Before Acquisition and the Track After Acquisition functions

The *Track Before Acquisition* function acquires a dedicated image after the specimen is tilted, before the Tilt Series image is acquired. The function then measures the tracking error and applies a corresponding corrective image shift before the Tilt Series image is acquired.

In a Low Dose experiment, the tracking image is acquired at a dedicated Tracking Area. The Tracking Area is located on the virtual tilt axis, at an offset from the Exposure Area.

The *Track After Acquisition* function uses the acquired Tilt Series image to measure the tracking error in the X and Y directions. The result is used:

- To cross-check the correction that is applied by the *Track Before Acquisition* function.
- To feed-forward an initial correction to the next tilt angle, so that the overall tracking correction performance becomes more accurate and reliable.

The use of the acquired Tilt Series image for calculation of the tracking correction has these advantages:

- The correction is based on a measurement at the Exposure Area itself instead of the nearby Tracking Area.
  - Although the Tracking Area is nearby, the appearance and condition of the specimen at the Tracking Area may be slightly different from the Exposure Area. This may cause small but noticeable inaccuracies.
  - The virtual tilt axis in the Search image can have a small offset relative to the physical tilt axis. If the *Rotate Tilt Axis* value is not accurate, then the measured shift of the Tracking Area may be slightly different than the actual shift of the Exposure Area. These differences typically become larger as the tilt angle increases.
- There is no tracking image acquisition prior to the Tilt Series image acquisition, so:
  - The specimen is not exposed to an additional dose.
  - No additional acquisition time is spent.

The most prominent drawback of the *Track After Acquisition* method is that the specimen is tilted to the next tilt angle *after* the correction has been calculated. If *Tracking Before Acquisition* is not enabled, then any incidental shift that is caused by the tilt step cannot be corrected.

7.5.2.2 Recommendations for the Tracking, Focus and Holder Prediction Corrections

The *Tracking Before Acquisition* function measures the tracking error (TE_{before}) at the Tracking Area, before the Tilt Series image is acquired.
The *Tracking After Acquisition* function measures the tracking error (TE_{after}) at the Exposure Area, after the Tilt Series image has been acquired.

These tracking functions complement each other as follows:

1. After a Tilt Series image is acquired, the *Tracking After Acquisition* function measures TE_{after}.
   If a TE_{before} correction has already been applied, then TE_{after} is the difference between the shift of the feature of interest and the Tracking Area.
2. The specimen moves to the next tilt angle.
3. The *Tracking Before Acquisition* function measures TE_{before} on the Tracking Area.
4. Tomography STEM applies a tracking correction for TE_{before} and adds a correction that corresponds to TE_{after} at the preceding tilt angle.

For the highest tracking accuracy:

- Tick **Tracking Before Acquisition** and **Tracking After Acquisition**
  Specify **Period**: **1**
- Tick **Focus Before Acquisition**
  Specify **Period**: **1**
- If the *Holder Prediction* calibration is completed, then tick **Predictions** and select the **XY and Focus** mode.
  See Perform the Holder Calibration on page 101 for instructions.

For higher throughput rates:

- Increase the **Period** values for *Tracking* and/or *Focus*
- If the *Holder Prediction* calibration is completed, then tick **Predictions** and select the **XY and Focus** mode.
  See Perform the Holder Calibration on page 101 for instructions.

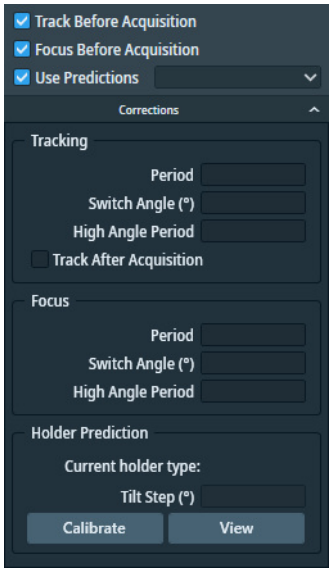
7.5.2.3 (Optional) Limit the tilt range for the Track Before Acquisition function

For Slab-like specimens, the time that is required to complete a Tilt Series can be shortened by limiting the tilt angle range in which *Track Before Acquisition* is active

- For small tilt angles, *Track Before Acquisition* is necessary to accurately correct for specimen shift. At small angles, specimen shift directly affects the achievable reconstructed volume.
  - If *Session Setup > Low Dose* is cleared, then Tomography automatically skips the *Track After Acquisition* function in the tilt range where *Track Before Acquisition* is active.
    In experiments that are *not* marked as Low Dose, there is no need to place a dedicated Tracking Area at an offset from the Exposure Area in the Template. The *Track Before Acquisition* function can acquire its tracking image on the Exposure Area. Using the acquired Tilt Series image from the same Exposure Area to cross-check the applied correction does not add significant value.
  - If *Low Dose* is ticked, then both tracking correction methods are active.
- For large tilt angles, the *Track After Acquisition* is sufficient. The effective field of view is already quite large due to foreshortening, so a small residual shift has a limited impact on the reconstructed volume. Spending time on *Track Before Acquisition* would not result in a significantly better reconstructed volume.

Follow the steps below to limit the tilt angle range in which *Track Before Acquisition* is active. It is not possible to limit the tilt angle range in which the *Tracking After Acquisition* function is active.

1. Tick **Corrections** > **Track Before Acquisition**
2. Select the **Corrections ribbon bar title** to display the detailed settings.



