Image series modules

The controls / information within these modules have the same meaning:

Slice: gives the serial number of shown image / number of all images

Image: Image resolution, dwell time, number of frames integration for this image

Microscope: Accelerating voltage, beam current, {detector type and its mode}

Result: Active item with the image series name – clicking it redirects you to the corresponding images folder.

Save as AVI…: clicking this button enables the creation of an AVI type movie from the image stack. A File dialog appears to specify a file name and select a directory for the resulting AVI file.

AF / ACB: informs that the auto focus / auto contrast brightness image processing is in progress for the actual slice.

Export to CSV…: used to export the drift correction data to the format readable by most table processor applications.

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UNIVERSITY OF FRIBOURG SWITZERLAND

Focused ion beam

Introduction

Version 3 – May 2026

TOMOGRAPHY – SLICE AND VIEW

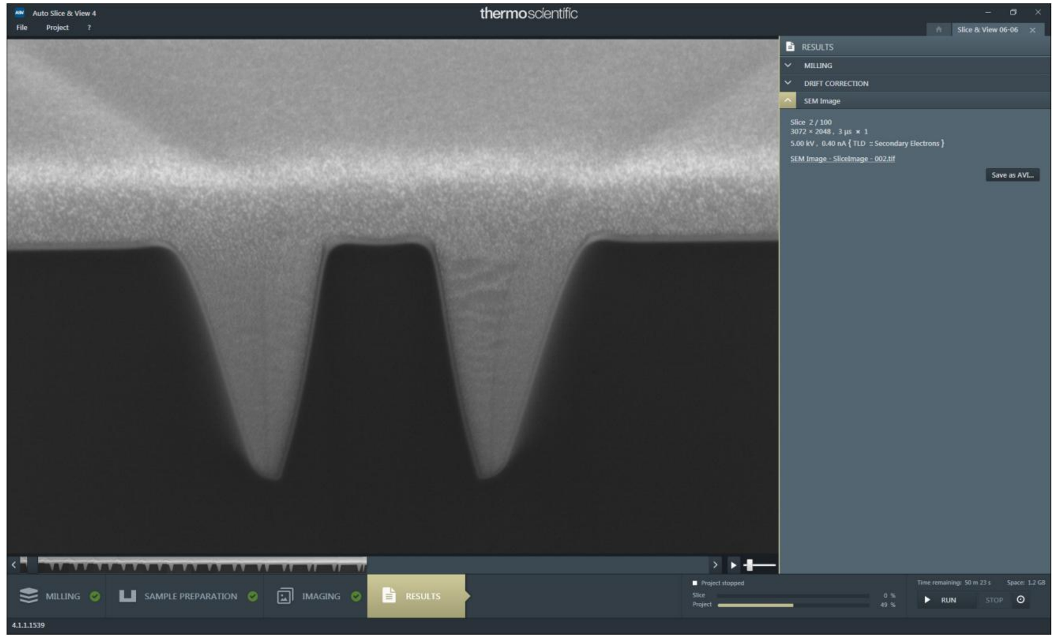
Demonstration: Results

Prerequisites:

Tomography running or finished

Collect your recorded data

This page is the same for viewing the results (images) as they come in during processing, as well as for viewing a previously executed project. The modules on the right side of the page show each resulting image series (as defined in the imaging page). The imaging area shows any image from the series by moving the slider at the bottom.



- Auto contrast brightness

When enabled, it applies ACB (Auto Contrast Brightness) to each image acquisition set in the Perform Every nth Acquisition. It determines whether or not an ACB is done <u>before</u> acquiring the image during the automated run. A reduced area is positioned over the image to visualize which part of the field of view will be used. Here, it is advised to use a part on the side of your cut face and not the fiducial marker, as the B/C signal of the marker (Pt) may not reflect the B/C signal of your object.

- Tiling

When activated, a set of tiles of the cut face area is grabbed instead of a single image.

Columns / Rows
The number of columns and rows of the grid used for tiling. An image/map will be acquired for each tile.

Tile Overlap
Defines the percentage of each tile that will be overlapped during image acquisition to provide a reference area in the images for reconstructing a composite image of all tiles.

Final Image Resolution (info field)
Shows the microscope UI settings of the image resolution.

Enable Stitching
When ticked; this service stitches the image tiles to produce one large image.

<u>Note: Tiling can only be enabled for SEM images and is recommended only for imaging with the electron beam perpendicular to the cut face.</u>

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Universal rules

Rule 1: Don't touch a control if you are not sure of the outcome of that action

Rule 2: Never, ever force anything beyond finger strength

Rule 3: Wear gloves when touching anything that goes into the chamber

Rule 4: If in doubt, ask for help

Best Practices

Location — The auto stigmator functionality slightly adjusts the working distance, therefore: place the reduced area FOV at a location having the same working distance as the cut face (see also remark autofocus).

Marker — The reduced area FOV should contain structures in various directions to ensure a good correction of astigmatism. Areas without any texture or areas with 1-directional patterns, such as lines, are not recommended. The fiducial cross is usually a good choice.

Size — The area must have an area at least 100×100 pixels.

