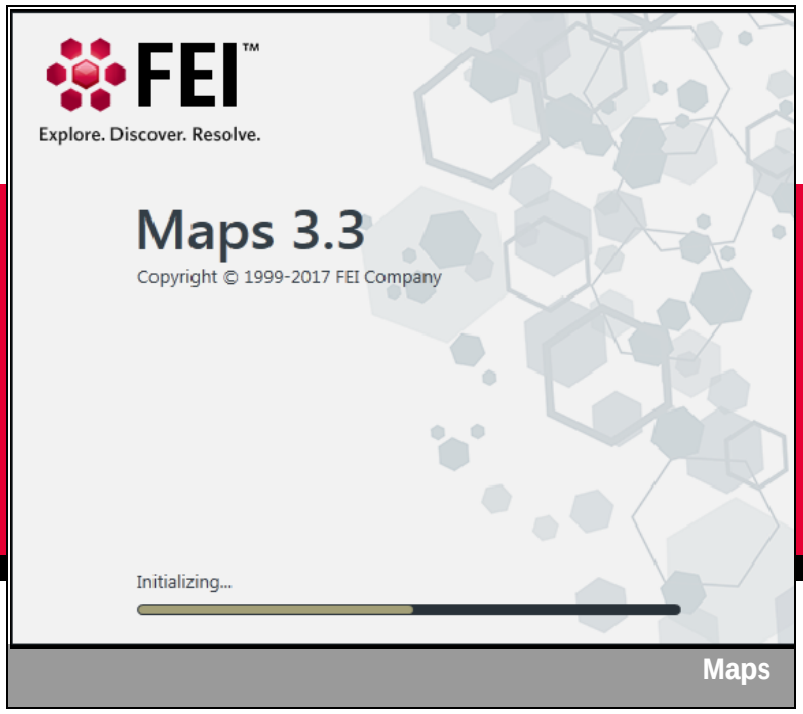
Maps 3.3 User Guide

PN 1148913-A



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1 Overview

Overview

This document describes the Maps 3.3 application software used on SEM/SDB, CorrSight™, and TEM systems.

Topics include:

- *"Introduction" on page 3*
- *"Terminology" on page 4*
- *"System Requirements" on page 5*
- *"Data Storage Requirements" on page 5*
- *"Project Directory" on page 6*
- *"Package Options" on page 7*

Introduction

The Maps software application provides users with the ability to focus on areas of interest within a sample region by organizing the sample into tiles and then navigating, selecting, and overlapping tiles so they can be viewed and analyzed in a higher level of detail than previously available.

The application will:

- Create a grid of a sample to enable efficient navigation and previews of selected tiles.
- Acquire tiled images in an automated fashion from multiple sample locations, with correlation between multiple layers.
- Find stitching information between tiles in an automated or semi-automated fashion using cross-correlation.
- Stitch and blend tiles into larger images with the capability to export them into common industry formats for viewing.
- Provide the capability to interactively display and investigate images constructed from multiple locations and layers of the sample.
- Process powerful workflows to do alignments or array tomography.

Maps complements the Correlative Workflow features that enable you to move samples between light microscopes and electron microscopes with the help of automated alignment on supported sample holders and a suite of powerful correlation tools.

Maps provides a solution for correlative microscopy by facilitating workflows that involve optical microscopy, SEM, and/or TEM. Both automated and manual alignments are used for correlation of the data to truly understand the sample.

NOTE	When upgrading from version 1 or 2 of Maps, a new license key is required to use Maps 3.

Terminology

- **Alignment**: In Maps, adjusts project data to the current stage position.
- **Blending**: The Maps software will create a smooth transition between images during stitching.
- **Correlation**: In Maps context, correlation is used for two things: 1. finding back areas of interest that show up in different modalities, and 2. for visually overlaying data sets that correspond to the same area on the sample.
- **CorrSight**: Fluorescent microscopy system for correlative workflows.
- **EM**: Electron microscopy
- **FIB**: Focused Ion Beam
- **HD View**: A Microsoft Research image data format used for gigapixel-resolution image data.
- **Holder Calibration:** This procedure is used to adjust the expected fiducial positions for automated holder alignment on individual systems. It must be performed the first time alignment is run on a supported holder. See the *"Automated Holder Alignment" on page 168* section on Calibration.
- **Image Registration**: The computational process Maps uses to determine the position of a single tile within the greater tile set. The Image Registration process is run during tile set acquisition.
- **Live Acquisition (LA)**: The CorrSight platform software.
- **LM**: Light microscope
- **LM**: Low Magnification mode on TEM.
- **Maps Offline Viewer**: A version of Maps that has read-only capabilities for Maps projects.
- **MIP**: *(CorrSight only)* Maximum intensity projection. Collapses a Z-Stack of images into a single image using the highest intensity pixels from the stack.
- **Nav-Cam**: Navigation Camera
- **SDB**: Small DualBeam; a family of microscopes that have both scanning electron and ion columns.

- **Segmentation**: In the context of images, segmentation is the process of assigning a label to specified pixels in an image such that pixels with the same label share certain visual characteristics, such as color.
- **SEM**: Scanning Electron Microscope
- **Stitching**: The computational task of combining a set of individual images to form a single image.
- **STEM**: Scanning Transmission Electron Microscopy. STEM mode exists on SEMs, SDBs and TEMs (depending on the exact configuration).
- **TEM**: Transmission Electron Microscope
- **TIA**: On TEM, the instrument user interface consists of two parts: TEM User Interface for instrument control and TEM Imaging and Analysis for acquisition and analysis.
- **Tile Acquisition**: The automated task of acquiring, aligning, and registering tiled images, as defined by the user.
- **Tile Grid:** The empty grid that defines where and how a set of tiles will be acquired.
- **Tile Set**: The output of a tile grid once acquisition is run, or the set of actual tiled images.
- **xT Software**: Platform software for the SEM/SDB microscopes.
- **xT UI**: xT software user interface
- **Z-Stack**: A sequence of images through the z-axis, used to build a 3D volume.
- **Z-Stack Browser**: The Maps UI component that allows you to view and select image planes from a z-stack.

System Requirements

- 3 GB of physical RAM
- 4.5 GB of memory paging allocated in Windows®
- Dual-core processor
- For Microscope Operation, microscope system software (xT, TEM Server, or LA) must be running before launching Maps

Data Storage Requirements

You may need additional internal or external storage capacity to acquire large tile sets. You will be prompted to select a default location for project data. It is suggested that you place the Project Data Directory on the D drive or a supplemental data storage device such as a network drive, external USB drive, or additional internal hard drive. Acquisition speed may be reduced when saving project data to USB or network locations.

The table below indicates the number of tile sets you will be able to acquire at various image resolutions and dimensions, provided that the Project Data Directory is configured to save to a standard empty 350 GBD drive partition.

NOTE	For multiple channel acquisitions, the storage requirements will increase incrementally with the number of channels.

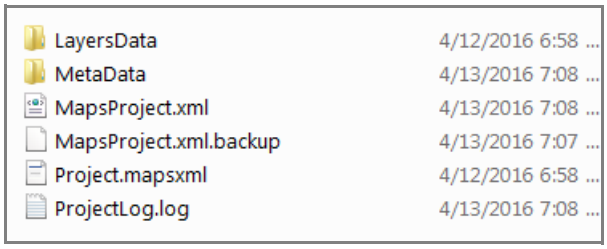
Table 1 Data Storage Requirements for Single Channel Image Acquisition

Resolution	Tile Set Size			
	5x5	10x10	50x50	100x100
512	15 MB	100 MB	1.25 GB	5 GB
1 K	55 MB	200 MB	5 GB	20 GB
2 K	200 MB	700 MB	20 GB	75 GB
4 K	750 MB	2 GB	75 GB	250 GB
8 K	4 GB	10 GB	250 GB	1.00 TB

Color Key:	
Red	< 1 Tile Set
Orange	1 to 2 Tile Sets
Yellow	2 to 5 Tile Sets
Green	> 4 Tile Sets

Project Directory

At the root directory, you will see the following:



The LayersData folder contains a directory structure that mimics the Layer tree in Maps, so you can manually locate the image files.

Launching Maps from a New File in the Project Directory

Double-click *Project.mapsxml* to launch Maps and automatically open the project from the directory in which this mapsxml file exists.

Package Options

This manual covers all options available with the Maps software. The About box lists the licensed options as determined by your product license key.

Contact your Thermo Fisher representative for licensing information.

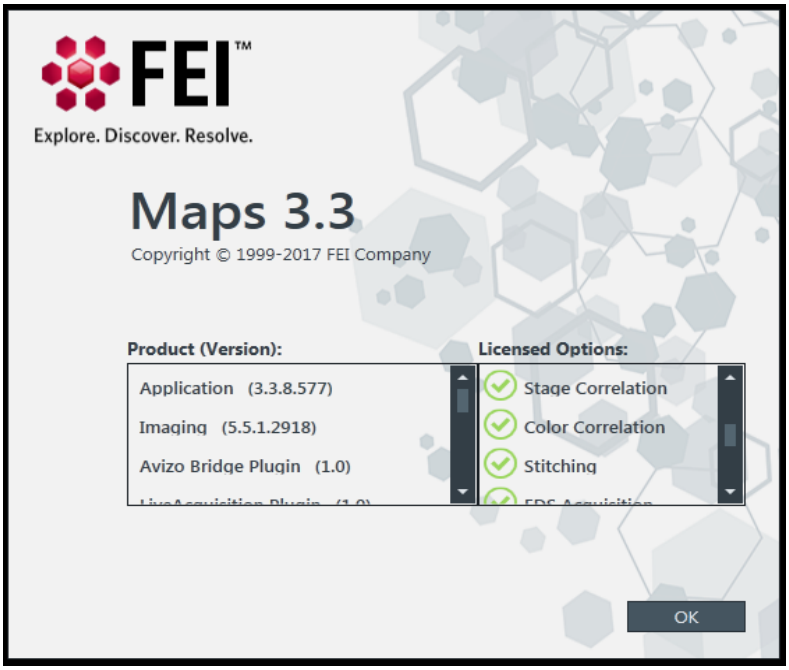


Table 2 Maps Licensed Options (1 of 2)

Option	Description
Big Snapshot	Licensed to acquire high resolution square images (4k and 8k) on supported xT microscopes.
xT Online	Licensed to run on the xT family of SEM and SDB microscopes, such as Helios NanoLab™, Quanta™, Versa, etc.
LA Online	Licensed to run on CorrSight LM.
TEM Online	Licensed to run on TEM family of microscopes, such as Titan, Talos, etc.
Array Tomography	Licensed to enable the Array Tomography workflow.
Annotations	Licensed to create annotations in the project.

Table 2 Maps Licensed Options (2 of 2)

Option	Description
Project Editing	Licensed to edit projects. Without this projects are opened read-only.
External Image Import	Licensed to allow importing of images from any source (for example, other microscopes, cameras, CAD exports, etc.)
Stage Correlation	Licensed to allow 1–3 point alignment.
Color Correlation	Licensed to allow correlation features including segmentation and color maps.
Stitching	Licensed to allow stitching of acquired tile sets.
Array Tomography	Licensed to enable features involved with the Array Tomography use case.
Maps Offline Viewer	Installations of Maps Offline Viewer are licensed only for viewing datasets that have been generated by the full version of Maps.

2 User Interface

Overview

This document describes the Maps 3.3 application software user interface.

Topics include:

- *"Default Shortcut Keys" on page 10*
- *"Viewer Shortcut Keys" on page 11*
- *"Main Screen" on page 13*
- *"Menus" on page 15*
- *"Tool Bar" on page 30*
- *"Layer/Holder Definition Control (SEM/SDB and CorrSight Only)" on page 33*
- *"Tile Set Tab" on page 33*
- *"Results Tab" on page 34*
- *"Layer Control" on page 41*
- *"Job Queue" on page 49*
- *"Viewer" on page 51*
- *"Rotation Control in the Viewer" on page 60*
- *"Micron Bar" on page 60*

Default Shortcut Keys

Table 1 Shortcut Keys

Keys	Description
F1	Opens the *Maps 3.3 SW Application User Guide.*
F2	Saves the screenshot with an automatically incremented file name. Same as selecting **File > Save Screenshot.** See *"Save Screenshot" on page 19*.
Space	View all objects (zoom out).
Ctrl+Shift+C	Centers the view on the current layer, same as *"Center View on Selection" on page 32* and *"Center View" on page 44*.
Ctrl+G	Shows/hides grid lines for the current layer, same as *"Show Grid Lines" on page 47* (Tile Set right-click menu selection).
Ctrl+H	Hides all graphic elements in the viewer, leaving only the images. Same as selecting **Options > Hide Annotations**. See *"Hide All Annotations" on page 27*.
Ctrl+L	Opens the Log Viewer. Same as selecting **Help > Open Logs**. See *"Open Logs" on page 29*.
Ctrl+O	Opens the project folder. Same as selecting **File > Open Project Folder**. See *"Open Project Folder" on page 19*.
Arrows	Moves selected tile set in small increments.
Shift + arrow	Moves selected tile set in finer increments.
Ctrl + left-click or drag	Selects tiles.
Shift + left-click	Selects tiles by row.

Viewer Shortcut Keys

There are three profiles defined for Viewer's shortcut keys (Application settings).

- *"Default Profile" on page 11*
- *"Classic Maps Profile" on page 12*
- *"Avizo Profile" on page 12*

Default Profile

Table 2 Viewer Default Profile Shortcut Keys

Keys	Description
Ctrl + mouse wheel +/-	Zooms in and out.
Mouse wheel	Zooms in and out slowly.
Shift + Ctrl + left-click and drag	Zooms the image to the selected region.
Left-click and drag	Pan (move)
Shift + left-click and drag	Rotates the viewer without moving the stage.
Alt + left-click and drag	Draw a feature of interest rectangle with a number of available options. See options. See *"Alt +Left-Click and Drag Selected Area" on page 52*.
Left-click	Selects an object.
Ctrl + left-click	Selects multi objects.
Escape	Deselects all objects.
Shift + left-click	Selects tiles by row.

Classic Maps Profile

This profile defines the input as in previous Maps 2.5 version.