3. In the **Tracking** section:
   a. Specify the **Period**
      This is typically **1**
   b. Specify the **Switch Angle**
   c. Specify a very large value for **High Angle Period**, for example **100**
      By specifying a value that is larger than the number of tilt steps between the **Switch Angle** and the **Max. Negative Angle** or **Max. Positive Angle** values, the **Track Before Acquisition** function will not be triggered.
   d. Verify that **Track After Acquisition** is ticked.
      If the tilt range for the *Track Before Acquisition* function is limited and *Track After Acquisition* is not ticked, then there will be no tracking correction at all outside the *High Tilt Angle* range. Execution of the *Track After Acquisition* function is independent of the *Switch Angle* and *High Angle Period* values.

7.5.2.4 Perform the Holder Calibration

The Holder Calibration is specific for the system mode. The results from a Holder calibration in TEM mode is not valid for STEM mode, and vice versa. If Tomography is used for TEM and for STEM experiments, then the Holder Calibrations must be performed for TEM mode and for STEM mode.

Within a system mode, Holder Calibrations are specific for:

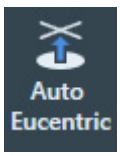
- All non-symmetric tilt schemes: the Start Angle and the holder type.
- Dose Symmetric Scheme: the Start Angle, the Tilt Step and the holder type.

The Holder Calibration is independent of the selected Prediction mode *XY*, *Focus* or *XY and Focus*, it always includes the *XY* as well as the *Focus* values.

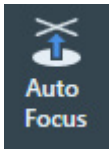
The Holder Calibration is not available when *Session Setup* > *Batch* is ticked.

To perform the Holder Calibration, follow the steps below:

1. Load the **Combined Test Specimen (Agar S142)**
   This specimen is a lacey carbon foil with gold particles and graphitized carbon. This specimen is delivered with the microscope.
2. Move the **specimen** to an area that has:
   - Flat, stable carbon foil.
   - Gold particles or other contrast-rich features that are easily recognizable at the Tracking Preset magnification.
3. Select **Auto Eucentric**

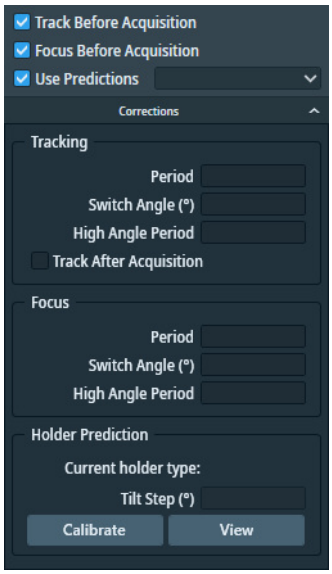


4. Select **Auto Focus**

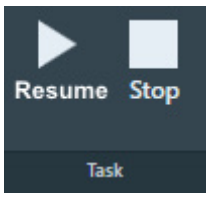


5. Set the **Max. Negative Angle** and **Max. Positive Angle** to the widest usable range.

6. In **Corrections** > **Holder Prediction**, specify **Tilt Step**: **5.00**
   If *Acquisition* > *Dose Symmetric Scheme* is selected, then Holder Calibration uses the same Tilt Step as specified in the Acquisition settings.



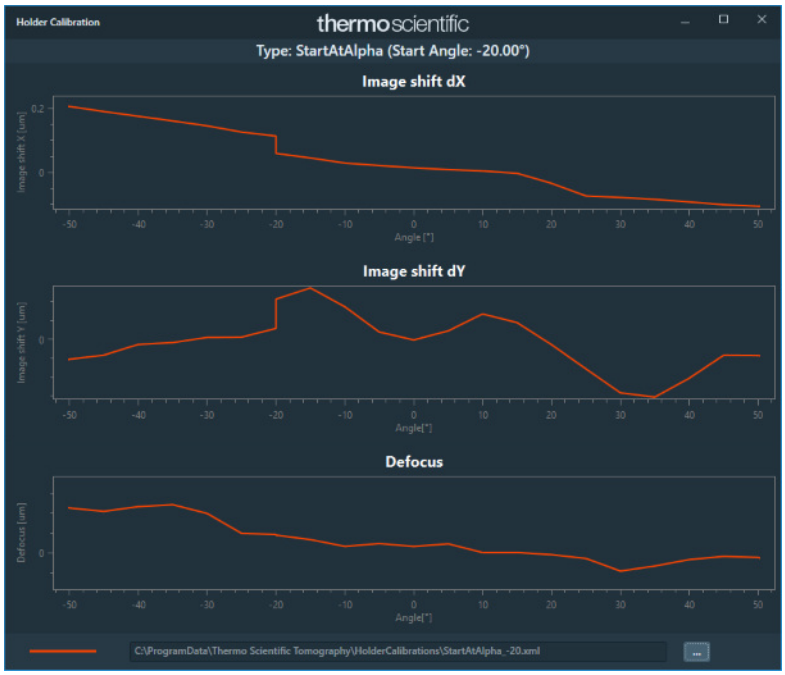
7. Select **Calibrate**
8. Monitor the progress and intermediate results in the **Status** side panel.
   The procedure may request to perform manual actions. If so, perform the requested action and select **Resume**



After the calibration is completed, Tomography STEM automatically stores the results in `C:\ProgramData\Thermo Scientific Tomography\HolderCalibrations` as a TXT file, a CSV file, and an XML file. If desired, use a spreadsheet application to visualize the calibration result in a graph.

7.5.2.5 View the Holder Calibration results

1. Select the **Correction ribbon title** to show the detailed Correction settings.
2. In the **Holder Prediction** section, select **View**
   The *Holder Calibration* viewer appears. By default, the Holder Calibration viewer opens the most recent calibration results that match with the current tilt scheme.



   If there are no matching calibration results, then the graphs are empty.
3. (Optional) View the Holder Calibration results for a different tilt scheme:
   a. Select the file picker

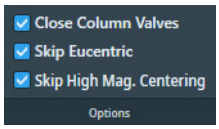
   

   b. Select the **XML file** for the desired Holder Calibration, then select **Open**

For a regular user or supervisor, Tomography STEM shows only one Holder Calibration file.