Scan Rotation — Untick this box to simulate the behavior of Auto Stigmator functionality in the UI (uses rotation), that changes scan rotation value.

Clicking the Run Now button starts the functionality immediately.

- Auto lens alignment

The auto lens alignment functionality is performed before every nth acquisition of a series. A square reduced area is laid over the image to visualise which part of the field of view will be used.

- Auto source tilt

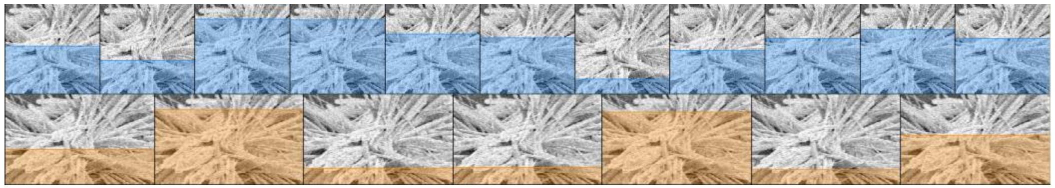
When activated, the auto source tilt functionality is performed before every nth acquisition of this series.

Best practices

There is always a trade-off between accuracy, speed, robustness, and sample exposure. The auto focus functionality is designed to work well even when the images appear “noisy” to the human eye. (low dwell times, low resolutions, high magnifications).

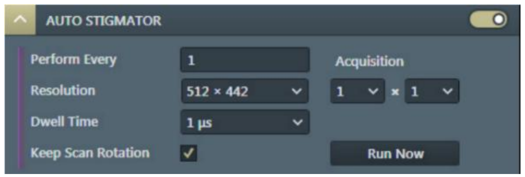
Time — is mainly determined by acquisition speed and number of steps.
Position — place your marker on line with your FOV, not in front or behind
Resolution — lower resolution and smaller dwell times will increase acquisition speed at the slight cost of accuracy and robustness.
Step number — higher number of steps increases the accuracy, but comes at the price of speed. Note that the coarse / fine sweep helps out here.
Range — rule of thumb: the range of the fine sweep should be a few times the step size (i.e., range divided by steps) of the coarse sweep.

The colored images obtained during the autofocus run indicate the success of the auto focus. The higher the colored bar, the better the focus. The application selects the best result (i.e., the highest bar) automatically. Ideally, both the orange and blue indicators follow a rough Gaussian curve.

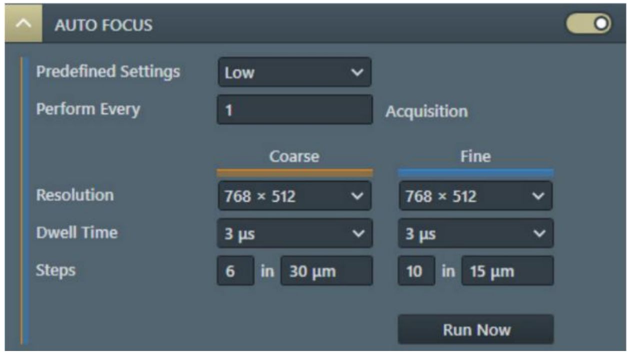


- Auto stigmator

Auto-stigmation is performed before every n[th] acquisition in the series. A squared reduced area is placed over the image to visualize the auto-stigmator FOV (which may be the same as the autofocus FOV).



sharpness. First, a Coarse sweep (orange color) to get close to focus is performed, followed by a Fine sweep (blue color) to optimize it. Tune the parameters – the autofocus needs a good quality image - and run the auto focus prior to starting a project execution.



Place the auftofocus window on a horizontal axis – same working distance – as your cut face

Predefined Settings
(Low / Medium / High / Custom) are different preset settings to the multiple controls.

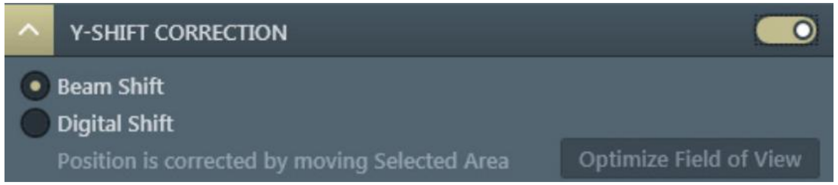
Perform Every # Acquisition
Specifies how often the autofocus should be performed.

Resolution
The pixel resolution of images acquired during the focus sweep.

Dwell Time
The amount of time the beam dwells on each pixel when images are acquired during the focus sweep. The higher, the less noisy the images are and the higher the chance for a good autofocus.

Steps
The number of working distance (WD) steps in which the range will be sampled: WD step size = range / steps.

Perform Sweep or run now button
performs an auto focus sweep with the configured settings and shows image series of both sweeps distinguished by the color.