Table 3 Viewer Classic Maps Profile Shortcut Keys

Keys	Description
Ctrl + mouse wheel	Zooms in and out.
Shift + mouse wheel	Zooms in and out slowly.
Shift + Ctrl + left-click and drag	Zooms the image to the selected region.
Shift + left-click and drag	Pan (move)
Shift + right-click and drag	Rotates the viewer without moving the stage.
Left-click and drag	Draw a feature of interest rectangle with a number of available options. See options. See *"Alt +Left-Click and Drag Selected Area" on page 52*.
Left-click	Selects an object.
Ctrl + left-click	Selects multi objects.

Avizo Profile

This profile defines the input as in Avizo® software.

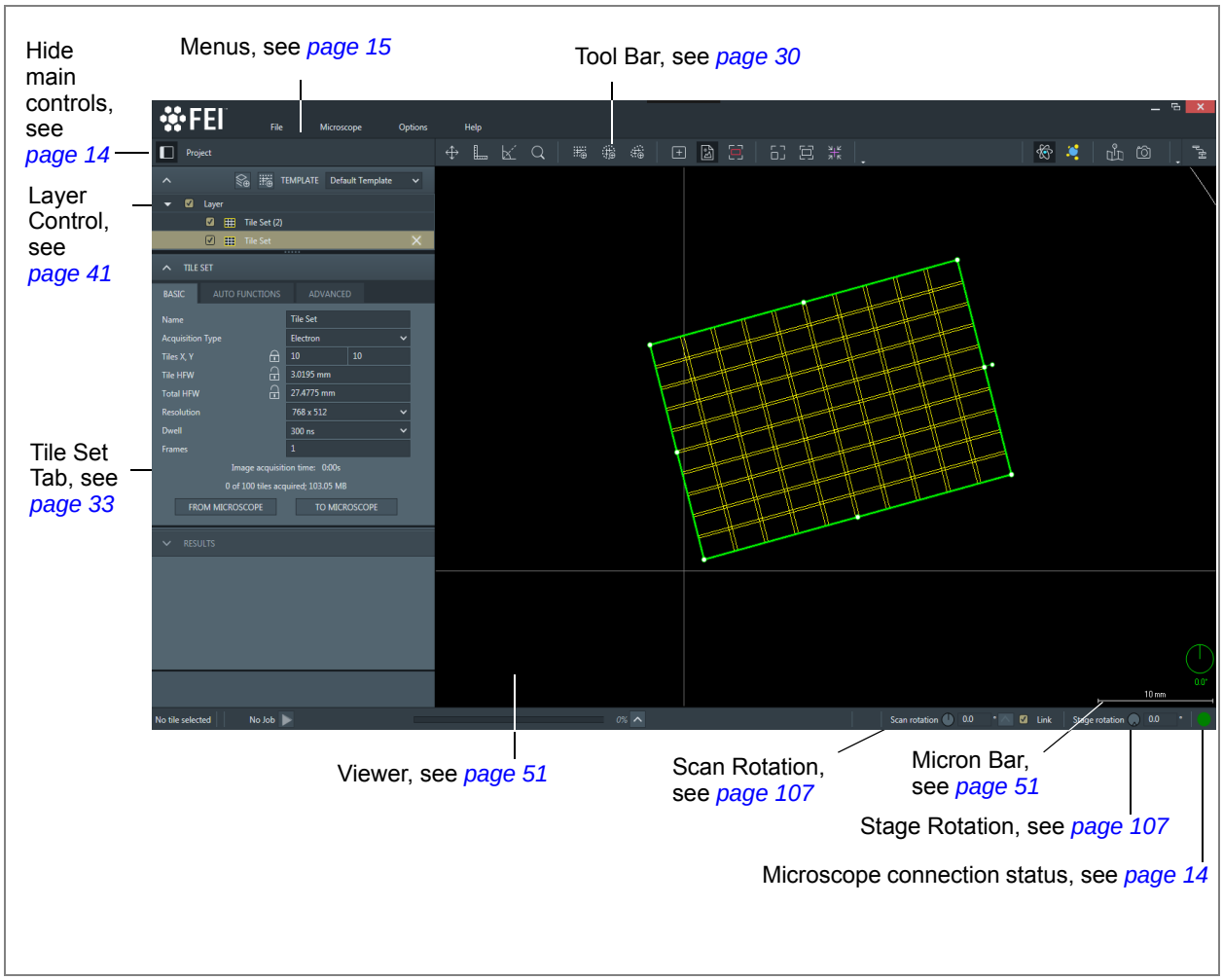
Table 4 Viewer Classic Maps Profile Shortcut Keys

Keys	Description
Ctrl + middle-click and drag	Zooms in and out.
Mouse wheel	Zooms in and out slowly.
Shift + Ctrl + left-click and drag	Zooms the image to the selected region.
Middle-click and drag	Pan (move)
Left-click and drag	Rotates the viewer without moving the stage.
Ctrl + Left-click and drag	Draw a feature of interest rectangle with a number of available options. See options. See *"Alt +Left-Click and Drag Selected Area" on page 52*.
'"P" + Left-click	Selects an object.
Ctrl + left-click	Selects multiple objects.

Main Screen

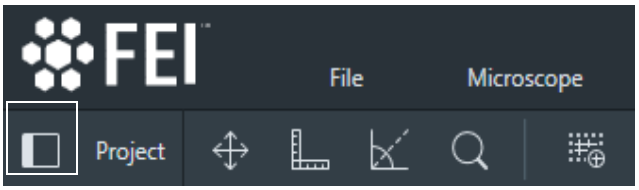
Figure 1 shows the main user interface.

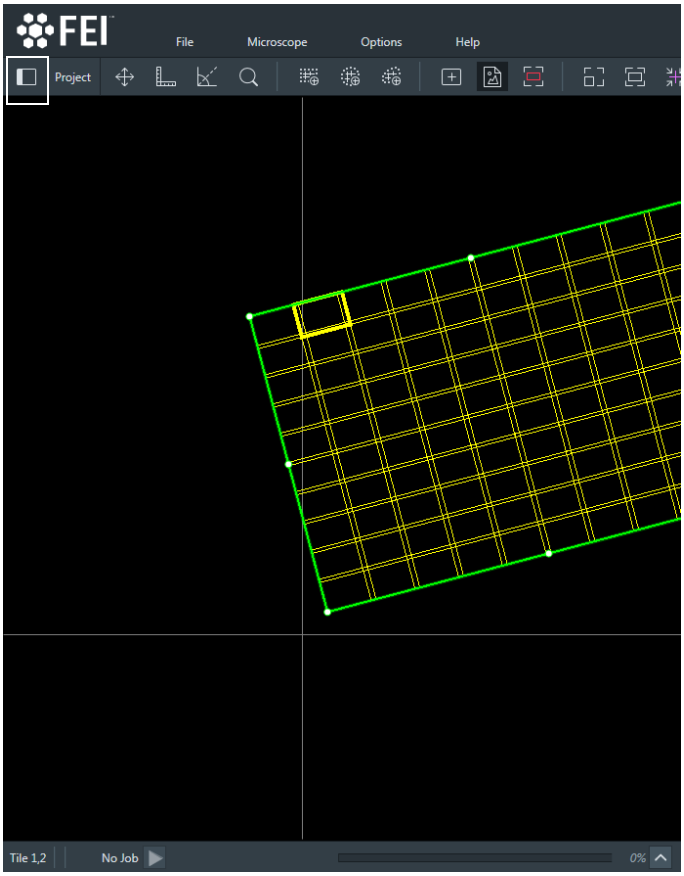
Figure 1 Maps User Interface, Control Panel Showing



Show/Hide Main Controls

Click the last icon on the left side of the tool bar to hide the main controls. Click the icon again to show the main controls.





Microscope Connection Status

The green dot in the lower right corner indicates that the application is connected to the microscope. Hover the mouse over the green dot to see a tool tip *Connected to Microscope*.

- If the application is started with no microscope, the dot will be red to indicate offline mode.
- If Maps is unable to connect to the microscope, verify that the microscope control software is running and restart Maps.

For the CorrSight, the yellow dot indicates that the microscope is trying to connect.

Interface Button States

- **Enabled**: White text on dark background
- **Disabled**: Gray text on darker background

Menus

Topics include:

- File, below
- *"Microscope Menu" on page 88*
- *"Options" on page 22*
- *"Help" on page 28*

File

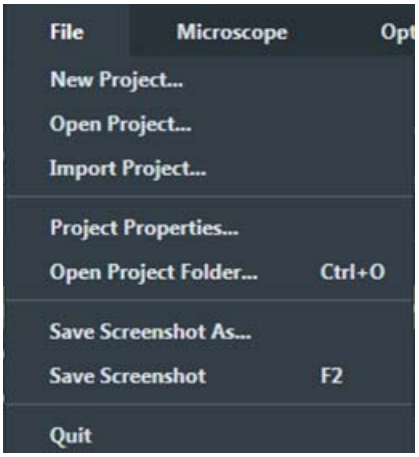


Table 5 File Menu Overview (1 of 4)

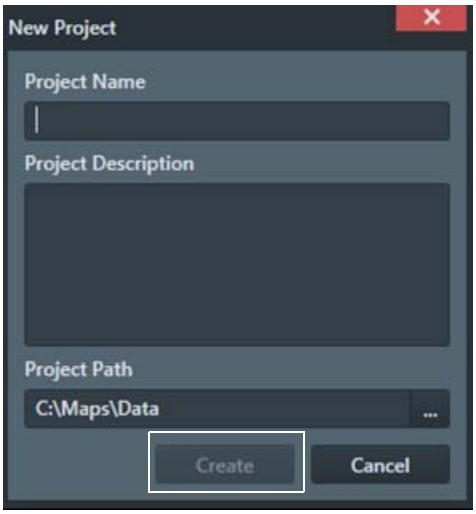
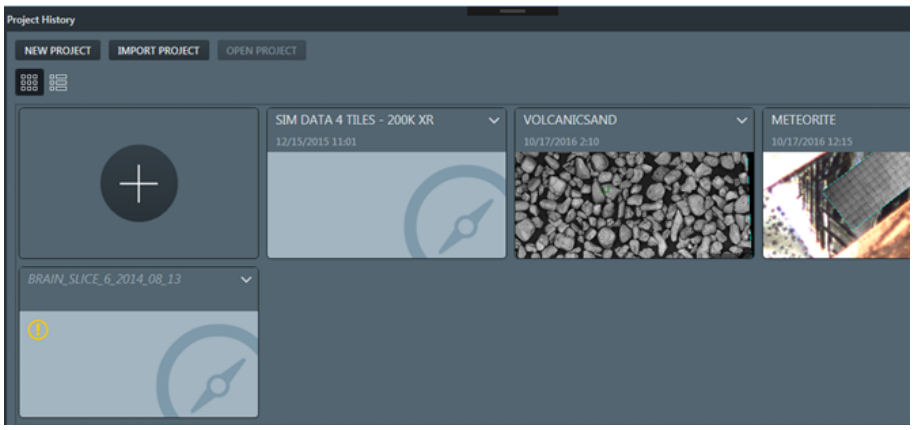
Menu Selection	Description
New Project	Displays the New Project window. Define a new Project Name and Description and click **Create**.  See *"New Project" on page 64*.
Open Project	Displays the Project History window showing all previous projects. The exclamation icon and a grey project title in italics indicates that the current project is not available (on a removable device). 

Table 5 File Menu Overview (2 of 4)

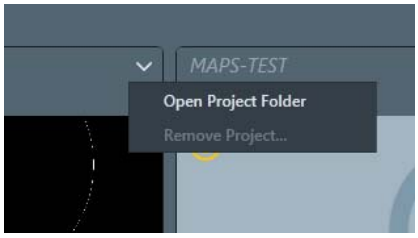
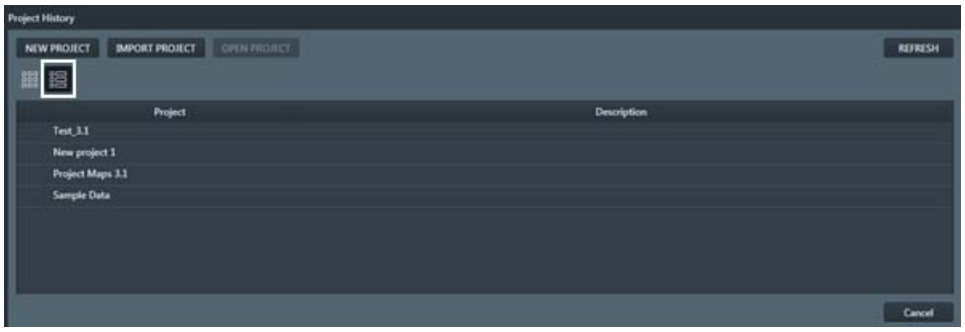
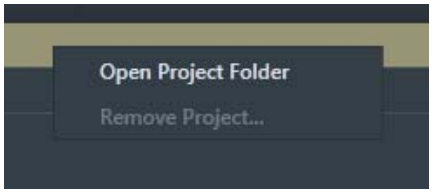
Menu Selection	Description
Open Project (Cont.)	• Click **New Project** to access the New Project window for creating a new project. • Click **Import Project** to display the Import Project window for importing a project. This is the same functionality as selecting **File > Import Project.** • Click **Open Project** to select an existing project. • Click the down arrow menu of the selected project to view two submenus: **Open Project Folder** and **Remove Project...**  • Click **Refresh** to refresh the status of the project list (e.g., Project not found). • Double-click on a thumbnail to select an existing project. • Click the **List View** icon to switch to a list view of projects.  • Right-click a project name in the list view to access the submenu for opening a project folder and removing a project.  • See *"Open Project" on page 66*.