For Thermo Fisher Scientific engineers, the Holder Calibration viewer can open up to three Holder Calibration files, so the results can be compared.

7.5.3 Description of the Options



The Options section of the Ribbon Bar provides the following functionalities.

Close Col. Valves
Closes the Column Valves after the Automated Acquisition run is completed.

Skip Eucentric
Only available when Session Setup > Batch is ticked.
When ticked, Tomography STEM skips the Auto Eucentric function during Batch Position refinement.

Skip High Mag. Centering
Only visible when Session Setup > Batch and Low Dose are cleared.

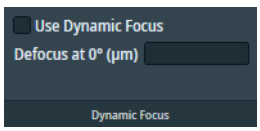
The automated centering procedure at the start of a new Tilt Series is as follows:

1. Center with the stage at 0 degrees at Search magnification.
2. Center with Beam Shift at the Start Angle at Search magnification.
3. Center with Beam Shift at the Start Angle at Tracking magnification.
   When *Skip High Mag. Centering* is ticked, this action is skipped. The availability and default value of the *Skip High Mag. Centering* option depend on the configured Tilt Scheme.

Stop / Reduce Emission
Only available on systems with a thermionic filament.
Not displayed in the screen image above.

Switches off the emission, or brings the electron source to a safe standby state after the Automated Acquisition run is completed.

7.5.4 Description of the Dynamic Focus settings



Use Dynamic Focus
Only available for slab-like specimens.
When a slab-like specimen is tilted, the areas that are farther away from the tilt axis go out of focus. The Dynamic Focus function adjusts the focus during the acquisition, so that a wider area around the tilt axis is accurately focused. This helps to create a higher quality 3D reconstruction. For instructions how to calibrate the Dynamic Focus functionality, see: The Dynamic Focus task on page 45.

Defocus at 0°
Specifies the focus value at 0° tilt.

7.5.5 Description of the Additional Detectors options

If there are multiple STEM detectors available on the system, then the Additional Detectors options ribbon bar shows the STEM detector(s) that are not selected for the Exposure Preset. To acquire images with more than one detector, tick the checkbox for the desired detector(s).

Make sure that the Gain and Offset are properly defined for each selected detector. For instructions, see Define the Exposure Preset on page 20.

7.5.6 Prepare for the acquisition of an EDS spectrum image at each tilt angle

If the microscope is equipped with EDX detectors, then Energy Dispersive X-Ray Spectroscopy (EDS) data can be recorded fin addition to the STEM images.

To do so, the EDS experiment must be prepared in Velox. For every tilt angle in the Data Acquisition run, Tomography notifies Velox to acquire a full spectrum image, extract elemental maps, and store these in the *Tomography* > *Session Setup* > *Storage Folder*.

Velox stores the following files in the Tomography session folder. The filenames indicate their content:

- The quantified elemental maps: `[tilt_series]_Velox_[element]_[quantification]`
  for example: `MyTiltSeries_Velox_Cu_Int`
- The STEM image(s): `[tilt_series]_Velox_Stem_[detector]`
  for example: `MyTiltSeries_Velox_Stem_HAADF`

The raw EDS data is also stored by Velox in the experiment file, at a storage location that is specified in Velox. For convenience, specify the same folder as the Tomography session folder, so that all data is collected in one location.

7.5.6.1 Prepare the EDS acquisition in Velox

During the Data Acquisition:

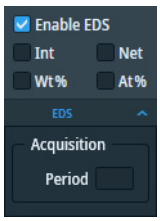
- Tomography controls:
  - The optics settings of the system.
  - The detector settings and acquisition settings for the STEM acquisitions.
- Velox controls the resolution and dwell time for the EDS acquisition,
  as defined in the Velox experiment.

To setup the EDS acquisition in Velox, follow the steps below:

1. In **Tomography**:
   a. Define the **Exposure Preset**.
      For instructions, see: Define the Exposure Preset on page 20.
   b. Select **Optics Settings** > **Set**
   c. Select **Detector Settings** > **Set**

2. In **Velox**, prepare an EDS experiment as described in the Velox User Manual.
   Unless it is unavoidable, do *not* adjust the optics settings, and do not adjust the detector settings for the STEM detector(s).
3. If it was necessary to adjust the optics settings or detector settings during the preparation of the EDS experiment in Velox, then apply the adjustments to the Exposure Preset in Tomography.
   In Tomography:
   a. Select the **Exposure** Preset.
   b. Select **Optics Settings** > **Get**
   c. Select **Detector Settings** > **Get**

7.5.6.2 Description of the EDS options



- **Enable EDS**: acquire Spectrum Images during the STEM acquisitions.
- Quantification options:
  - **Int**: integrated intensities (no deconvolution, no background correction).
  - **Net**: net intensities (deconvoluted, background corrected).
  - **Wt%**: weight fraction (deconvoluted, background corrected).
  - **At%**: atom fraction (deconvoluted, background corrected).
- **Period**: the tilt step interval between Spectrum Image acquisitions.
  An EDS acquisition can take a while. The *Period*

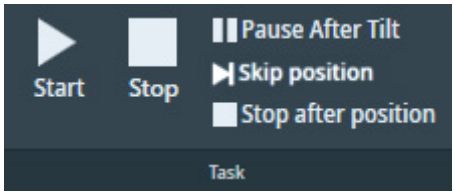
The quantification options that are selected in Tomography override any quantification methods that are enabled in Velox during the preparation of the EDS experiment.

For a detailed description of the maps and fractions, and how to prepare these, see the Velox User Manual.