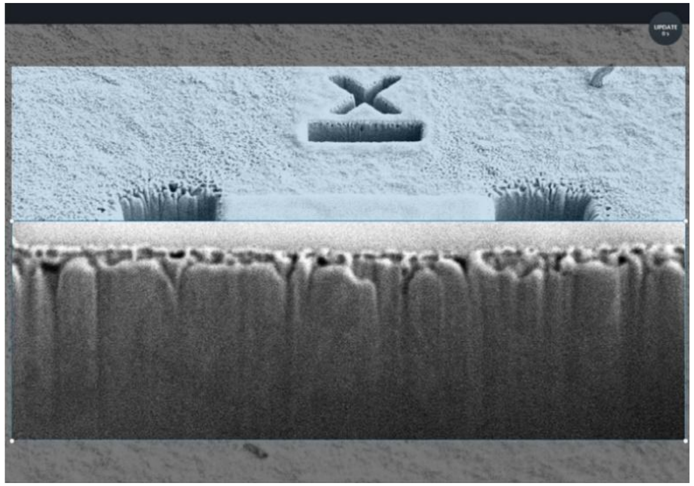


Beam Shift

compensates for an image shift by changing the beam focus. There is limited beam shift available, and the detector mode also affects the available beam shift.

Digital Shift

starts the Selected Area Scan for a corresponding image series, and compensates an image shift by shifting of this area. Below: the lower (clear) area is the actual selected area, the area above (bluish) delineates, where the digital shift will take place.



Dimensions of these areas are set by an application, they are influenced by AOI dimensions. The image above shows the situation after clicking the Optimize Field of View button, causing extention of the AOI to fill the display optimally.

Auto focus module

The autofocus functionality is performed before every nth acquisition in a series. An orange area with a blue border is positioned over the image to visualize the aufofocus FOV. Position and size of the reduced area can be adjusted. The autofocus uses a two-step "sweeping" of the working distance over a specified range to measure image

Demonstration: Starting up Auto Slice and view

Prerequisites:

Running xT server

Running UI

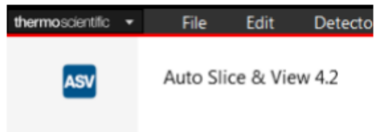
Action:

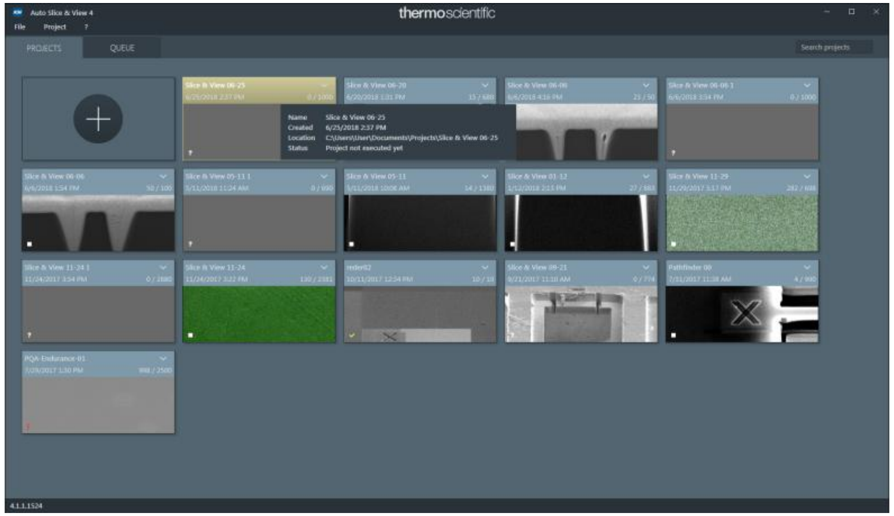
Slice and View software

Load the dedicated tomography software

To launch the application:

- Double-click the ASV icon on the Windows desktop,
- Or select the Thermo Scientific toolbar button / Auto Slice & View





The software opens. Place the window on the rightmost screen

• Starting a new project

Prerequisites:

- At Eucentric heigth
- Z is Linked
- HFW between 10 – 150 µm

Experiment: starting a new project

1. Load a sample onto the stage
2. Navigate to the feature of interest and place it in the center of the field of view.
3. Set the sample to the **eucentric position**.
4. Tilt the stage to 52°.
5. Ensure you have set the **beam coincidence point**
6. Verify that the image shift, when changing the ion column aperture, is minimized.
7. Optimize electron beam focus.
8. Run the Link Z to FWD procedure.
9. Set the ion beam horizontal field width within the range 10 – 150 µm.
10. Select the Stage menu / Enable Safe Stage Moves item on the microscope UI
11. In the slice and view software, click the big + to start the new project effect.

- Enter a unique project name.
- Save on a folder in the **SharedData drive**. NOT on the local drive.

The alignment module

An automatic image stack alignment. It is used to get all of the images aligned with one another. The user specifies a unique feature to look for (tip: use the fiducial point) and then for each slice it beam shifts the image so that the fiducial point always appears in the same spot.

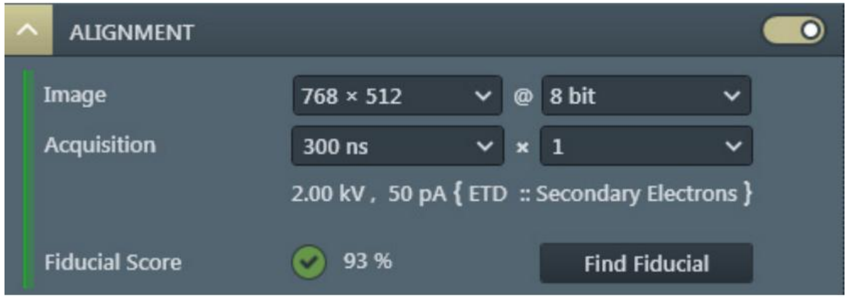


Image
The image resolution and its bit depth (16 bit!).