Table 5 File Menu Overview (3 of 4)

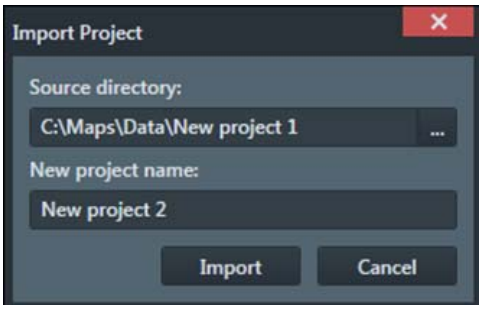
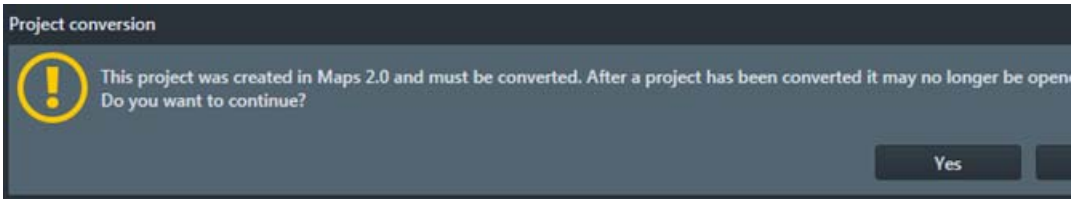
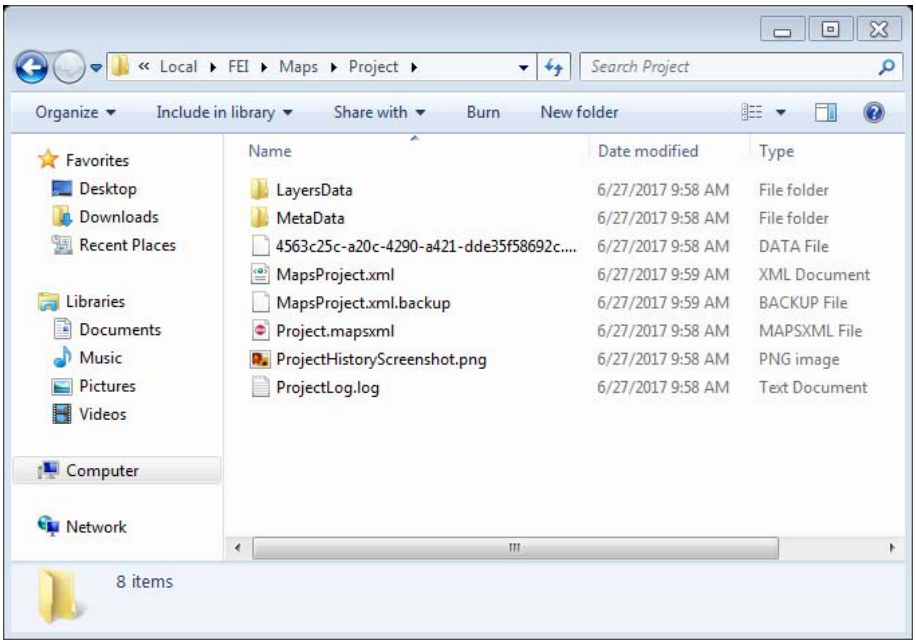
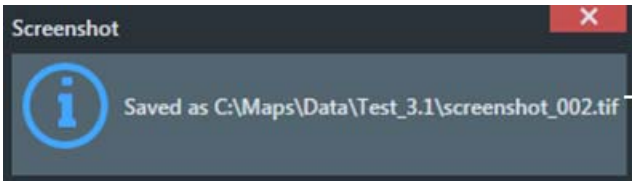
Menu Selection	Description
Import Project	Displays the Import Project window. Browse to a Source directory and select a project, rename it if appropriate, and then click **Import**. The imported project name will appear in the Project History list. This can be used for passing projects between computers. 
Import Project (Cont.)	**Note:** *The import process is only one-way. Older software projects must be converted. When a project is imported from Maps v1.1 and 2.0, a warning is displayed telling the user that the import is one-way and the project will no longer be compatible with v1.1 and 2.0.*  See *"Import Project" on page 67*.
Project Properties	Displays the Project Properties window for selecting the Project Path, Sample Holder, and a project description. See *"Project Properties" on page 20*.

Table 5 File Menu Overview (4 of 4)

Menu Selection	Description
Open Project Folder (Ctrl+O)	Displays the project folder. This can also be accessed by selecting **Open Folder** in the Project Properties window. 
Save Screenshot As	Displays a standard Windows Save As dialog box for saving a screen capture of the Maps viewer. Used for selecting a file name and a file type for saved viewer screenshots.
Save Screenshot (F2)	Saves the screenshot with an automatically incremented file name. Briefly displays a dialog box with the saved (automatically incremented) file name. 
Quit	Closes the Maps software.

Project Properties

View the attributes of the current project and modify the selected sample holder and project description here.

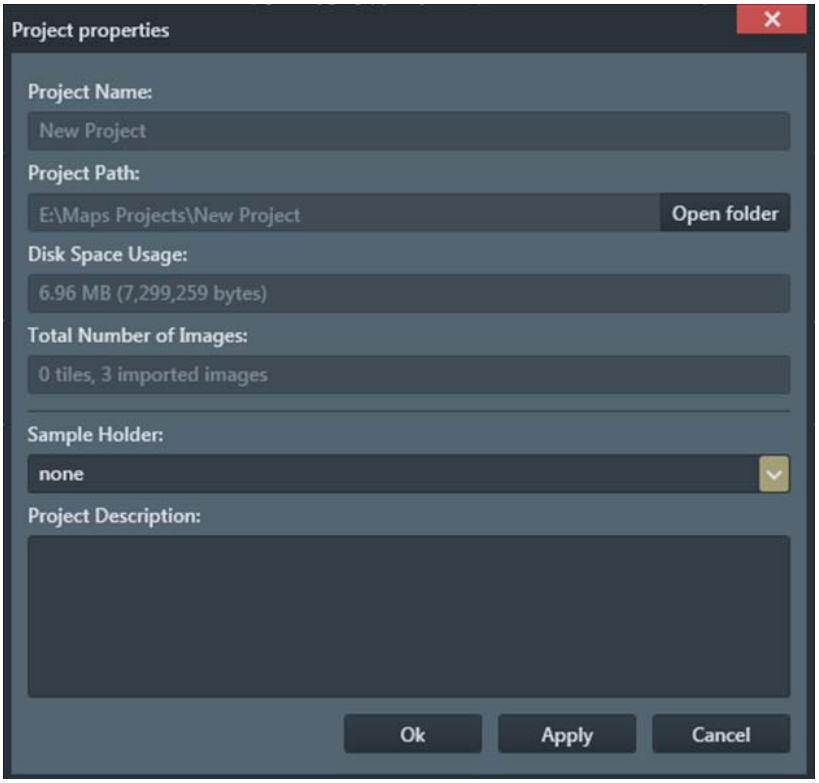
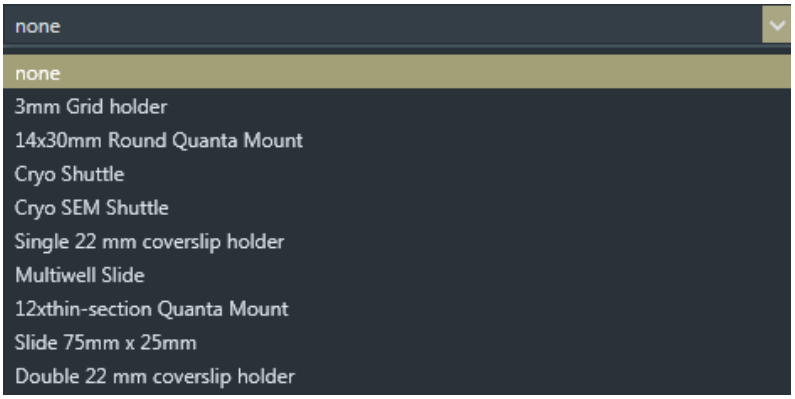


Table 6 Project Properties Overview

Interface Item	Description
Project Name	Displays the name given to the project.
Project Path	Displays the folder and location of the selected project.
Open Folder	Displays the project folder. This can also be accessed by selecting **File > Open Project Folder**.
Disk Space Usage	Displays the amount of disk space used by the project.
Total Number of Images	Displays the number of tiles and imported images for the project.
Sample Holder	Displays a dropdown list of possible sample holders that support automated alignment on SEM/SDB or CorrSight microscopes.  Make a selection and then click **Apply** to see the holder outline displayed on the main screen. *See "Sample Holders" on page 157 for more information.*
Project Description	Displays the description of the current project.
OK	Applies the selections and closes the window.
Apply	Applies the selection only.
Cancel	Cancels any changes to the Project Properties and closes the window.

Sample Holders

When a holder is selected and applied to the project:

- The outline displays in green on the viewer. The holder outline is red until it is aligned.
- The name, drawing, version, etc. display on the Holder tab.
- A new Layer is added to the Layer Control. This is where you choose to show or hide the Holder outline and Fiducials. The Fiducials only show after image acquisition. The default is to not show them. Enable the check box to view your fiducial images.

Refer to your application specialist for procedural information for each sample holder type.

Options

System Views

The common selections are described in the following table. For system-specific selections, see:

- SEM/SDB: *"Options Menu" on page 89*
- CorrSight: *"Options Menu" on page 113*
- TEM: *"Options Menu" on page 135*

Figure 2 Options Menu, Specific Selections by System

Table 7 Options Menu, All Selections Overview (1 of 4)

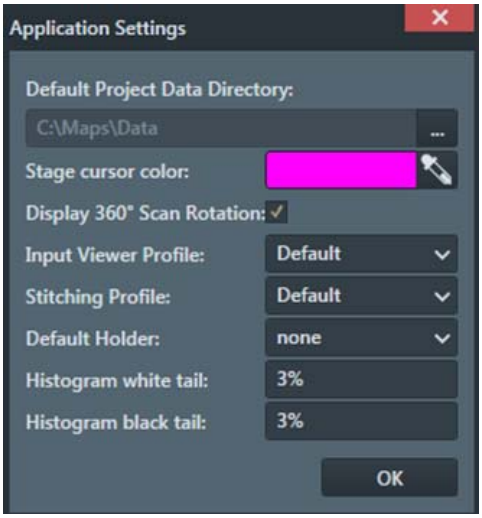
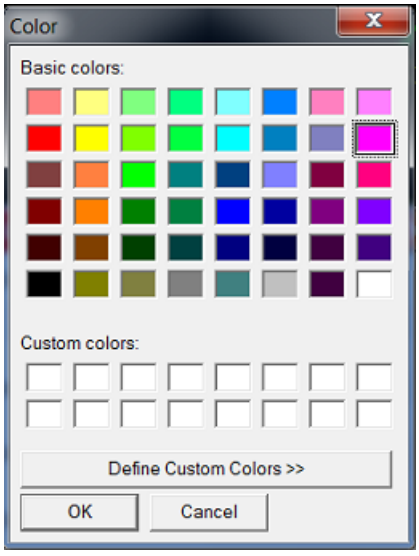
Menu Selection	Description
Application Settings:	Displays the Application Settings window for changing defaults for all new projects. 
Default Project Data Directory	Displays the default path with a browse button to allow you to choose a different path.
Stage cursor color	Click the browse button to access the Color picker to optionally change the stage cursor color. 
Display 360° Scan Rotation	• When selected, displays 360° scan rotation. • When not selected, displays –180° to 180° scan rotation.

Table 7 Options Menu, All Selections Overview (2 of 4)

Menu Selection	Description
Input Viewer Profile	Displays a dropdown list of viewing choices.  Choices are: • **Default:** *See "Default Profile" on page 11 for detailed inputs.* • **Avizo**: The inputs defined for the viewer are based on Avizo/Amira® software. *See "Avizo Profile" on page 12 for detailed inputs.* • **Classic Maps**: The inputs defined for the viewer are based on the previous Maps version (v2.5). *See "Classic Maps Profile" on page 12 for detailed inputs.*
Stitching Profile	Displays a dropdown list of stitching choices.  Choices are: • **Default:** The settings in this profile are intended for stitching a broad range of samples. One should generally start with this profile. • **Natural Structures**: This profile is intended for life science or geology samples. The main difference with the default profile is more tolerance in its assessment on alignment validity. Use this profile if the default settings mark too many tiles as invalid while the alignment is OK. • **Repetitive Structures**: This profile is intended for stitching of electronic devices, wafers, or repetitive structures. These settings force the algorithm to be more sensitive to edges and uses a different method of assessing alignment validity (as repetitive structures may mislead the image recognition algorithms). • **No Stitching**: The profile will not attempt to align the images. This will prevent any kind of stitching. Use this to save CPU resources when stitching is not desired, or if stitching will be done by another application. See *"Stitching Profile Best Practices" on page 27*.

Table 7 Options Menu, All Selections Overview (3 of 4)

Menu Selection	Description
Default Holder	Displays a pulldown list of possible sample holders. Select a holder to be used as a default for all new Maps projects. 
Histogram white tail	Trims a percentage of histogram pixels to eliminate noise.
Histogram black tail	Trims a percentage of histogram pixels to eliminate noise.
Tile Set Templates:	These are system-specific: • SEM/SDB: *"Tile Set Templates:" on page 90* • CorrSight: *"Tile Set Templates:" on page 113* • TEM: *"Tile Set Templates:" on page 136*
Microscope Settings:	These are system-specific: • SEM/SDB: *"Microscope Settings:" on page 90* • TEM: *"Microscope Settings" on page 137*
Snapshot Settings:	SEM/SDB: See *"Options Menu, SEM/SDB Overview" on page 90*
Do Not Change Lens Mode	SEM/SDB: See *"Do Not Change Lens Mode" on page 92*.
Snapshot Settings	TEM: See *"Snapshot Settings" on page 138*.
Prefer scan rotation	If there is viewer rotation when creating a new tile set, the rotation will either be applied as stage rotation or scan rotation. • **When selected**: Uses scan rotation. • **When not selected**: Uses stage rotation.
Stitch Tile Sets When Done	Automatically adds a stitching job to the end of the job queue for each completed acquisition job.
Turn Off Beam When Done	SEM/SDB: See*"Turn Off Beam When Done" on page 92*.
Retract Detectors when Done	SEM/SDB: See *"Retract Detectors when Done" on page 92*.

Table 7 Options Menu, All Selections Overview (4 of 4)

Menu Selection	Description
Sleep When Done	SEM/SDB: See *"Sleep When Done" on page 92*.
Pump to HiVac When Done	SEM/SDB: See *"Pump to HiVac When Done" on page 92*.
Close Column Valves when Done	TEM: See *"Close Column Valves When Done" on page 138*.
Switch off Emission when Done	TEM: See *"Switch off Emission when Done" on page 27*.
Hide All Annotations (Ctrl+H)	Hides all graphic elements in the viewer, leaving only the images.
Show Layer name Annotations	Displays an additional annotation of the tile set names to help identify data during viewing.
Animate Pan & Zoom	Allows a smoother transition of panning and zooming. *Note that this is a slower process and takes more memory.* • **Pan**: To make a sweeping movement • **Zoom**: To simulate movement rapidly away from or toward a subject using a zoom lens
Avizo Settings	See *"Maps-Avizo/Amira Connectivity Bridge" on page 213*.