7.5.7 Setup and start the automated Data Acquisition run

1. Select or specify the parameters for the Data Acquisition run.
   See:
   - Description of the Acquisition Settings on page 93.
   - Description of the Corrections Settings on page 97.
   - Description of the Options on page 104.
   - Description of the Dynamic Focus settings on page 104.
   - Description of the Additional Detectors options on page 105.
2. (Optional) Prepare the EDS acquisition.
   For instructions, see:Prepare for the acquisition of an EDS spectrum image at each tilt angle on page 105.

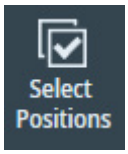
3. (Optional) Select **Pause After Tilt** to inspect the image after each tilt step and, if necessary, to make adjustments before the next Tilt Series image is acquired.



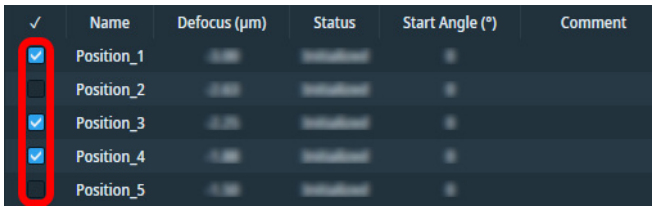
Depending on the session settings, the Start button can have the following functions:

- *Start* to acquire data for a single Tilt Series.
- *Start Batch* to acquire data for all Batch Positions.
- *Acquire [N] Position(s)* to acquire data for a selected subset of the Batch Positions.

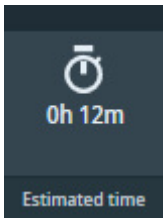
4. (Optional) If *Session Setup* > *Batch* is ticked and if *not all* Batch Positions must be processed, then select a subset of the Batch Positions:
   a. Select **Select Positions**



   b. In the Batch Positions list, tick the desired **Batch Positions**



5. Select **Start**, **Start Batch** or **Acquire [N] Position(s)**
   - Tomography STEM shows the estimated remaining duration of the Automated Acquisition run.



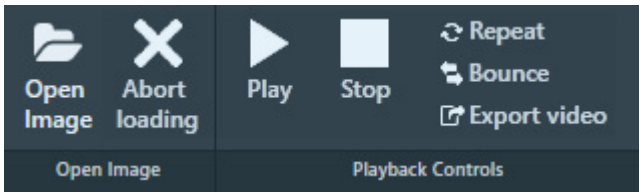
     - The estimated time is updated during acquisition.
     - The tooltip shows the estimated end date and time.
   - The acquired data is stored as a stacked MRC file. Additionally, Tomography STEM stores the metadata as MDOC files.
   - If the system has a CFEG, then the tip is flashed automatically at convenient times during the Data Acquisition run to ensure optimal illumination.
   - If a Gatan filter with Gatan K3 camera is used to acquire the Tilt Series, then Tomography STEM automatically refreshes the Dark Reference at the start of each Tilt Series.

6. Wait until the Data Acquisition run is completed.
   At any time during the Data Acquisition run, it is possible to:
   - Select **Pause** to make adjustments.
     After the adjustments are made, select **Resume** to continue the Data Acquisition run.
   - Select **Stop** to abort the Data Acquisition run.
   - Select **Pause After Tilt** to activate or de-activate *Pause After Tilt* mode.
   - If the Data Acquisition run acquires data for multiple Batch Positions,
     select **Skip position** to abort data acquisition for the current Batch Position.
   - Select **Stop after position** to abort the Data Acquisition run after Tilt Series at the current Batch Position is completed.

7.6 The Movie Player task

The Movie Player tasks offers functionalities to view the images of a Tilt Series as a movie. It is possible to view a Tilt Series that has just been acquired in an automated Data Acquisition run, or to load the MRC file from a previously acquired Tilt Series.

7.6.1 Description of the Movie Player functionalities



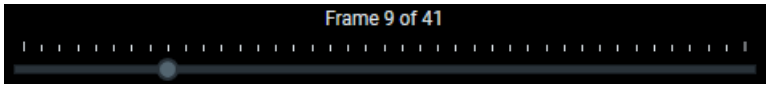
To open an image, image series or frame series:

- **Open Image**
  Open an MRC file.
- **Abort loading**
  Stop loading the selected MRC file.

If the opened MRC file contains an image series or frame series, then the following playback functions become available:

- **Play**
  Start the playback of a frame series.
- **Stop**
  Stop the ongoing playback of a frame series.
- **Repeat**
  Continuously repeat the playback of a frame series until Stop is selected.
  The Repeat function can be toggled before and during the playback of a frame series.
- **Bounce**
  Reverse the playback direction when the last frame is reached.
  The *Bounce* function can be toggled before and during the playback of a frame series.

- **Export video**
  Export the frame series as a video file.
  See Export a frame or image series to a video file on page 110.
- **Frame Selection Slider**
  The Frame Selection slider is available at the bottom of the Image Display.



  Drag the slider with the mouse to display the desired frame number.

7.6.2 View the Tilt Series images for a single feature of interest

Use the **Slider** or the functions in the **Playback Controls** ribbon to inspect the most recently acquired Tilt Series images.

7.6.3 View the Tilt Series images in a Batch Mode Session

If Tilt Series have been acquired for multiple Batch Positions, then follow the steps below to inspect the acquired images:

1. In the **Positions** list, select the desired **Batch Position**

Id	Name	Defocus (µm)	Status	Start Angle (°)	Comment
1	Position_1	-3.00	Acquired	0	Comment_1
2	Position_2	-1.50	Acquired	0	Comment_2
3	Position_3	-5.00	Acquired	15	Comment_3
4	Position_4	-5.00	Acquired	15	Comment_4

2. The Movie Player will automatically show a scaled down version, a *thumbnail*, of your tilt series movie if it was acquired in Batch mode. The thumbnail file is saved in a Thumbnails folder found in the corresponding sessions's folder.
3. Use the **Slider** or the functions in the **Playback Controls** to inspect the Tilt Series frames.