Acquisition
the dwell time, number of frames to be integrated for the images used to align the final cut face area during the automated run.

Find Fiducial
Clicking this button positions the sample, performs auto-contrast and brightness if activated, and finds the fiducial marker. **Alignment configuration is not complete until you click this button.**

Y-shift correction

In perpendicular SEM imaging, the sample is tilted in such a way, that the plane of the cut face is perpendicular to the electron beam, that results in the most accurate image. Normally an electron beam imaging is under 52°, so each slice causes a little image shift. To compensate this behavior for every slice, activate this module and select a desired method by checking the radio button.

Detector setup

Type
The beam used by this acquisition type.

Voxel cube
The size dimensions are dependent on the resolution, HFW, slice thickness, stage position and tilt correction settings. A tooltip gives a hint about setting of the isometric (cubic) voxel.

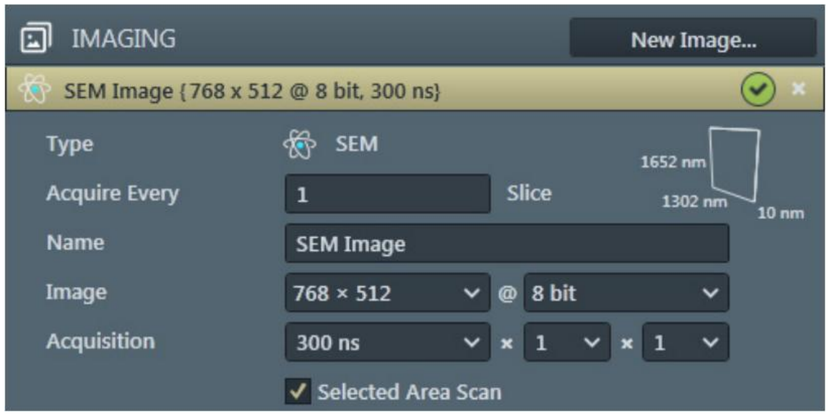
Acquire Every # Slice
The interval between slices to be acquired. You can e.g. only record every nth slice.

Name
A meaningful name for the actual image series.

Image
The image resolution and its bit depth (always 16 bit!)

Acquisition
the dwell time, number of lines integration and number of frames integration to be used for this image series.

Selected Area Scanning
if checked, only selected area will be scanned for imaging (see below)



Together with the time reported in Milling > Area of interest, milling and imaging should take ~1 minute. Much longer will make the experiment unnecessarily long, shorter will make it unnecessarily noisy.

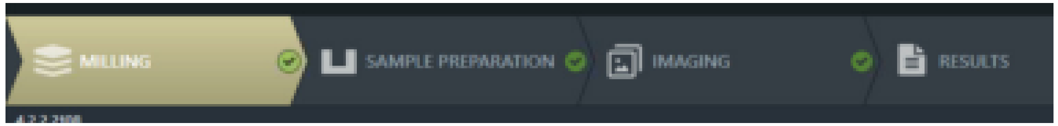
Demonstration: The milling page

Prerequisites:

Area of interest located, 52°

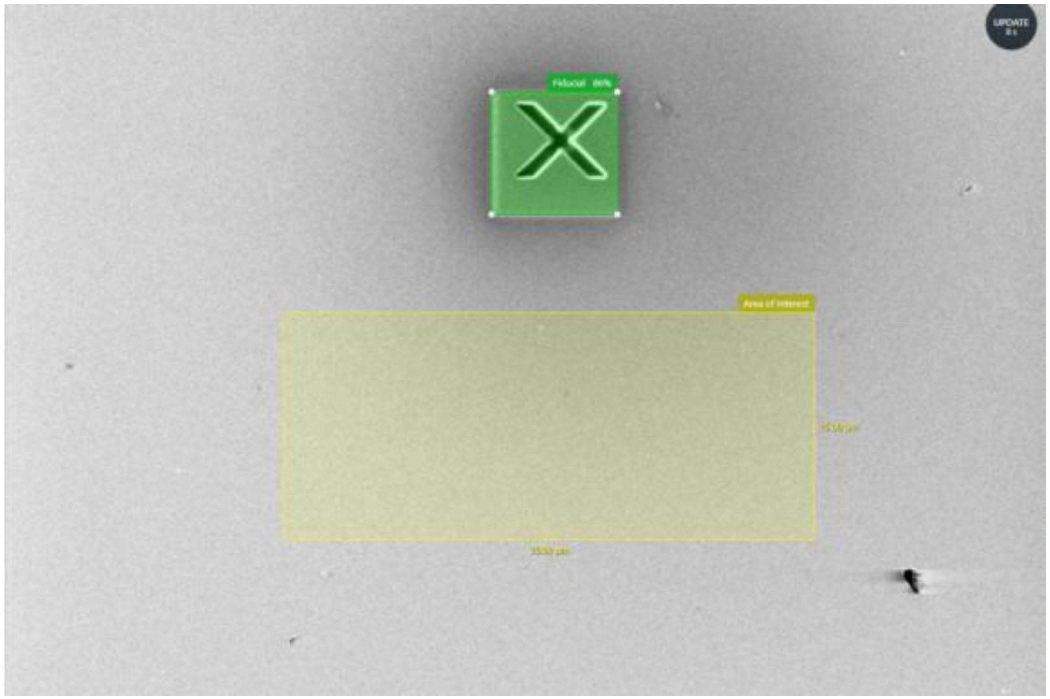
xT location focused and stigmated

Background on the ion beam



Set up the milling controls and the drift correction / fiducial parameters.

In the **ion image**, the slicing/milling area is indicated by a yellow rectangle. The fiducial point area is indicated by a red rectangle; when the fiducial point is defined, this area changes to green.



Controls summary

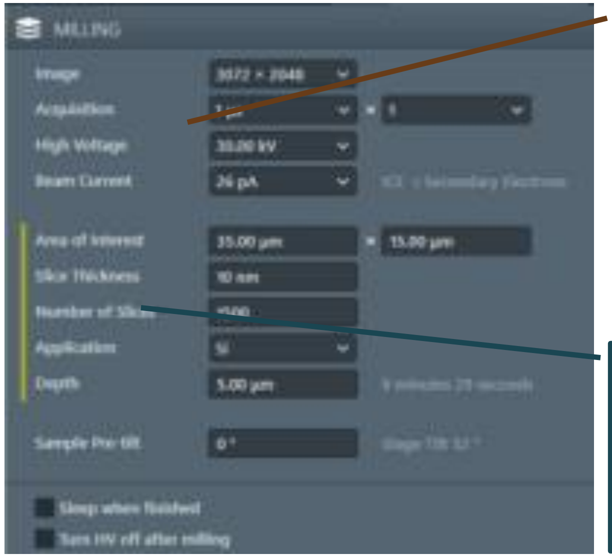


Image. Ga Ion imaging settings.

Beam current: will be used for both milling and imaging. Typically around 100 – 500 pA

Area of interest. Defines the area you will process/image

Application: Si (ie. Values for Si). Time should be <1 minute

Image

The resolution of the image is to be used to grab the drift correction images. It is described by the pixel count in an image as: number of pixel columns × number of pixel rows. The higher the resolution, the more image detail can be seen. Consider selecting a higher resolution if milling < 20 nm slice thickness.

Acquisition

The dwell time is used to acquire the images and the number of frames.

High Voltage

The ion beam accelerating voltage. Always 30 kV!

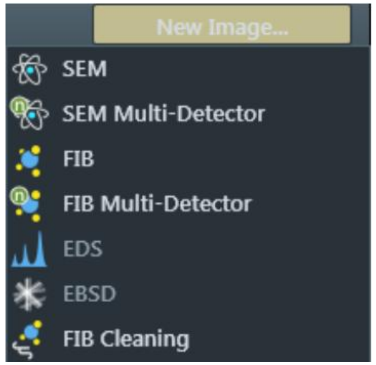
Beam Current

The ion beam current to be used for milling (and imaging!). Select a current high enough to mill the sample in a reasonable time, but low enough to guard against sample damage and loss of image resolution. The estimated milling time for one slice is indicated at the bottom right of the module. The rule of thumb is that milling time + imaging time should be about 1 minute. Typically, currents between 0.5 and 5 nA are used.

Area of Interest (width × height)

The width and height of the slicing area as defined by the yellow rectangle. Changing the height will adapt the

Imaging controls



Acquisition types

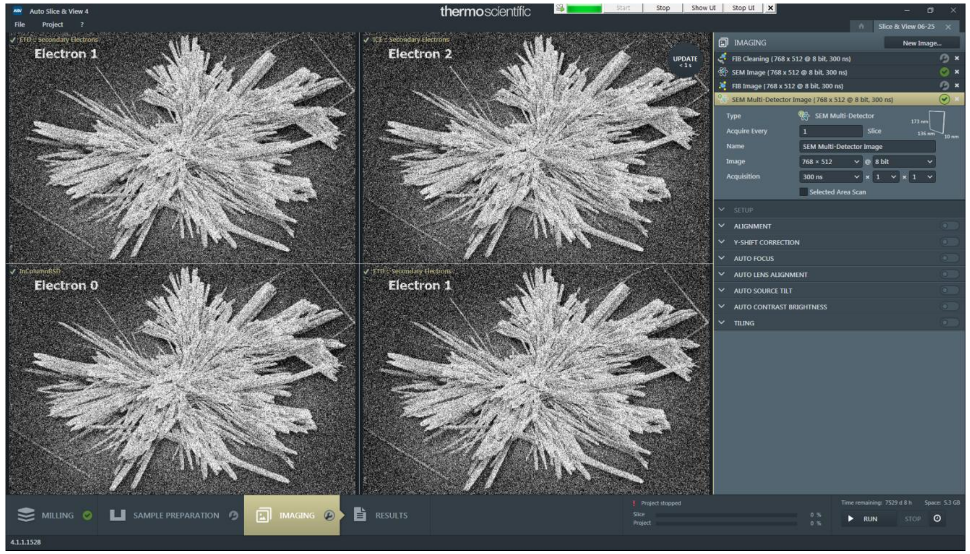
There are several possible acquisition types available in the drop-down menu when clicking the New Image... button. By selecting any item, a new line (an image series to be taken at every slice) is added to the list, along with the corresponding settings.