Stitching Profile Best Practices

- Start with the Default profile and only change it if the default settings are not working well enough.
- Before starting a large job, to do a few small runs (e.g., 4 x 4 tiles) and **visually inspect** the result to find out what the best profile and acquisition settings are. Then use the same acquisition setting on the small runs as on the large run.
- If neither profile gives satisfactory results, try to improve the image quality via the acquisition settings; high quality image data has better chance to be successfully stitched. On SEM, consider using larger dwell times as higher image quality may reveal small features that facilitate image recognition
- If the image data is highly repetitive or very sparse, use a tile size such that there are enough distinct features present in each tile.

- When the success rate of the alignment is not sufficient, try to look at it from an image recognition point-of-view: the algorithm looks at the pixel data in the overlapping border regions; if there are not enough distinct features in that area, the alignment is very difficult. Also think about ambiguity. For example, if the overlapping area only contains a horizontal line, the vertical alignment is trivial but horizontally any position is a valid solution. The tile size, overlap, or the image resolution may need to be increased in order to get data that aligns well.
- On SEM/SDB, recommended overlap for systems with a mechanical stage (e.g., Quanta FEG):
  - HFW > 50 µm overlap: 10%
  - HFW 20–50 µm overlap: 15%
  - HFW < 20 µm overlap: 20%

  If stitching is still unsuccessful, consider increasing the overlap even more.
- On TEM, recommended overlap:
  - Low Magnification: 20%
  - Magnification 3000x–50000x: 10%–15%
  - Magnification > 50000: 15%–20%

NOTE	The amount of required overlap is strongly related to how well the system is aligned.

Help

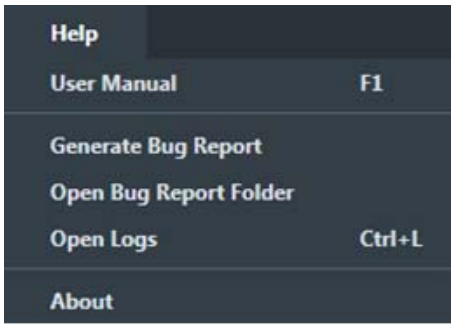
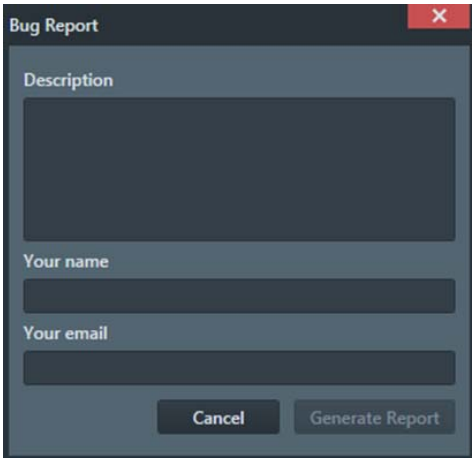
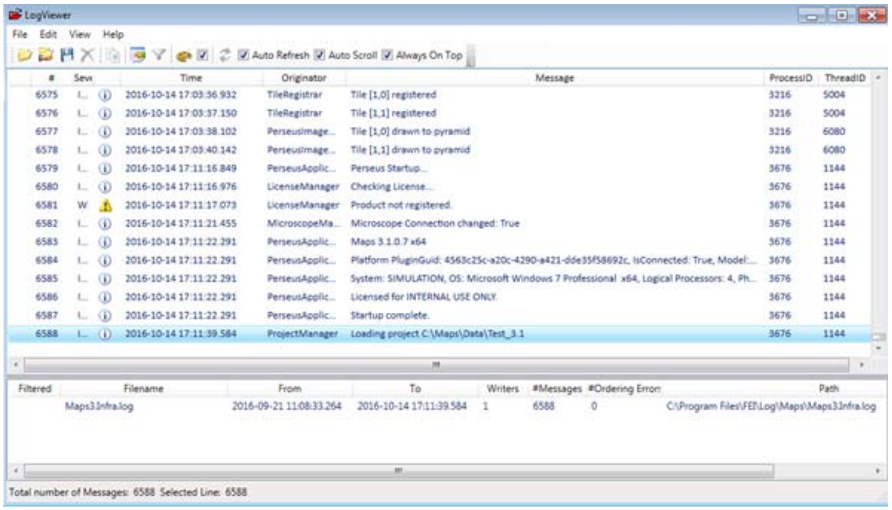


Table 8 Help Menu Overview

Menu Selection	Description
User Manual (F1)	Displays a PDF of this user guide.
Generate Bug Report	Displays the Bug Report dialog used to generate a bug report.  Enter a description of the issue, your name, and email address. Then click **Generate Report**. The Bug Report folder will automatically open. The Maps development team will contact you if more details are needed.
Open Bug Report Folder	Displays the folder where the generated bug reports are stored.
Open Logs (Ctrl+L)	Displays the Log Viewer. 
About	Displays the About box with information about the software versions and a list of installed licensed options. For descriptions of the options, see *"Package Options" on page 7*.

Tool Bar

The common selections are described in the following table. For system-specific selections, see:

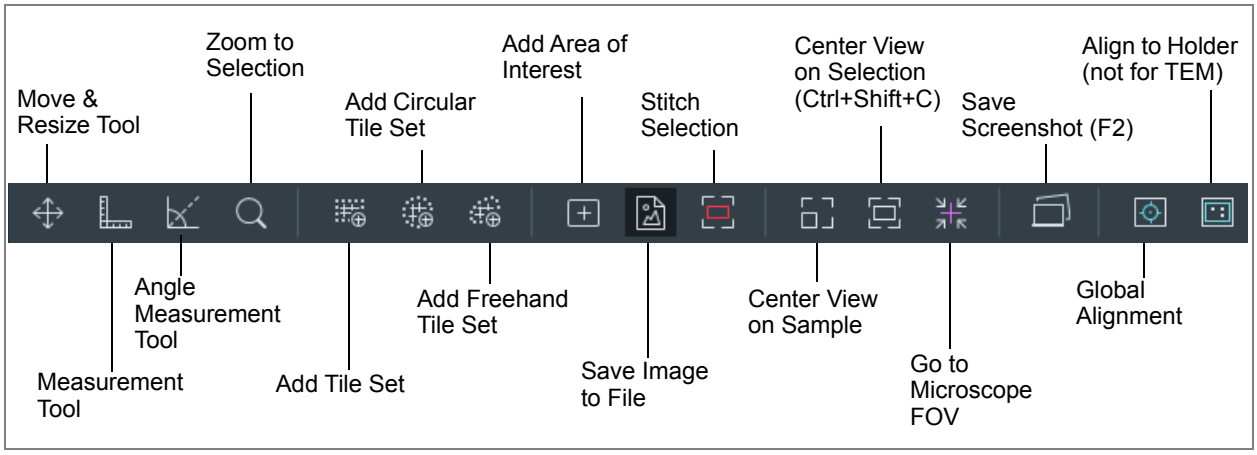
- SEM/SDB: *"Tool Bar, Right Side" on page 93*
- CorrSight: *"Tool Bar, Right Side" on page 114*

Topics in this section include:

- Left Side, All Systems, below
- *"Secondary Menu" on page 30*
- *"Tool Bar Descriptions" on page 31*

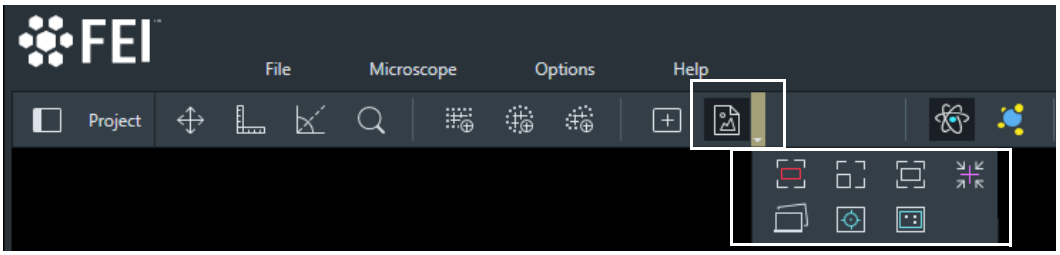
Left Side, All Systems

Figure 3 Tool Bar, Left Side, All Systems



Secondary Menu

If the window is resized too small to display all tools, some of them are stored in a secondary menu. Click the down arrow to access them.



Tool Bar Descriptions

Table 9 Tool Bar Descriptions (1 of 2)

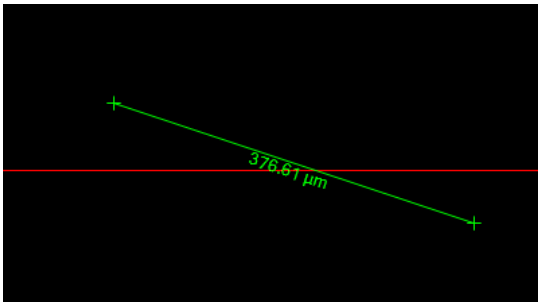
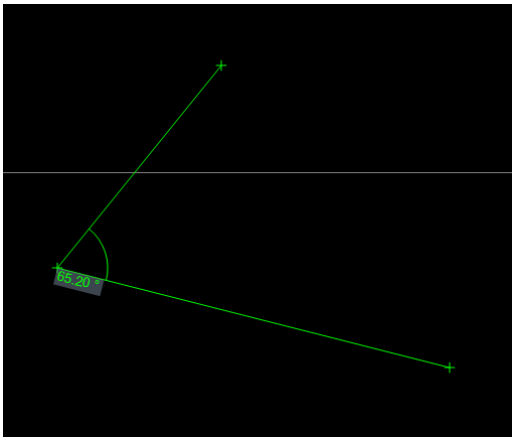
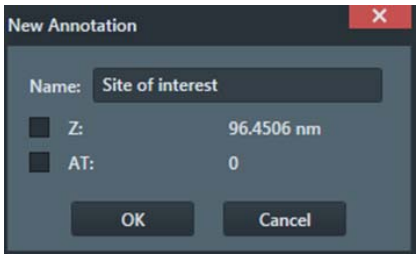
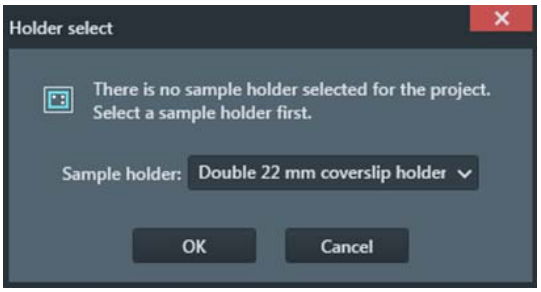
Tool	Description
Move & Resize Tool	Moves or resizes the tile grid with click and drag.
Measurement Tool	Draws a line and displays the length as a callout. 
Angle Measurement Tool	Draws a line and a third point; displays the angle as a callout. 
Zoom to Selection	Zooms the field of view to the selected area.
Add Tile Set	Adds a tile set over a selected area in the viewer.
Add Circular Tile Set	Adds a circular tile set over a selected area in the viewer.
Add Freehand Tile Set	Adds a freehand drawn tile set over a selected area in the viewer.

Table 9 Tool Bar Descriptions (2 of 2)

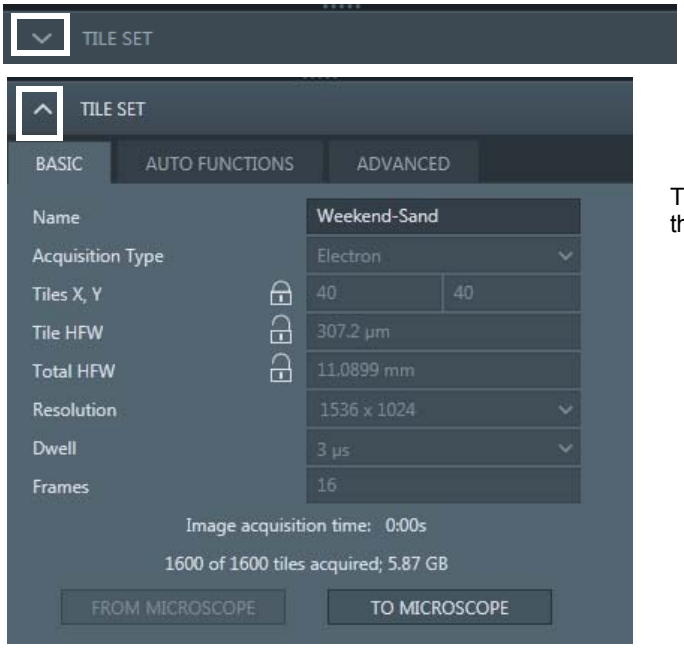
Tool	Description
Add Area of Interest	Displays the New Annotation dialog box for adding the drawn selected area of interest (AOI).  The new AOI becomes a new Annotation layer in the Layer control.
Save Image to File	Saves an image to file. See *"Save Image to File" on page 69*.
Stitch Selection	Stitches a subset of tiles, as selected by the rectangle. See *"Stitching" on page 99*.
Center View on Sample	Zooms out to the full sample view.
Center View on Selection (Ctrl+Shift+C)	*Disabled until a project is open.* Pans & zooms the viewer so that the currently selected layer item is centered.
Go to Microscope FOV	Pans and zooms the view to match the microscope (electron beam) field of view (FOV).
Save Screenshot (F2)	Briefly displays a dialog box with the saved (automatically incremented) file name. Also available from **File > Save Screenshot**.
Global Alignment	Performs a 1-, 2-, or 3-point alignment to align all previously acquired data to the stage. It is useful if the sample was removed from the tool and you want to continue with it. See *"1-, 2-, or 3-Point (Coarse) Alignment" on page 196*.
Align to Holder *(SEM/SDB and CorrSight only)*	Performs an Automated Holder Alignment; requires a specific holder hardware. If no sample holder has been selected for the project, a dialog box appears to prompt you to select a holder.  See *"Automated Holder Alignment" on page 168*.

Layer/Holder Definition Control (SEM/SDB and CorrSight Only)

- SEM/SDB: See *"Layer/Holder Definition Control" on page 94*.
- CorrSight: See *"Layer/Holder Definition Control" on page 115*.