7.6.4 View the Tilt Series images from a previous Tomography session

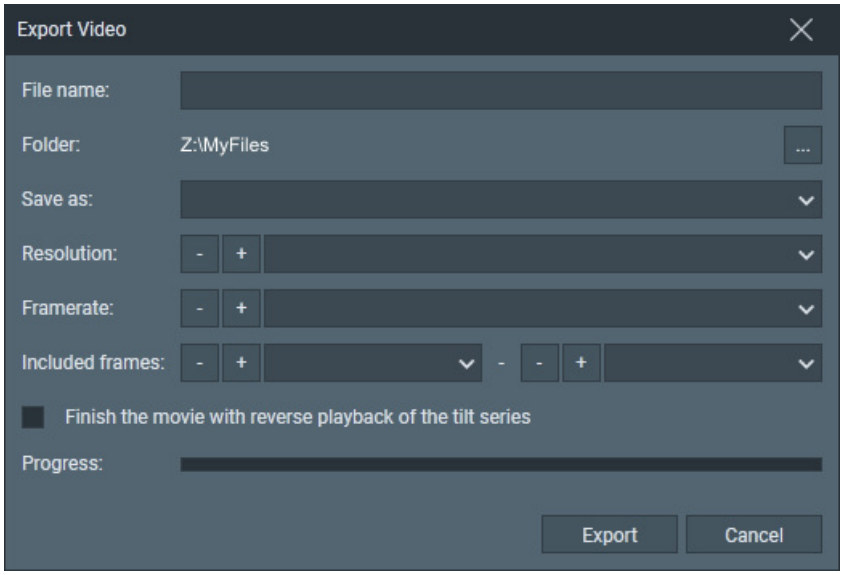
1. Select **Open Image**, then navigate to the desired **MRC file** and select it.
2. Use the **Slider** or the functions in the **Playback Controls** to inspect the Tilt Series frames.

7.6.5 Export a frame or image series to a video file

An acquired frame series can be exported as an H.264 (MPEG 4 part 10) video file. To do so, follow the steps below:

1. Either:
   - Right-click in the Image Display and select **Export video**
   - From the Image Display video controls, select **Export video**

     
2. In **Export Video**:



   a. Specify the **File name**
   b. Select the **Folder** where the video will be saved.
   c. In **Save as**, select the video format.
      Currently, **H.264 (*.mp4)** is the only option.
   d. Select the **Resolution**
   e. Select the **Framerate**
   f. In **Included frames**, select the first frame and the last frame to be included in the exported video file.
   g. (Optional) Tick **Finish the movie with reverse playback of the tilt series**
      When ticked, the exported video will show the frame series from the first to the last selected frame and back again. If the exported video is played in a loop, then the frame series will bounce back and forth between the first and last included frames.
   h. Select **Export**

8 Inspect the Acquired Images

8.1 View and post-process MRC images with Thermo Scientific Velox software

MRC images can be viewed and post-processed:

- On the Microscope PC with the Thermo Scientific Velox Online Processing software.
- On any other computer with the Thermo Scientific Velox Offline software.

To open an MRC image in Velox:

1. Open **Velox Online Processing** or **Velox Offline**.
2. Drag and drop the **MRC image file** in the Velox window.

For detailed descriptions and instructions of the viewing and processing functionalities in Velox, see the Velox User Manual.

The Velox software does not provide 3D reconstruction functionalities.

8.2 Install the Thermo Scientific Imaging Codec Pack to add native support for common microscopy formats to Windows

The Thermo Scientific Imaging Codec Pack adds commonly used microscopy image formats to the natively supported image formats in Windows. After installation, the supported microscopy image formats can be opened in the Windows Photo Viewer, and the thumbnails and previews in Windows Explorer show the actual image, instead of a generic icon.

For an overview of the supported image formats, see the Imaging Codec Pack User Guide.

A The MRC2014 Image Format

A.1 The Main Header and Extended Headers in an MRC file

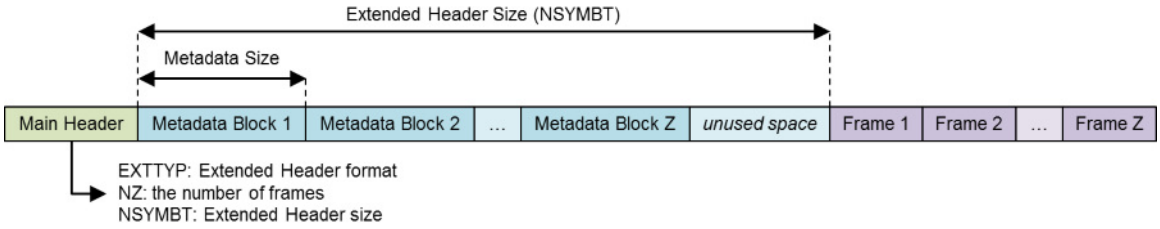
MRC files have a generic Main Header and an optional Extended Header.

- The Main Header contains generic image information, such as the image dimensions and the pixel format. For the specification of the main header,
  see MRC/CCP4 File Format for Images and Volumes.
- The Extended Header contains application specific metadata.
  For Thermo Scientific and FEI products, the extended header contains information about:
  - The microscope state at acquisition time, such as magnification, accelerating voltage, stage position, beam shift and many other relevant parameters.
  - Image acquisition information, such as binning and exposure time.

Among others, the Main Header contains the following fields:

- `NZ`: the number of frames in the MRC file.
- `NSYMBT`: the reserved size for the Extended Header.
- `EXTTYP`: the format of the Extended Header: `FEI1` or `FEI2`.
  The FEI2 format is an extended version of the FEI1 format.