For the xT UI detectors (ETD, T1, T2,...), select the **SEM Multi-Detector**

Multi-detector

This allows the selection of the quadrant containing meaningful detectors.

Assuming the detectors are set up in the microscope UI, select the desired display(s) to include by checking their checkboxes in the top-left corner. All selected displays must have the same beam (e-beam). By clicking the update button, the settings are updated, and the image(s) is/are acquired in their associated display.



Demonstration: Imaging

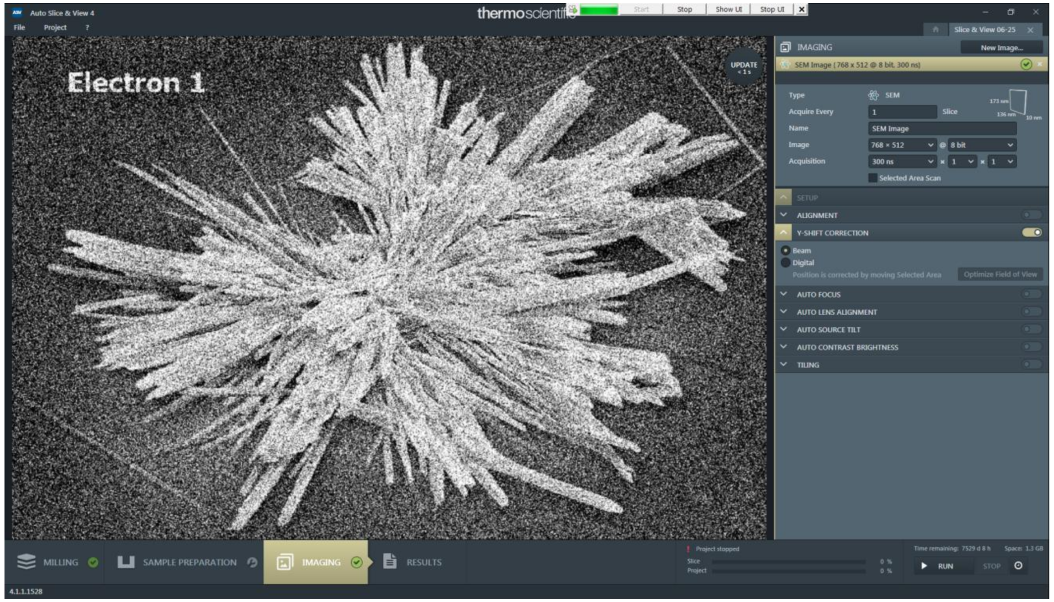
Prerequisites:

At least trenches and rough-cut milled.

Set up the detectors

Before proceeding with the items on the Imaging page, make sure the site is prepared for imaging. In the xT UI:

1. Check and, if needed, adjust the microscopy conditions such as accelerating voltage, beam current, and detectors for each display.
2. Optimize the imaging parameters such as lens alignment, focus, astigmatism correction, and brightness & contrast.



Start by removing the pre-loaded Imaging (click the X next to the green check). You want SEM multi-detector.

number of slices to height/slice thickness.

Slice Thickness

The targeted thickness of each slice. Changing this value will adapt the number of slices to match the new thickness.

Number of Slices

The number of slices to be made in the automated slicing phase. Changing this value adjusts the milling site height to match the slice thickness and the number of slices.

Application

A GIS material application that is appropriate for the sample under investigation. Typical Pt Deposition.

Depth

The relative depth of the slicing area, depending on the kind of sample, sample geometry, etc. The depth is set by application, but it can be changed by typing a value, typically between 0.5 and 100 µm

Sleep when finished

Force the system to sleep after the project has been finished.

Turn HV off after milling

Only used with a rocking mill. It switches off the ion beam accelerating voltage during long-lasting electron beam imaging (when the screen resolution and dwell time are set high).

Note Y-shift correction and compensation for the change in the Working Distance in the SEM images through the slicing job will fail, if a sample Pre-tilt is not set up correctly

Drift correction module

FIB drift correction is needed for accurate processing results: a higher slice thickness accuracy and placement of individual slices can be achieved. It refers to the placement of the pattern for milling, not image drift.

A fiducial point is used for FIB drift correction during the entire slicing process.