Tile Set Tab

When you create a new tile set, the parameters for basic tile acquisition are populated with defaults. Information is grouped by category and opened by clicking the down arrow.



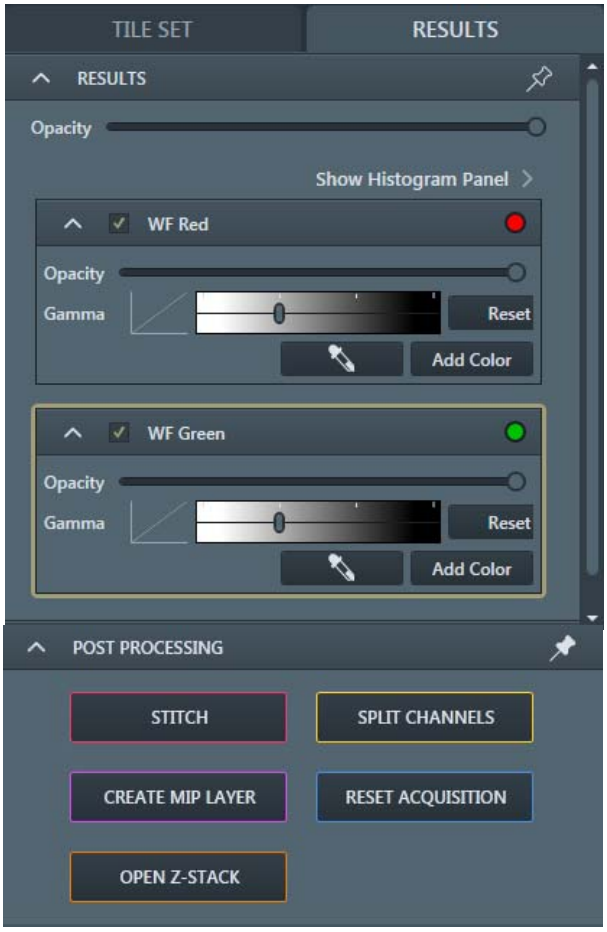
This example shows the SEM/SDB version.

The selections are system specific:

- SEM/SDB: *"Tile Set Tab" on page 95*
- CorrSight: *"Tile Set Tab" on page 116*
- TEM mode: *"Tile Set Tab, TEM Mode" on page 140*
- STEM mode: *"Tile Set Tab, STEM Mode" on page 144*

Results Tab

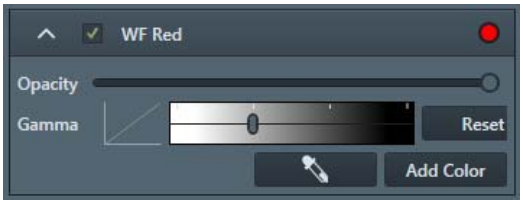
Use the controls on this tab to apply simple coloring to the tile set images when acquired and to do post processing functions. Some controls will appear disabled if they are not applicable to the system type. See *"Correlation" on page 202* for more information.



Topics in this section include:

- *"Results Tab Overview" on page 35*
- *"Post Processing Group" on page 35*

Table 10 Results Tab Overview

Control	Description
Opacity	Sets the measure of opaqueness for the entire image. The less opaque, the more transparent it becomes. Transparent images are useful when overlaying on image over another.
Show Histogram Panel	Shows/hides the histogram panel. See *"Histogram" on page 206*.
Channels	 • **Channel color selector**: Enables display of that channel. Shows the name and color. • **Opacity slider**: Adjusts opacity for this channel only. • **Gamma**: Displays the gamma curve adjustment. • **Reset**: Resets the Gamma slider to the default. • **Color picker:** Displays the color picker. Choose a unique color for each channel to display in the histogram. • **Add Color**: Launches Segmentation. Displays an additional histogram every time you click the button. See *"Segmentation" on page 208*.

Post Processing Group

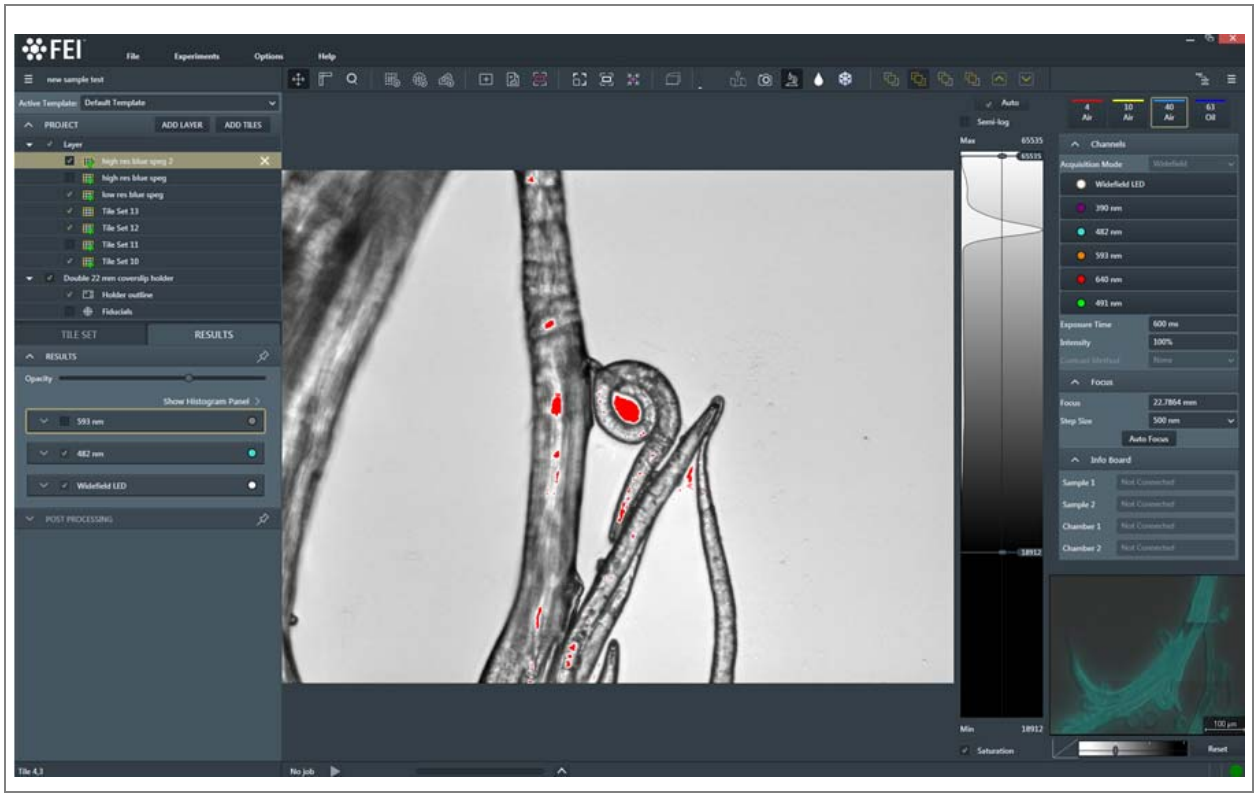
Table 11 Results Tab, Post Processing Group Overview

Control	Description
Stitch	Stitches the entire tile set.
Split Channels	Divides the individual channels of a tile set into separate tile sets. ***Note:*** *This operation is permanent and cannot be undone.*
Create MIP Layer	Collapses the Z-stack into a single image using the highest intensity pixels from the stack.
Reset Acquisition	Clears all acquired image data in the selected tile set and unlocks the parameter fields in the Basic tab for editing.
Open Z-Stack (*SEM/SDB and CorrSight only*)	Opens the Z-Stack Browser to view the focus stack of the tiles. See*"Z-Stack Browser Controls" on page 36*.

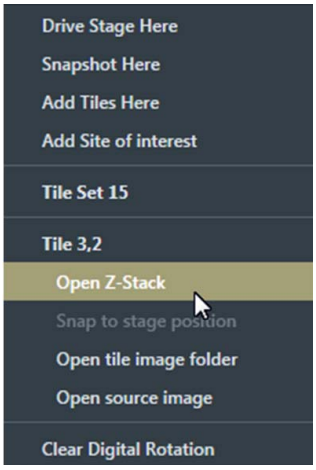
Z-Stack Browser Controls

The Z-Stack Browser allows you to select and view individual image planes in the Z-Stack.

Figure 4 Z-Stack Data Set in Viewer Before Opening Z-Stack Browser

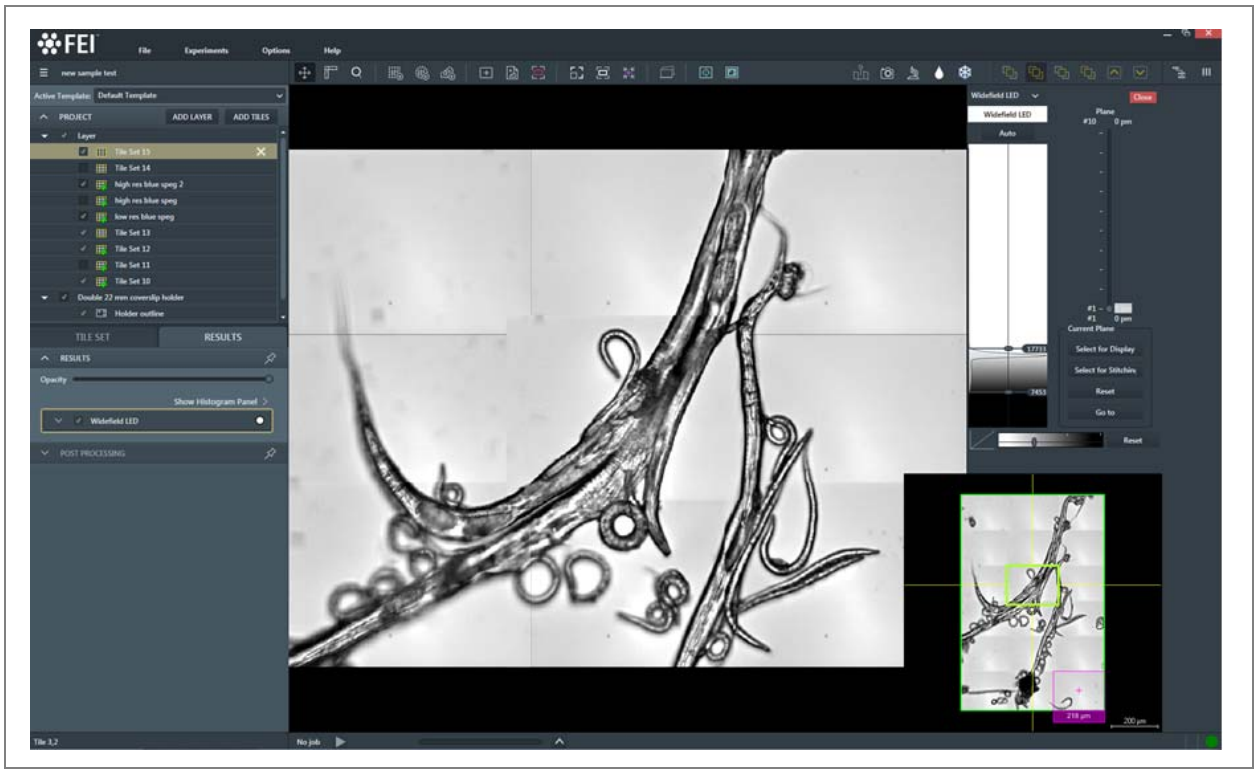


After acquiring a Z-stack tile set, right-click on a single tile in the tile set and then click **Open Z-Stack** in the menu that appears.



The Z-Stack Browser appears on the right-side of the interface.

Figure 5 Z-Stack Browser Open with Controls on the Right



Z-Stack Browser Controls

Figure 6 Z-Stack Browser Controls

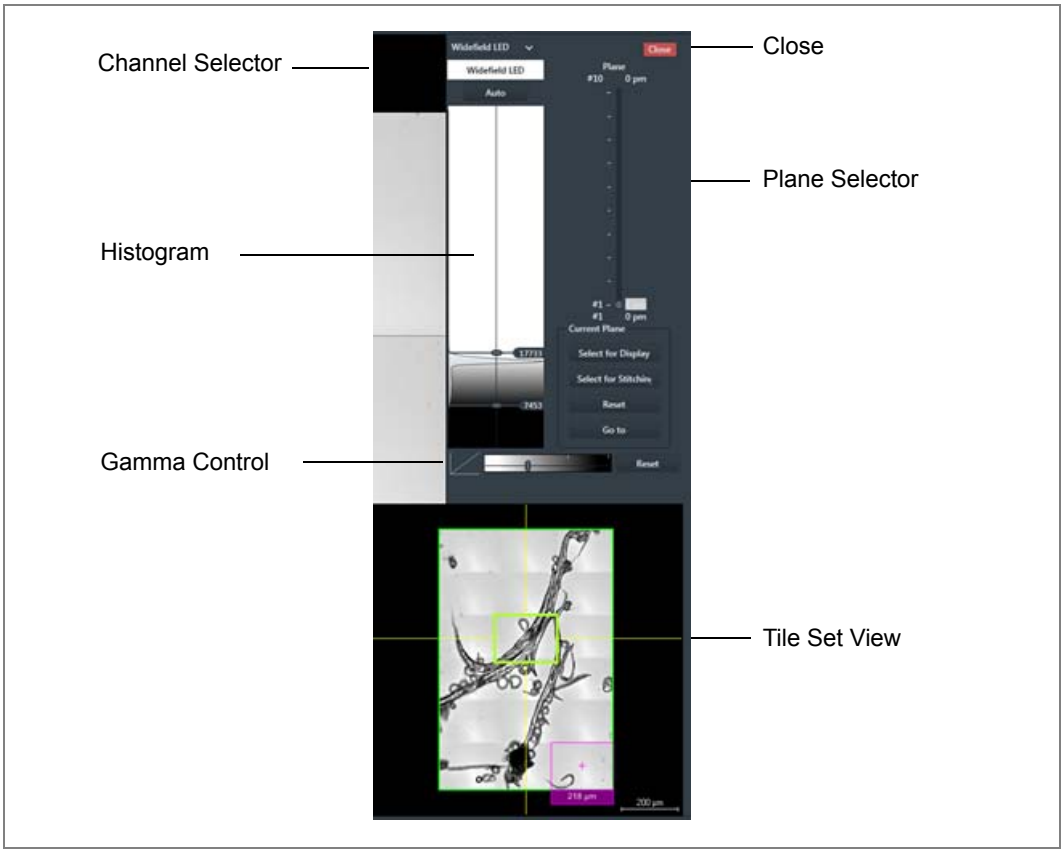


Table 12 Z-Stack Browser Controls Overview (1 of 3)

Interface Item	Description
Channel Selector	Selects the channel to be displayed in the multi-channel Z-Stack. The pulldown list contains the same choices as in the Microscope controls.
Histogram	Displays the same histogram as in the Microscope controls. See *"Histogram" on page 206*.
Close	Closes the Z-Stack Browser.