For every frame in the file, the Extended Header contains one Metadata Block. The first element of each block contains the Metadata Block size. All Metadata Blocks in the Extended Header have the same size and contain the same fields. The sum of the Metadata Block sizes fits within the reserved size for the Extended Header.



A.2 The Extended Header specification

The FEI1 and FEI2 Extended Header formats allow for the addition of new fields without breaking compatibility. When a new field is added, the `Metadata Size` and `Metadata Version` fields are updated. Image reading and processing software can use the `Metadata Size` value from the first Metadata Block to index the blocks for the other frames in the MRC file.

With the introduction of the FEI2 format, the format of the FEI1 Extended Header is frozen. For MRC files with an FEI1 Extended Header, image reading and processing software can assume the values of the `Metadata Size` and `Metadata Version` fields are 768 bytes and version number 0.

The tables below specify the content of the FEI1 and FEI2 Extended Headers for the MRC2014 file format. In these tables, the **Format** and **'IsPresent' flag** columns have to the following values:

- **Format**:
  - Bool: Boolean of 1 byte (0 = false, other value = true).
  - Int32: Signed integer of 4 bytes.
  - Int64: Signed integer of 8 bytes (only used in FEI2 Extended Header).
  - UInt32: Unsigned integer of 4 bytes.
  - Float64: Floating point number of 8 bytes.
- **IsPresent**:
  UInt32 value that is used as a 32-bit / little-endian bitmask. If a metadata field is set, then the value of the *IsPresent* bit in the bitmask is 1.

A.2.1 FEI1 Extended Header specification

Image, System and Application

Name	Offset (dec)	Offset (hex)	Format	IsPresent	Description
Metadata size	0	0x0000	Int32	NA	Metadata size [bytes] All Metadata Blocks in the file have the same size. • FEI1: 768 bytes • FEI2: updated for each version.
Metadata version	4	0x0004	Int32	NA	Version ID of the metadata format. All Metadata Blocks in the file have the same format. • FEI1: 0 • FEI2: initial value: 2 The value is updated for each new version.
Bitmask 1	***8***	***0x0008***	***UInt32***	***NA***	***Individual bits indicate which metadata fields are set.***
Timestamp	12	0x000C	Float64	Bitmask 1 – #0	Time when the image was taken. The used format is the DATE data type that is used in OLE automation by Microsoft: Microsoft OLE DATE data type specification
Microscope type	20	0x0014	16 chars	Bitmask 1 – #1	Identifier for microscope type (Krios, Talos, Titan, Metrios, etc.)
D-Number	36	0x0024	16 chars	Bitmask 1 – #2	Microscope identifier

Application	52	0x0034	16 chars	Bitmask 1 – #3	Application name
Application version	68	0x0044	16 chars	Bitmask 1 – #4	

Gun

Name	Offset (dec)	Offset (hex)	Format	'Is Present' flag	Description
HT	84	0x0054	Float64	Bitmask 1 – #5	High tension [Volt]
Dose	92	0x005C	Float64	Bitmask 1 – #6	Dose [electrons/m²]

Stage

Name	Offset (dec)	Offset (hex)	Format	'Is Present' flag	Description
Alpha tilt	100	0x0064	Float64	Bitmask 1 – #7	Holder Alpha tilt along axis [degr.]
Beta tilt	108	0x006C	Float64	Bitmask 1 – #8	Holder Beta tilt along axis [degr.]
X-Stage	116	0x0074	Float64	Bitmask 1 – #9	Stage X position [m]
Y-Stage	124	0x007C	Float64	Bitmask 1 – #10	Stage Y position [m]
Z-Stage	132	0x0084	Float64	Bitmask 1 – #11	Stage Z position [m]
Tilt axis angle	140	0x008C	Float64	Bitmask 1 – #12	Angle of tilt axis in image [degr.]
Dual axis rotation	148	0x0094	Float64	Bitmask 1 – #13	Measured rotation angle after b flip [degr.] (Tomography only)

Pixel Size

Name	Offset (dec)	Offset (hex)	Format	'Is Present' flag	Description
Pixel size X	156	0x009C	Float64	Bitmask 1 – #14	Pixel size X [m]
Pixel size Y	164	0x00A4	Float64	Bitmask 1 – #15	Pixel size Y [m]

Optics

Name	Offset (dec)	Offset (hex)	Format	'Is Present' flag	Description
Defocus	220	0x00DC	Float64	Bitmask 1 – #22	Defocus [m]
STEM Defocus	228	0x00E4	Float64	Bitmask 1 – #23	STEM defocus [m]
Applied defocus	236	0x00EC	Float64	Bitmask 1 – #24	Relative defocus applied by application [m]

Instrument mode	244	0x00F4	Int32	Bitmask 1 – #25	• 1: TEM • 2: STEM
Projection mode	248	0x00F8	Int32	Bitmask 1 – #26	• 1: Diffraction • 2: Imaging
Objective lens mode	252	0x00FC	16 chars	Bitmask 1 – #27	• LM • HM • Lorentz
High magnification mode	268	0x010C	16 chars	Bitmask 1 – #28	• Mi • SA • Mh
Probe mode	284	0x011C	Int32	Bitmask 1 – #29	• 1: NanoProbe • 2: MicroProbe
EFTEM On	288	0x0120	Bool	Bitmask 1 – #30	TRUE when the magnifications are adapted to the energy filter
Magnification	289	0x0121	Float64	Bitmask 1 – #31	Nominal magnification
Bitmask 2	***297***	***0x0129***	***UInt32***	***NA***	***Individual bits indicate which metadata fields are set.***
Camera length	301	0x012D	Float64	Bitmask 2 – #0	Nominal camera length [m]
Spot index	309	0x0135	Int32	Bitmask 2 – #1	-
Illuminated area	313	0x0139	Float64	Bitmask 2 – #2	• TEM: beam diameter in meters • STEM: not used • Undefined on 2 lens condenser systems
Intensity	321	0x0141	Float64	Bitmask 2 – #3	Uncalibrated measure of beam diameter on 2 lens condenser systems
Convergence angle	329	0x0149	Float64	Bitmask 2 – #4	[degr.] Undefined on 2 lens condenser systems
Illumination mode	337	0x0151	16 chars	Bitmask 2 – #5	• None • Parallel • Probe • Free • Undefined on 2 lens condenser systems
Wide convergence angle range	353	0x0161	Bool	Bitmask 2 – #6	Undefined on 2 lens condenser systems