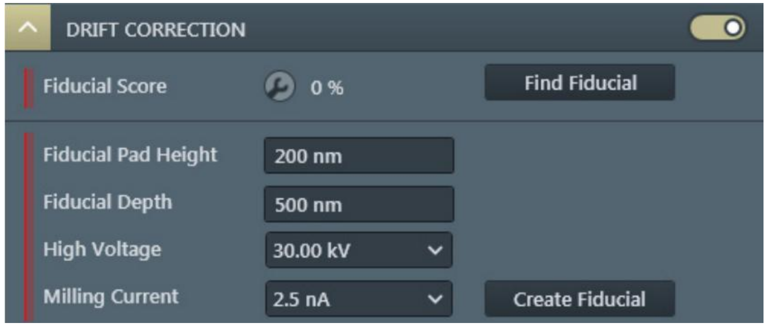
Position
The position of the fiducial point has to be some distance apart from the milling, but also within the imaging area borders. Ideally, it is placed right or left of the region of interest.

Fiducial Pad Height
The deposited height of the protective Pt layer, wherein the fiducial point will be milled. Defaults are rather low and can be increased to 1-1.5 µm.

Fiducial Depth
The depth of the cross patterns. If increasing the Pad Height, also increase this to about 30-50% more than the Pad Heigth (e.g. 1.5 – 2.0 µm).

High Voltage
The ion beam voltage used to mill the cross patterns, ie. 30 kV

Milling Current
The application should suggest a suitable milling current according to the fiducial rectangle size



Experiment: Creating a fiducial marker

1. In the microscope UI, optimize the ion beam imaging parameters using the current you have set in the AS&V beam current settings (usually 0.5-5 nA). Adjust stage position and magnification so the region of interest is centered and sufficient space around it is available for a fiducial marker.

2. In the AS&V, click Create fiducial.

After completion, the fiducial marker will be searched for; if found, the software will proceed to the Sample Preparation page.

- The height of the green box is unrestricted, but typically it will be very small (10-20 slices). It represents a Slice Count – number of slices (text box), that will be milled.

- The AOI Overlap Slice Count text area specifies the number of GCP slices that overlap the bottom of the area of interest.[1] If you stop the milling within the area of interest, the software will know where to begin during the automated slicing process.

High Voltage / Beam Current

The ion beam voltage / current to use when milling the Trench cuts, Rough cut, and Anti-Shadow Side Wall. (Note: the voltage and Beam current of the green clean pattern are set in the Milling > Beam Current)

Depth

The depth of the selected milling pattern area

Anti-Shadow Side Wall

Mills an anti-shadow angle trench for EBSD processing.

Experiment

Click the Prepare Sample button to start the milling of all selected options.

[1] If you are watching the milling process on the microscope UI and you decide that enough of the GCP has been milled, you can stop the milling at the end of the actual slice by clicking the Abort button.

Rough Cut

The rough cut exposes the front face (= the cross section) of the feature of interest for electron beam imaging. Its size and location are represented as a blue rectangle on the ion image.

Experiment: long rough cut workaround

- Make sure your region of interest is covered with Pt.

- In Milling > Beam Current, set the value to an acceptable current for a large, rough cut, e.g. 3-5 nA (going over 7 nA activates the T1 protector).

- Record a new image in the Milling page (Update) and find the fiducial (Find fiducial).

- Now run the sample preparation with the left trench, right trench and rough cut checked (nothing else).

After the process, revert the Milling > Beam Current back to the original setting (100-500 pA) and repeat the Find fiducial step.

Green clean pattern

A Green Clean Pattern (GCP) is an optional polishing step for the sample cut-face for immediate data acquisition by milling away a user-defined number of slices at low voltage and current. The depth is the same as the standard slice depth (as set in the milling page). The GCP area is shown as a green box in the image.

- The milling occurs at the voltage, current, and depth specified on the Milling > Beam Current setting.

- The width of the green box is restricted to be the same width as the yellow area of interest (AOI) box.

If the fiducial marker could not be found, optimize the fiducial point image by using the microscope UI and manually find the fiducial (see below).

Manually finding the fiducial point

If the marker could not be found automatically, try a slightly more manual approach.

Experiment: Manually finding a fiducial marker

1. In xt UI, optimise the ion beam imaging parameters using the current you have set in the AS&V beam current settings (usually 0.5-5 nA). If needed, increase the scan time.

2. In the AS&V UI, click the update button to refresh the ion beam image.

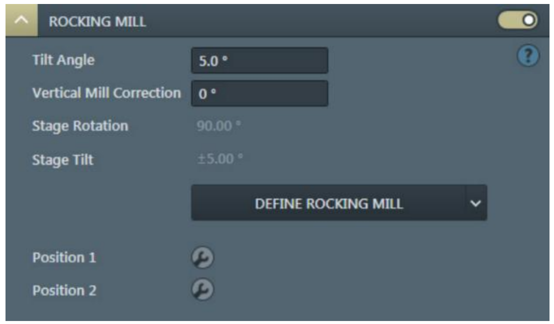
4. Manually resize and reposition the yellow box to cover the cut face area of interest.

5. Then click the Find Fiducial button. A new image will be grabbed to determine the Fiducial Score (in %) of the selected fiducial point. The higher the score, the better the fiducial point.

If the fiducial point is found, the software stores the acquisition settings to use when locating it during the automated run. From now on, the fiducial point is locked in; the yellow box can be resized but cannot be moved.