Table 12 Z-Stack Browser Controls Overview (2 of 3)

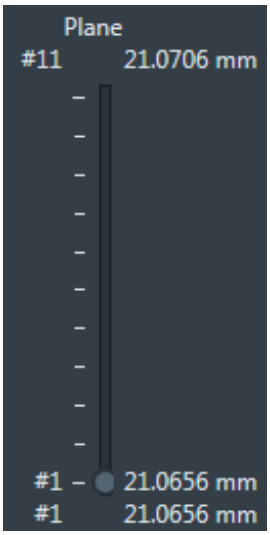
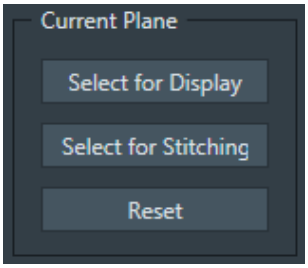
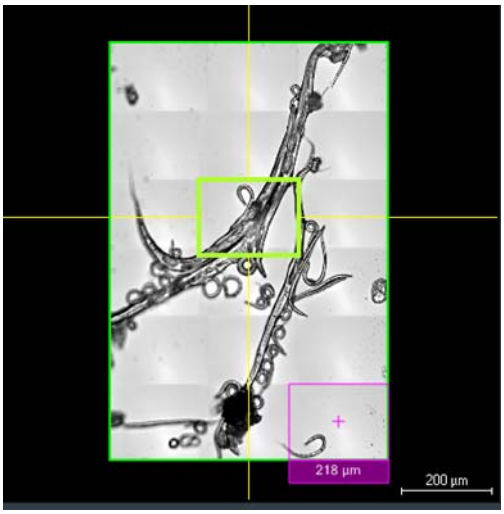
Interface Item	Description
Plane Selector	Selects the plane from the Z-Stack to be displayed with the channel selection. Move the slider or use the mouse scroll wheel to step through the planes. 
Current Plane Control:	
Select for Display	Selects the plane to be displayed in the main viewer after you close the Z-Stack Browser. ***Note****: Only one plane can be displayed in the main viewer.*
Select for Stitching	Selects the current plane to be used for the registration step for stitching. Choosing a plane with more image content is best.
Reset	Returns to the default gamma setting.
Go To	Sets the microscopes current focus to the value in the z stack viewer control.
Gamma Control	Adjusts the gamma for the live image.

Table 12 Z-Stack Browser Controls Overview (3 of 3)

Interface Item	Description
Tile Set View	Displays the selected tile (with a thick green outline) from the main viewer and its neighboring tiles. 

Layer Control

Use the controls to view existing layer items and create, delete, reorder, select, hide, and show layers. When a check box is not selected, that layer item will not be displayed in the viewer.

Double-click on an item in the Layer control to center it in the viewer. Topics in this section include:

- *"Layer Icons" on page 42*
- *"Running Layer Tasks Progress" on page 43*
- *"Annotation Right-Click Menu" on page 43*
- *"Layer Right-Click Menu" on page 44*
- *"Tile Set Right-Click Menu" on page 46*
- *"Preview Image Right-Click Menu" on page 48*
- *"Nav-Cam Right-Click Menu" on page 49*

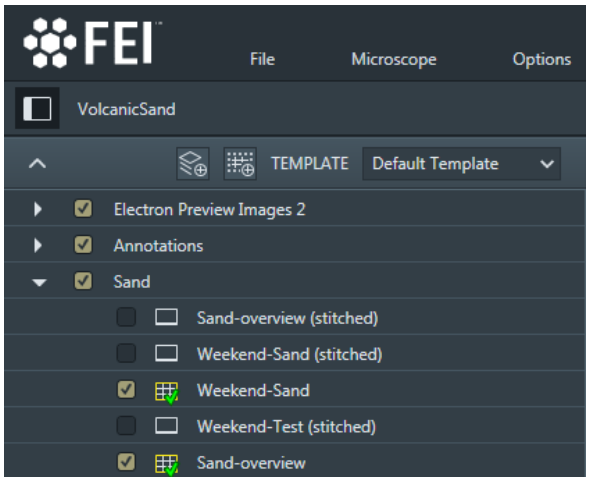
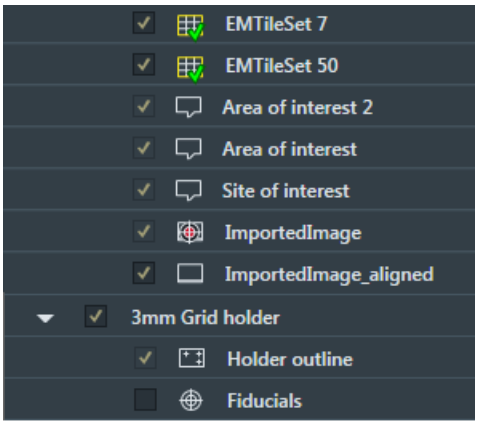


Table 13 Layer Control Overview (1 of 2)

Control	Description
Active Template	Displays the name of the default template for the connected microscope.
Add Layer	Adds another Layer to the tree. When you have multiple layers, the selected layer will be green and the non-selected layers will be yellow. 
Add Tiles	Creation of a tile set is only allowed if a microscope is connected. In that case, the tile set will use the type corresponding to the microscope, and the template specified in the **Active Template** field. 

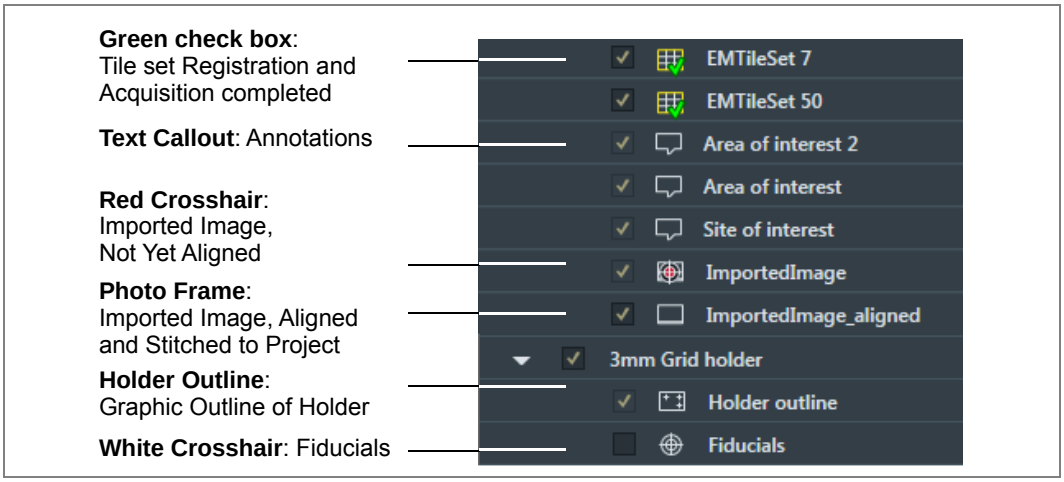
Table 13 Layer Control Overview (2 of 2)

Control	Description
Layer	Displays a list of existing tile sets, annotations (areas of interest), imported images (aligned and unaligned), sample holder, and fiducials for each layer.  See *"Layer Icons" on page 42*. Annotations are directly created in the layer selected at the time of creation.

Layer Icons

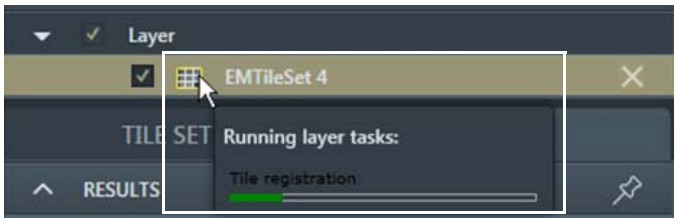
Each created layer item has a unique icon.

Figure 7 Layer Control Icons

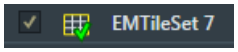


Running Layer Tasks Progress

- **Acquisition progress** is reported in the *"Job Queue" on page 49*.
- **Registration progress** is reported by hovering the mouse over the tile set symbol.



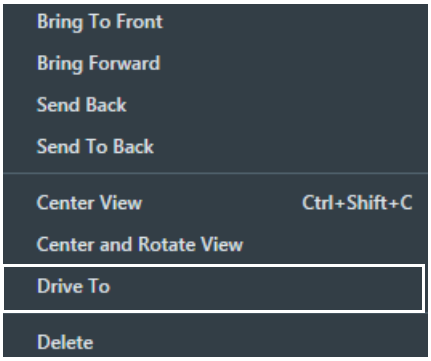
When both Registration and Acquisition have completed, a green check mark appears in the Tile set icon.



Annotation Right-Click Menu

Right-click on an Annotation in the Layer tree to access a context menu for moving the annotation layer forward or backward, centering and rotating the view, driving to, and deleting.

All of these menu selections, except **Drive To,** are common to most all other layer item context menus.



Unique selection for Annotation.

Table 14 Annotation Right-Click Menu Overview (1 of 2)

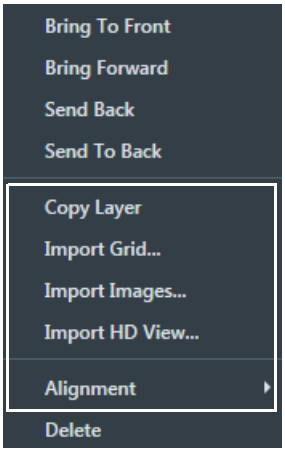
Menu Selection	Description
Bring to Front	Moves the (annotation) layer to the front (topmost) layer.
Bring Forward	Moves the (annotation) layer forward one layer.
Send Back	Moves the (annotation) layer back one layer.
Send to Back	Moves the (annotation) layer to the lowest layer.

Table 14 Annotation Right-Click Menu Overview (2 of 2)

Menu Selection	Description
Center View (Ctrl+Shift+C)	Centers the view on the created (annotation).
Center and Rotate View	Pan, zooms, and digitally rotates the viewer so that the currently selected (annotation) is centered and shown at its natural rotation.
Drive To	Drives to the created annotation.
Delete	Deletes the created (annotation).

Layer Right-Click Menu

Right-click on any Layer selection to access a context menu.



Unique selections for Layer.

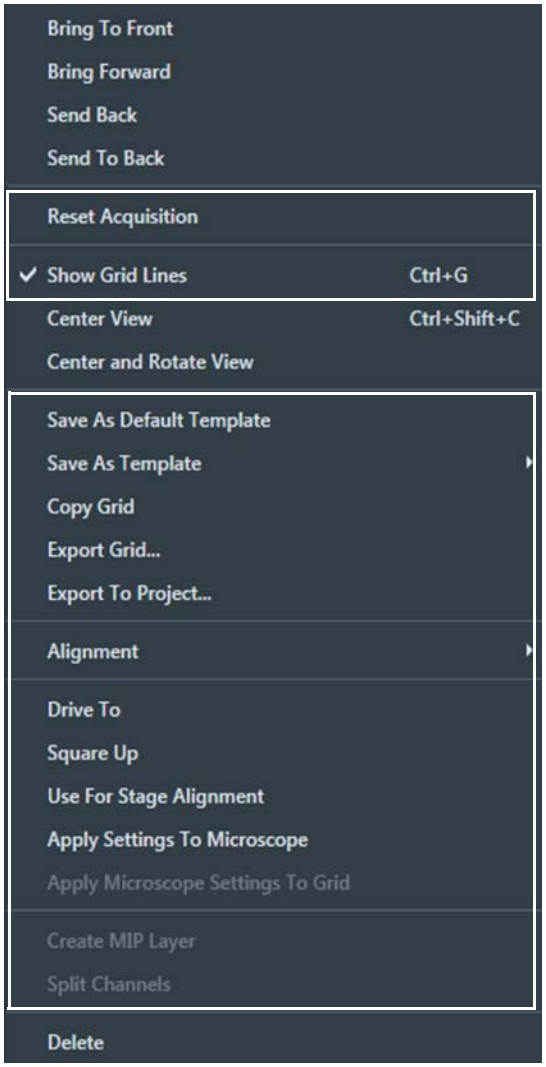
- For descriptions of common selections to all systems, see *"Annotation Right-Click Menu" on page 43*.
- For descriptions of Layer-specific selections, see the table below.

Table 15 Layer Right-Click Menu Overview

Menu Selection	Description
Copy Layer	Copies all tile sets in the layer to a new layer.
Import Grid	Displays a standard Open window for browsing to a saved tile grid file.
Import Image	Displays a standard Open window for browsing to a saved image file from another source, such as an optical microscope. These images, once aligned to the stage, can provide a navigation guide for you to find areas of interest, like the Nav-Cam does. See *"Importing Images from Other Sources" on page 77*.
Import HD View	Displays a standard Browse window for selecting an HD View image to import into Maps.
Alignment:	Displays a submenu of alignment choices. See *"Aligning a Tile Set and Its Magnification" on page 149*.
Align	Aligns a single tile set or image. Similar to Global Alignment, but for a single item. See *"1-, 2-, or 3-Point (Coarse) Alignment" on page 196*.
Fine Alignment	Use Fine Alignment to fine-tune the alignment of layers and tile sets visually. The Fine Alignment controls only display after clicking **Next** after a 1-, 2-, or 3-point alignment. See *"Fine Alignment" on page 200* for descriptions of the controls.
Clear Alignment	Clears any tile set alignment that has been applied in Maps.