EFTEM Imaging

Name	Offset (dec)	Offset (hex)	Format	'Is Present' flag	Description
Slit inserted	354	0x0162	Bool	Bitmask 2 – #7	-
Slit width	355	0x0163	Float64	Bitmask 2 – #8	Slit width [eV]
Acceleration voltage offset	363	0x016B	Float64	Bitmask 2 – #9	[Volt]
Drift tube voltage	371	0x0173	Float64	Bitmask 2 – #10	[Volt]
Energy shift	379	0x017B	Float64	Bitmask 2 – #11	[eV]

Image Shifts

Name	Offset (dec)	Offset (hex)	Format	'Is Present' flag	Description
Shift offset X	387	0x0183	Float64	Bitmask 2 – #12	Corrective image or beam shift relative to exposure preset (in logical units) ● TEM: pure image shift ● STEM: image-beamshift-
Shift offset Y	395	0x018B	Float64	Bitmask 2 – #13	
Shift X	403	0x0193	Float64	Bitmask 2 – #14	Applied shift due to optimized position and tracking (in logical units) ● TEM: image beam shift ● STEM: beam shift-
Shift Y	411	0x019B	Float64	Bitmask 2 – #15	

Camera

Name	Offset (dec)	Offset (hex)	Format	'Is Present' flag	Description
Integration time	419	0x01A3	Float64	Bitmask 2 – #16	Camera or dose fraction exposure time
Binning Width	427	0x01AB	Int32	Bitmask 2 – #17	-
Binning Height	431	0x01AF	Int32	Bitmask 2 – #18	-
Camera name	435	0x01B3	16 chars	Bitmask 2 – #19	Name of the camera
Readout area left	451	0x01C3	Int32	Bitmask 2 – #20	-
Readout area top	455	0x01C7	Int32	Bitmask 2 – #21	-
Readout area right	459	0x01CB	Int32	Bitmask 2 – #22	-
Readout area bottom	463	0x01CF	Int32	Bitmask 2 – #23	-

Ceta noise reduction	467	0x01D3	Bool	Bitmask 2 – #24	-
Ceta frames summed	468	0x01D4	Int32	Bitmask 2 – #25	Number of frames summed for dynamic range
Direct detector electron counting	472	0x01D8	Bool	Bitmask 2 – #26	-
Direct detector align frames	473	0x01D9	Bool	Bitmask 2 – #27	-
Camera param reserved 0	474	0x01DA	Int32	Bitmask 2 – #28	-
Camera param reserved 1	478	0x01DE	Int32	Bitmask 2 – #29	-
Camera param reserved 2	482	0x01E2	Int32	Bitmask 2 – #30	-
Camera param reserved 3	486	0x01E6	Int32	Bitmask 2 – #31	-
Bitmask 3	***490***	***0x01EA***	***UInt32***	***NA***	***Individual bits indicate which metadata fields are set.***
Camera param reserved 4	494	0x01EE	Int32	Bitmask 3 – #0	-
Camera param reserved 5	498	0x01F2	Int32	Bitmask 3 – #1	-
Camera param reserved 6	502	0x01F6	Int32	Bitmask 3 – #2	-
Camera param reserved 7	506	0x01FA	Int32	Bitmask 3 – #3	-
Camera param reserved 8	510	0x01FE	Int32	Bitmask 3 – #4	-
Camera param reserved 9	514	0x0202	Int32	Bitmask 3 – #5	-
Phase Plate	518	0x0206	Bool	Bitmask 3 – #6	Indicates whether phase plate was used for data acquisition

STEM Detector

Name	Offset (dec)	Offset (hex)	Format	'Is Present' flag	Description
STEM Detector name	519	0x0207	16 chars	Bitmask 3 – #7	-
Gain	535	0x0217	Float64	Bitmask 3 – #8	-

Offset	543	0x021F	Float64	Bitmask 3 – #9	-
STEM param reserved 0	551	0x0227	Int32	Bitmask 3 – #10	-
STEM param reserved 1	555	0x022B	Int32	Bitmask 3 – #11	-
STEM param reserved 2	559	0x022F	Int32	Bitmask 3 – #12	-
STEM param reserved 3	563	0x0233	Int32	Bitmask 3 – #13	-
STEM param reserved 4	567	0x0237	Int32	Bitmask 3 – #14	-

Scan settings

Name	Offset (dec)	Offset (hex)	Format	'Is Present' flag	Description
Dwell time	571	0x023B	Float64	Bitmask 3 – #15	Dwell time per pixel [sec]
Frame time	579	0x0243	Float64	Bitmask 3 – #16	Frame time [sec] (currently it will not be used)
Scan size left	587	0x024B	Int32	Bitmask 3 – #17	-
Scan size top	591	0x024F	Int32	Bitmask 3 – #18	-
Scan size right	595	0x0253	Int32	Bitmask 3 – #19	-
Scan size bottom	599	0x0257	Int32	Bitmask 3 – #20	-
Full scan FOV X	603	0x025B	Float64	Bitmask 3 – #21	Field of view [m]
Full scan FOV Y	611	0x0263	Float64	Bitmask 3 – #22	-