Rocking mill (optional)

Vertical direction milling is a common FIB practice for milling a series of 2D slices. It alternates between two directions (under/overtilt) with a defined angle away from the vertical milling direction to suppress a curtain effect.

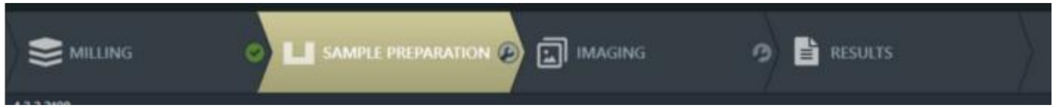


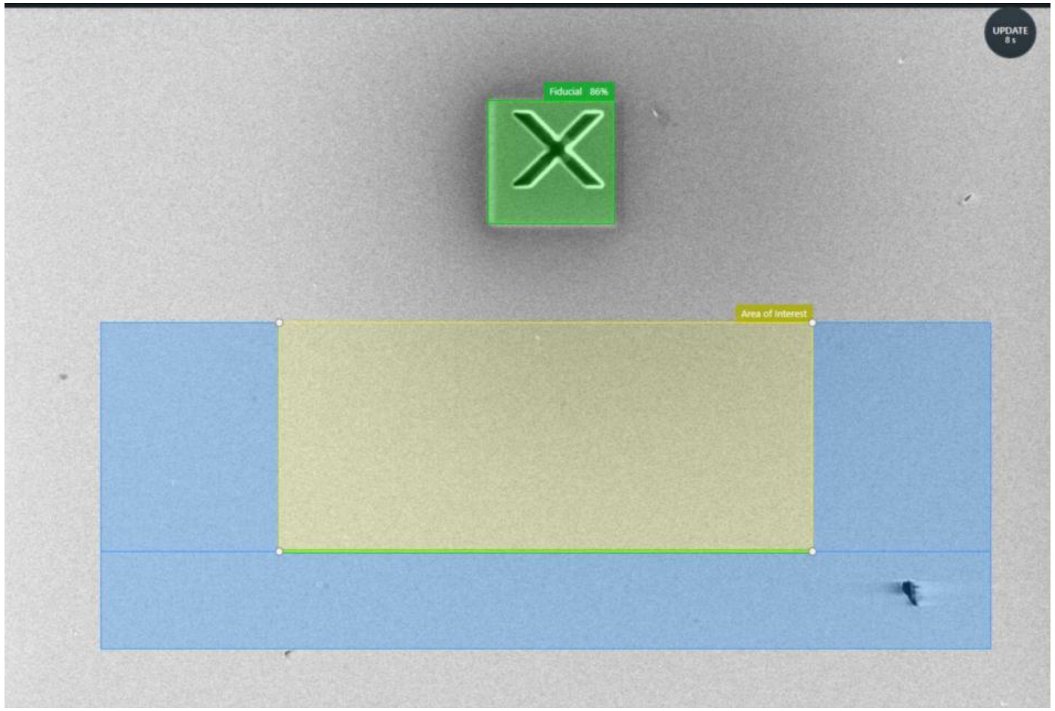
Demonstration: Sample preparation

Prerequisites:

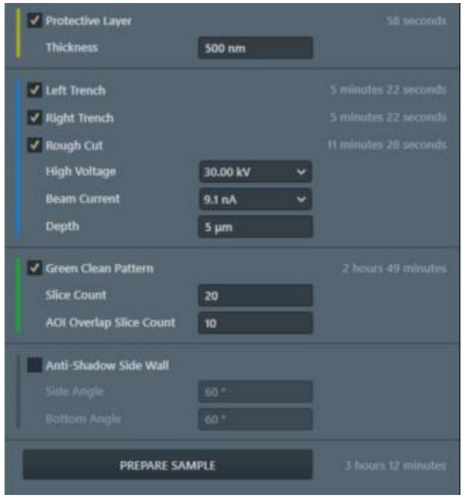
Fiducial marker placed and found
Area of interest defined

Mill the trenches





- Ticking the check box(es) enables the option(s), corresponding areas within the image are colored in the same way as in the module.



- The time value on the right side next to the corresponding items shows the estimated milling time. The Beam current and milling area dimensions are the main factors which influence the time.
The blue rough cut and trench cut areas, just as the yellow area of interest (or Protective layer area) can be modified graphically by dragging and dropping the selection boxes on the corners and sides of each rectangle. The Left Trench and Right Trench can not be used simultaneously with the Anti-Shadow Side Wall.

Protective Layer

Selecting this option deposits a protective Pt coating over the intended milling area. Typically, this is done manually before. If so, you do not need to repeat it. The protective layer should be as wide as your cross section (=Y) and as high as you want the tomogram to run (=X).

Left / Right Trench

The trench cuts provide a place for material to be deposited as slices are milled away from the area of interest during the automated run. They are represented as blue rectangles to the right and left of the yellow milling area rectangle. Mills standard cross sections to produce the rough cut and trench cuts.

Important note

Changing ion beam current will (slightly shift) the position of the image. Therefore, you want the Beam current in the Rough Cut to be the same as in Milling > Beam current (i.e. between 100 and 500 pA). This will create extremely long rough cuts.