Tile Set Right-Click Menu

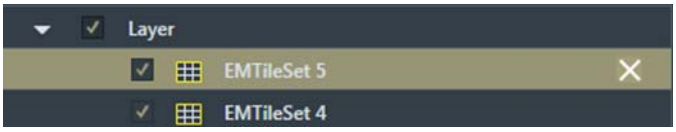
Right-click on any tile set to access a context menu.



Unique selections for Tile Set.

- For descriptions of common selections to all systems, see *"Annotation Right-Click Menu" on page 43*.
- For descriptions of Tile Set-specific selections, see the table below.

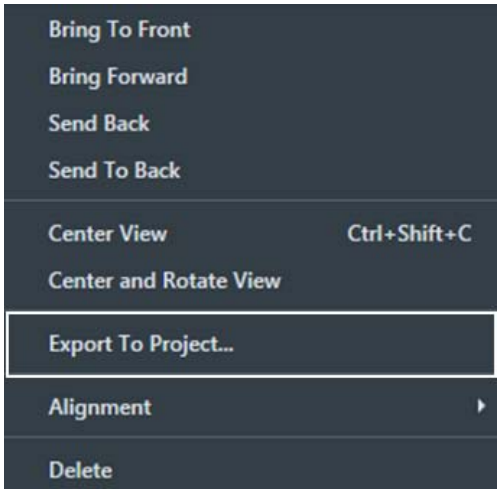
Table 16 Tile Set Right-Click Menu Overview

Menu Selection	Description
Reset Acquisition	Clears all acquired image data in the selected tile set and unlocks the parameter fields in the Basic tab for editing.
Show Grid Lines (Ctrl+G)	Toggles to show/hide the tile grid lines onscreen.
Save As Default Template	After editing the defaults, saves the edits as the new default template.
Save As Template	Displays a submenu **New Template**, that allows saving the current tile layers template as a new template or overriding a previous template.
Copy Grid	Creates a grid above the one that is copied, and incrementally numbers the name. In this example, EMTileSet 5 is a copy of EMTileSet 4. 
Export Grid	Displays a standard Save As window for browsing to a folder in which to save the exported grid. Grids are saved in the **.gridx* format.
Export to Project	Copies the selected tile sets into a new or existing Maps project.
Alignment:	Displays a submenu of alignment choices. See *"Alignment:" on page 45* for descriptions.
Drive To	Drives to the selected tile set.
Apply Settings to Microscope	Applies all defined tile set settings to the microscope.
Apply Microscope Settings to Grid	Applies all microscope settings to the tile set.
Create MIP Layer	Collapses the Z-stack into a single image using the highest intensity pixels from the stack. This menu selection is enabled for tile layers with more than one plane.
Split Channels	Divides the individual channels of a tile set into separate tile sets. ***Note:*** *This operation is permanent and cannot be undone.*

Preview Image Right-Click Menu

NOTE	This menu is SEM/SDB and CorrSight only.

Preview images are directly stored in the selected layer. Right-click on a preview image to access a context menu.



Unique selection for Preview Image.

- For descriptions of common selections to all systems, see *"Annotation Right-Click Menu" on page 43*.
- For descriptions of Preview Image-specific selections, see the table below.

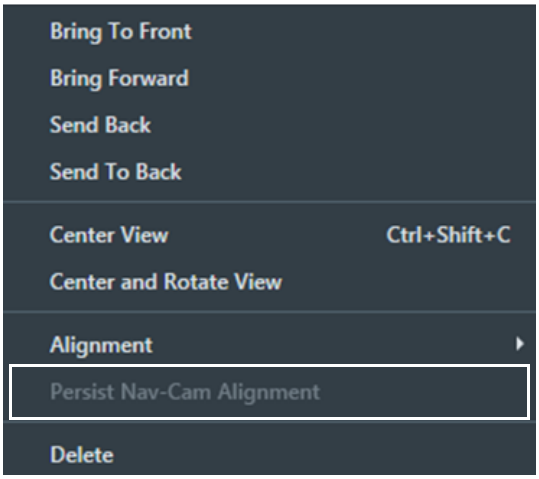
Table 17 Preview Image Right-Click Menu Overview

Menu Selection	Description
Export to Project	Copies the data to a new or existing project.

Nav-Cam Right-Click Menu

NOTE	This menu is SEM/SDB and CorrSight only.

The Nav-Cam image is stored in the selected layer. Right-click on a Nav-Cam entry to access a context menu.



Unique selection for Nav-Cam.

This is only applicable to SEM/SDB and CorrSight systems.

- SEM/SDB: See *"Nav-Cam Right-Click Menu" on page 102*.
- CorrSight: See *"Preview Layer, Nav-Cam Right-Click Menu" on page 121*.

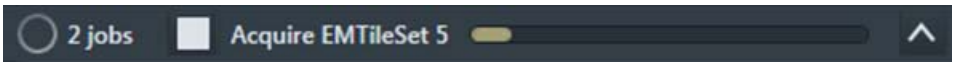
Job Queue

Topics include:

- *"Summary" on page 50*
- *"Floating Window" on page 50*
- *"Right-Click Menu" on page 51*

Summary

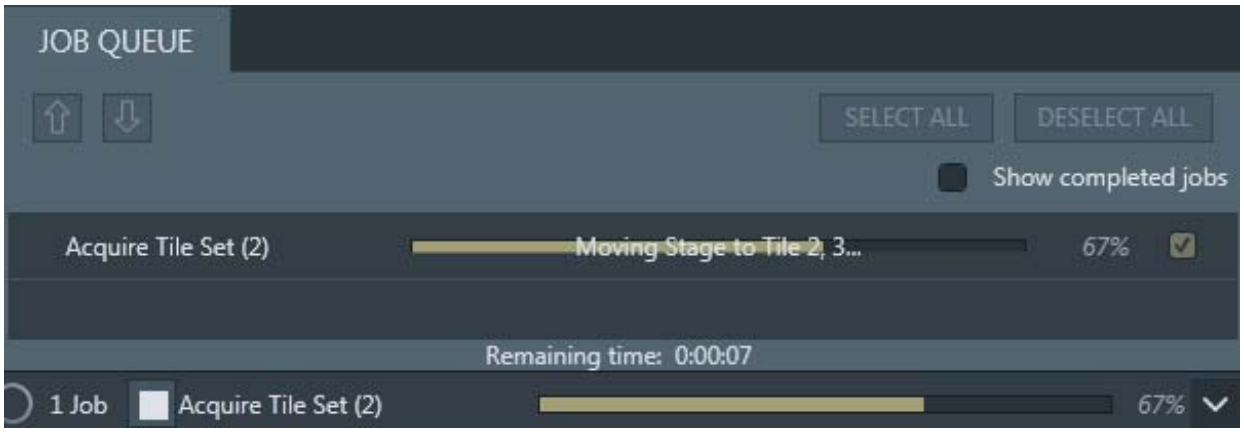
The Summary is always visible in the status bar, and indicates the status of the current acquisition, when a job is running. The progress bar is relative only to the tile set being acquired.



When a job is not running, it looks like this.

Floating Window

The Job Queue is a floating window that appears when you click the arrow on the right.



To dismiss this window, click anywhere outside the Job Queue window.

Table 18 Job Queue Floating Window Overview

Control	Description
Tile Sets selected for processing	Displays the list of selected tile set(s) for acquisition or stitching.
SELECT ALL	Selects all jobs for acquisition or stitching.
DESELECT ALL	Deselects all jobs for acquisition or stitching.
Show completed jobs	Displays completed jobs for acquisition or stitching.
Job Processing	Displays the percentage of progress for the acquisition or stitching process.
Up and Down Arrows	Reorders the non-running jobs.
Remaining Time	Displays the time remaining for processing.

Right-Click Menu

Remove: Removes only the selected tile set from the job queue, if it is not already in process.

Viewer

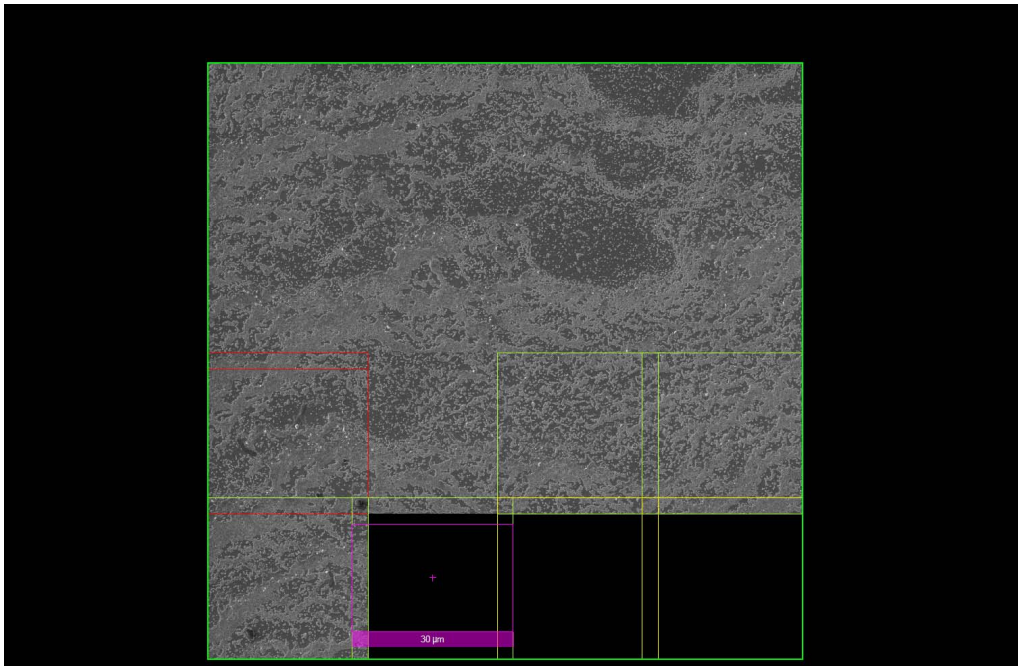
Overview

This example shows a 4 x 4 tile set grid.

- Double yellow lines indicate the overlap between tiles.
- The purple FOV (field of view) rectangle displays the HFW (horizontal field width).

NOTE	The coloring and labels of the FOV rectangle may change depending on the context.

- The *scale bar* (not shown) in the bottom right corner shows the distance (e.g., in microns) in relation to the current magnification of the displayed image.
- The **Delete** key works in Maps for any selected item. You will receive a Confirmation request.



Topics in this section include:

- *"Tile Set Grid Color Key" on page 52*
- *"Alt +Left-Click and Drag Selected Area" on page 52*
- *"Viewer Tile Right-Click Menu" on page 56*

Tile Set Grid Color Key

- **Yellow** = Unacquired tiles. Visually indicates the tile overlap.
- **Grey** = Tiles that have been disabled; they will not be acquired
- **Lime Green** = Acquired tiles that are awaiting registration
- **No outline** = Acquired tiles that have been successfully registered
- **Red** = Acquired; but these tiles failed registration and will require manual alignment (see *"Manual Alignment" on page 80*)
- **Bright Red** =Edges of groups of red tiles are highlighted with a brighter red, to show where the actual break in the connection is. Often, fixing a bright red connection (via *"Manual Alignment" on page 80*) will allow previously red tiles to become successful.

Alt +Left-Click and Drag Selected Area

Alt+left-click and drag a rectangle in the viewer to create a selected area. A menu appears with selections for that area.