EDX Elemental Maps

Name	Offset (dec)	Offset (hex)	Format	'Is Present' flag	Description
Element	619	0x026B	16 chars	Bitmask 3 – #23	-
Energy interval lower	635	0x027B	Float64	Bitmask 3 – #24	-
Energy interval higher	643	0x0283	Float64	Bitmask 3 – #25	-
Method	651	0x028B	Int32	Bitmask 3 – #26	-

Dose Fractions

Name	Offset (dec)	Offset (hex)	Format	'Is Present' flag	Description
Is dose fraction	655	0x028F	Bool	Bitmask 3 – #27	-
Fraction number	656	0x0290	Int32	Bitmask 3 – #28	-
Start frame	660	0x0294	Int32	Bitmask 3 – #29	-
End frame	664	0x0298	Int32	Bitmask 3 – #30	-

Reconstruction

Name	Offset (dec)	Offset (hex)	Format	'Is Present' flag	Description
Input stack filename	668	0x029C	80 chars	Bitmask 3 – #31	-
Bitmask 4	***748***	***0x02EC***	***UInt32***	***NA***	***Individual bits indicate which metadata fields are set.***
Alpha tilt min	752	0x02F0	Float64	Bitmask 4 – #0	
Alpha tilt max	760	0x02F8	Float64	Bitmask 4 – #1	

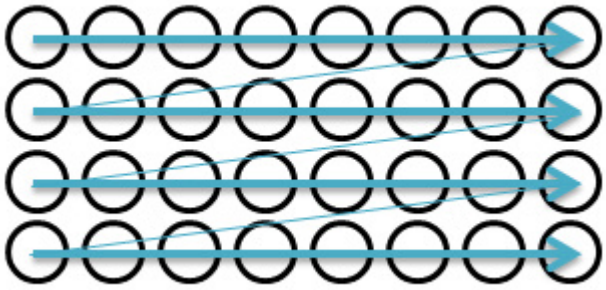
A.2.2 FEI2 Version 2 Extension to the Extended Header specification

Name	Offset (dec)	Offset (hex)	Format	IsPresent	Description
Scan rotation	768	0x0300	Float64	Bitmask 4 – #2	Rotation of the scan pattern in STEM mode [radians]
Diffraction pattern rotation	776	0x0308	Float64	Bitmask 4 – #3	Rotation of the diffraction pattern in diffraction mode [radians]
Image rotation	784	0x0310	Float64	Bitmask 4 – #4	Rotation of the image in imaging mode [radians]
Scan mode enumeration	792	0x0318	Int32	Bitmask 4 – #5	• 0: Other • 1: Raster • 2: Serpentine raster
Acquisition time stamp	796	0x031C	Int64	Bitmask 4 – #6	Microseconds since 1970-01-01T00:00:00Z at which the image was acquired
Detector commercial name	804	0x0324	16 chars	Bitmask 4 – #7	Commercial name of the detector or camera
Start tilt angle	820	0x0334	Float64	Bitmask 4 – #8	Start tilt angle of a tomography series [degr.]

End tilt angle	828	0x033C	Float64	Bitmask 4 – #9	End tilt angle of a tomography series [degr.]
Tilt per image	836	0x0344	Float64	Bitmask 4 – #10	Tilt increment per image in a tomography series [degr.]
Tilt speed	844	0x034C	Float64	Bitmask 4 – #11	Tilt speed in a tomography series [degr./sec]
Beam center X pixel	852	0x0354	Int32	Bitmask 4 – #12	Beam center X on image [pixels]
Beam center Y pixel	856	0x0358	Int32	Bitmask 4 – #13	Beam center Y on image [pixels]
CFEG flash timestamp	860	0x035C	Int64	Bitmask 4 – #14	Microseconds since 1970-01-01T00:00:00Z of the most recent CFEG flashing
Phase plate position index	868	0x0364	Int32	Bitmask 4 – #15	Position index of the phase plate aperture
Objective aperture name	872	0x0368	16 chars	Bitmask 4 – #16	Name of the inserted objective aperture

A.3 Pixel sequence in the MRC2014 format

In the MRC2014 files, the image pixel data is stored as rows from top to bottom, where each row is stored from left to right.



Most image viewers and image processing applications use the same pixel position sequence as the MRC file. Some image viewing and processing applications such as IMOD and Fiji/ImageJ use a pixel position sequence. In these applications, the image display may be mirrored and/or rotated.

A.3.1 The MRC image pixel data encoding for Thermo Scientific Ceta cameras

If the image is acquired with a Ceta camera, then the MRC image pixel data encoding depends on the presence of the Ceta Speed Enhancement (Ceta-2).

Camera	MRC Pixel Data
Ceta without Speed Enhancement	32-bit floating point
Ceta with Speed Enhancement	16-bit signed integer

9 Copyright, Limited Rights and Revision History

Copyright

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Revision Table

Revision	Date	Description of Changes
5.9	JAN-2022	Initial Release of this User Manual.
5.10	APR-2022	Update for Tomography 5.10
5.10.A	APR-2022	Secondary update for Tomography 5.10
5.11	OCT-2022	Update for Tomography 5.11
5.12	OCT-2022	Update for Tomography 5.12
5.13	JAN-2023	Update for Tomography 5.13
5.14	MAR-2023	Update for Tomography 5.14
5.15	JUL-2023	Update for Tomography 5.15
5.16	OCT-2023	Update for Tomography 5.16
5.17	JAN-2024	Update for Tomography 5.17
5.18	APR-2024	Update for Tomography 5.18
5.19	JUL-2024	Update for Tomography 5.19
5.20	OCT-2024	Update for Tomography 5.20
5.21	JAN-2025	Update for Tomography 5.21

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