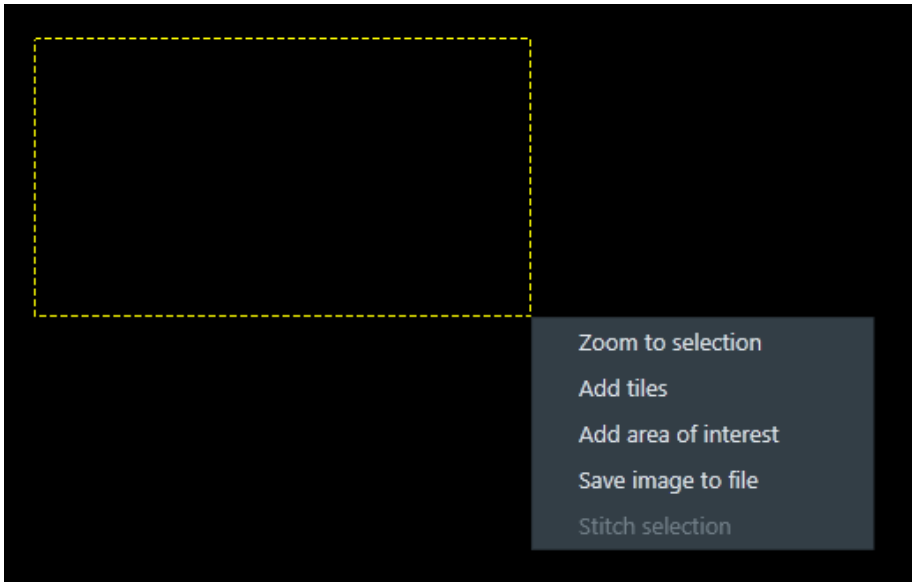
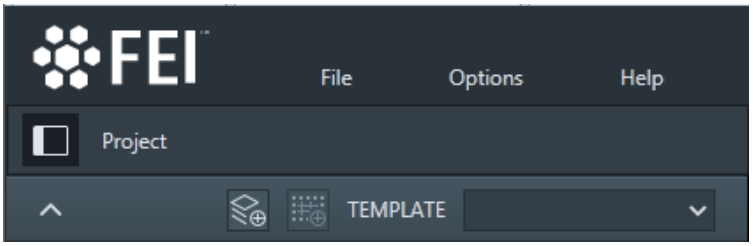
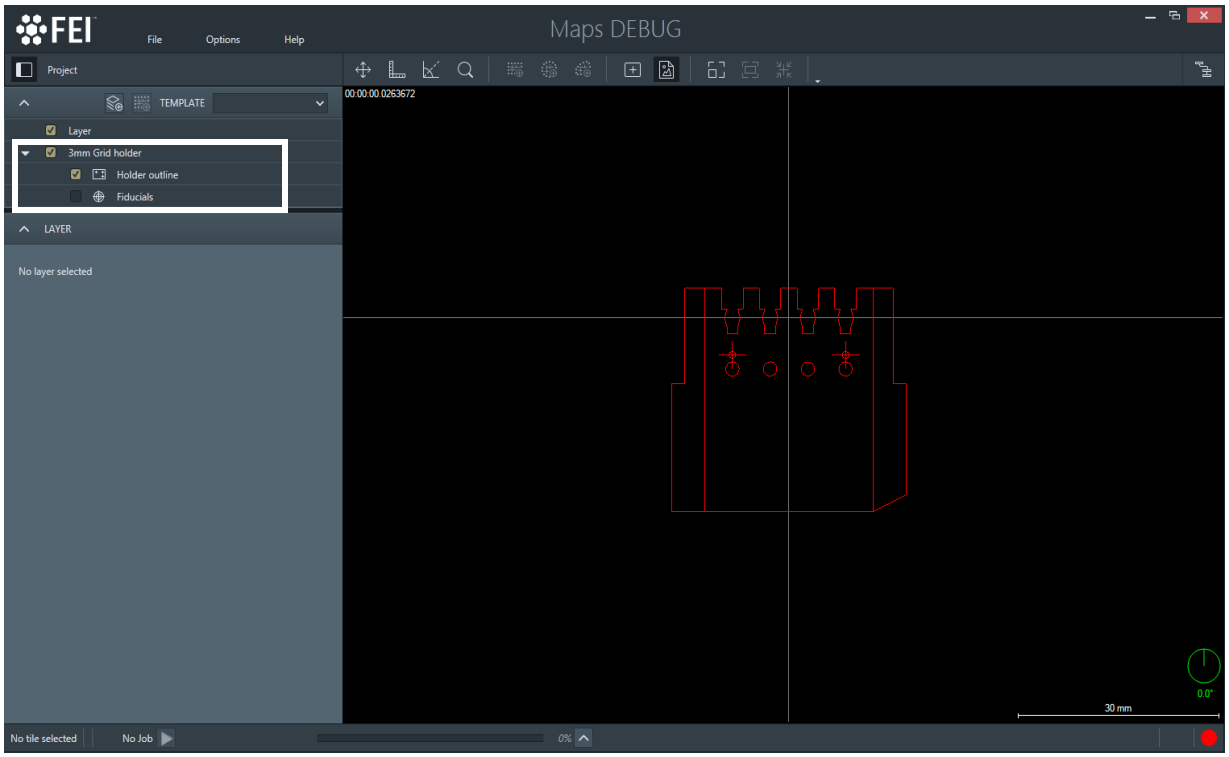
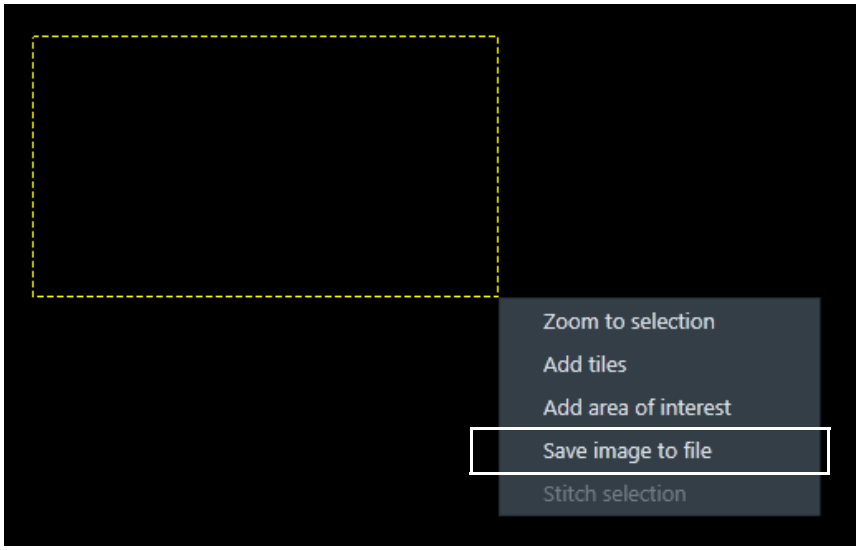


Table 19 Viewer Selected Area Overview

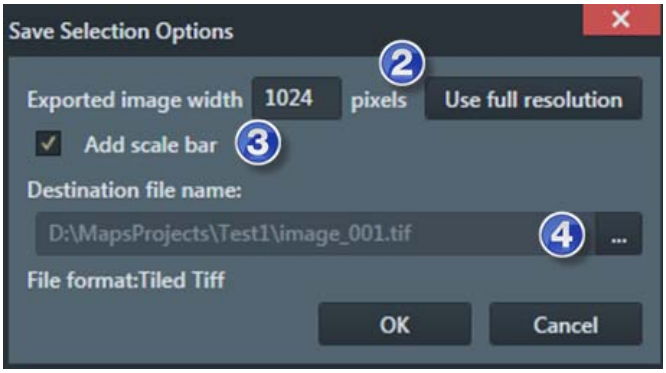
Menu Selection	Description
Zoom to selection	Zooms the field of view to the selected area.
Add tiles	Places a new tile set in the selected area, using the Active Template. 
Add area of interest	Places a new Annotation layer in the Layer control. 
Save image to file	Saves an image to file. See *"Save Image to File" on page 54*.
Stitch selection	Stitches a subset of tiles, as defined by the rectangle area of interest. See *"Stitching" on page 79*. This menu item is disabled if no tile set is selected.

Save Image to File

When you use Alt+left-click and drag to create a new tile set, the menu selection **Save Image to File** appears.



1. Click **Save image to file**. The Save Selection Options dialog box appears.

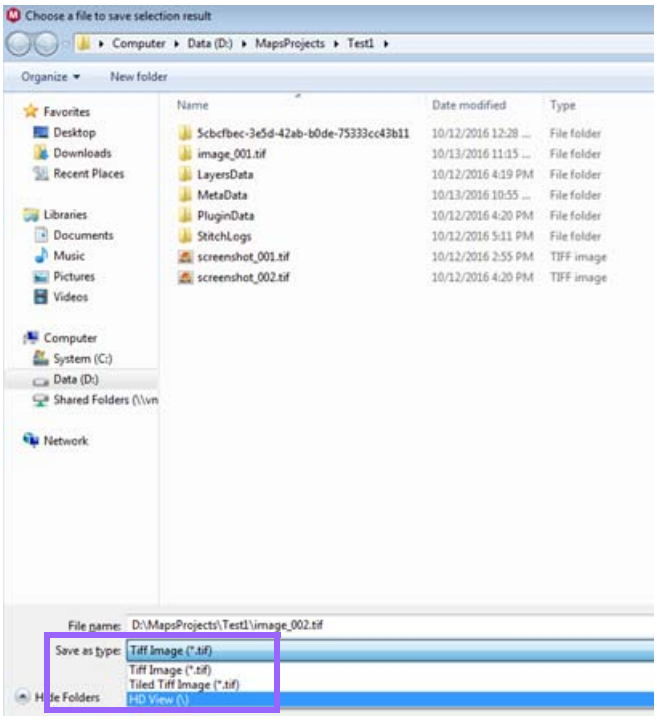


2. Enter a desired resolution (**Export Image Width** in pixels) or click **Use Full Resolution** to set the exported image width to the native pixel size.

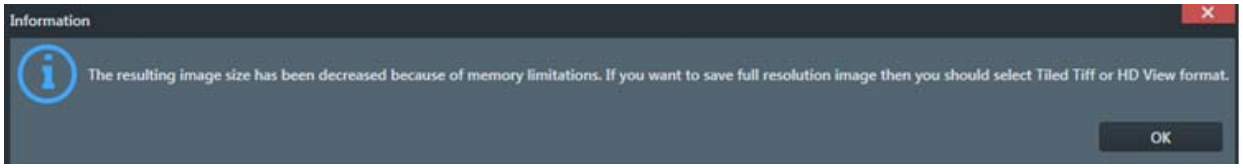
NOTE	Full resolution exports for large selections of high-resolution data are not available for all image types.

3. Select the **Add Scale Bar** check box to place the viewer scale bar on the exported image.
4. Click the browse button (**...**) to display the browser window.

5. File type options include TIFF Image and Tiled TIFF Image, and HD View. Select a file type, file name, and location and then click **Save**.



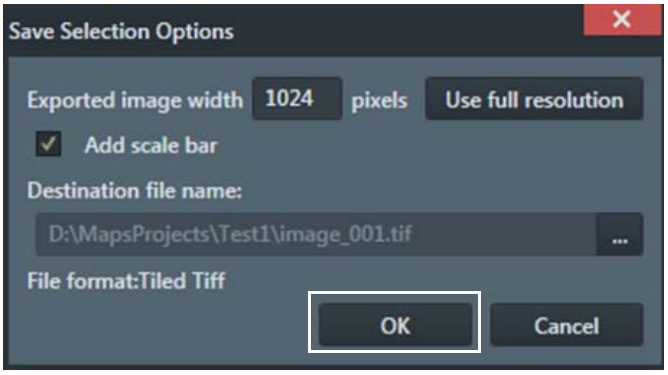
There are limitations for the TIFF file type. If TIFF is desired, but the resolution of the selected area is too large for TIFF export, a warning appears and the exported image width in pixels will be automatically reduced to the maximum for TIFF export.



To save large resolution images, use the Tiled TIFF format.

NOTE	Not all image processing software supports the Tiled TIFF format. It is recommended to use the TIFF format to ensure compatibility with external image processing packages.

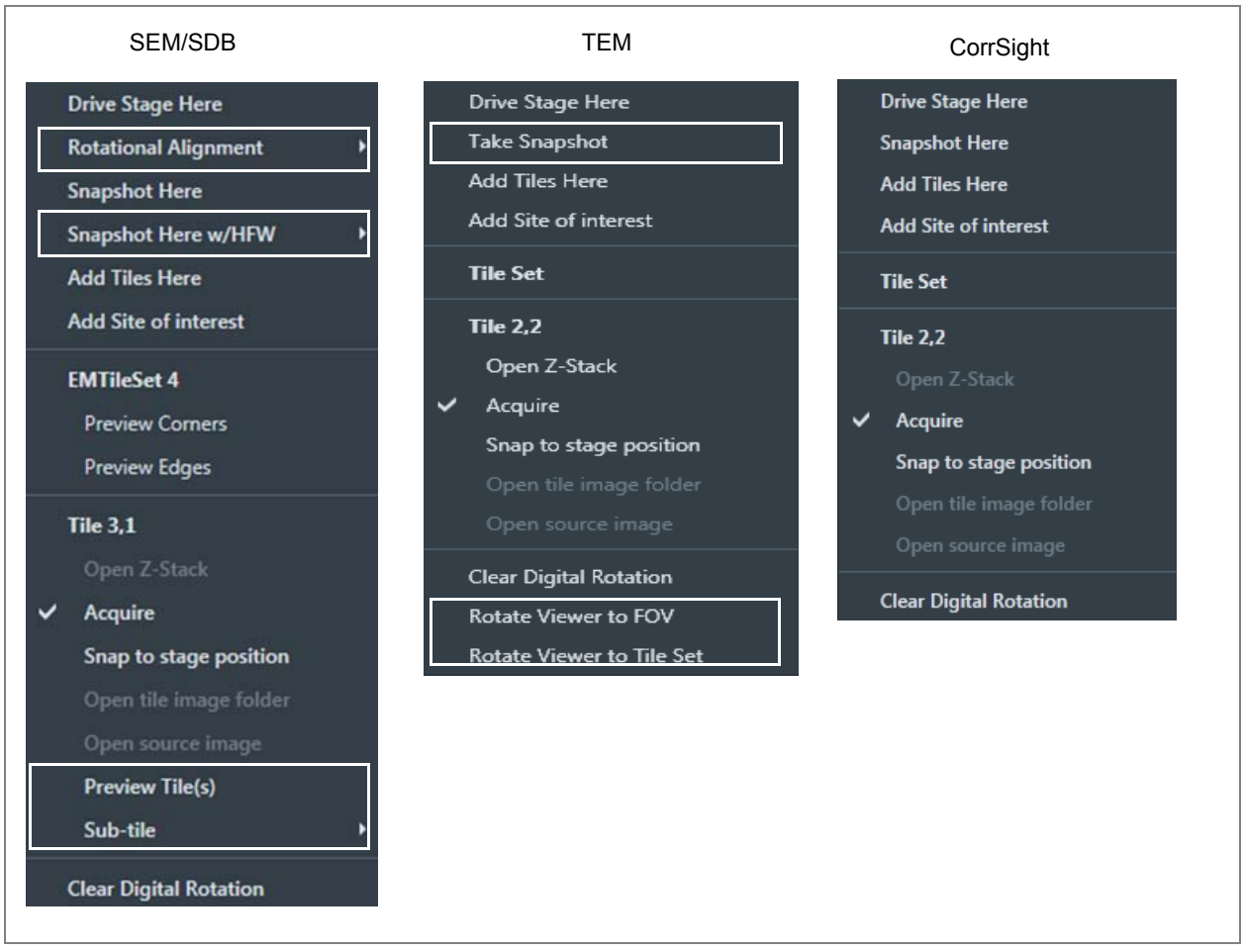
6. The Save Selection Options window is still open. Click **OK** to close it.



Viewer Tile Right-Click Menu

Right-click on a tile within the tile grid to access a context menu.

Figure 8 Viewer Tile Right-Click Menu, All Systems



The common selections are described in the table below. For system-specific selections:

- SEM/SDB: See *"Viewer Tile Right-Click Menu" on page 103*.
- CorrSight: See *"Viewer Tile Right-Click Menu" on page 122*
- TEM: See *"Viewer Tile Right-Click Menu" on page 148*.

Table 20 Viewer Tile Right-Click Menu Overview (1 of 3)

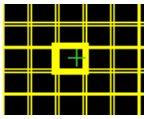
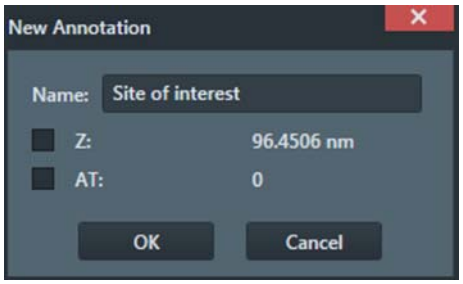
Menu Selection	Description
Drive Stage Here	Moves and centers the stage to the selected tile (see the green cross). 
Snapshot Here	Acquires a preview image at the selected stage coordinate and current HFW.
Add Tiles Here	Adds a new Tile Grid centered on the clicked location.
Add Site of Interest	Displays a New Annotation dialog box for creating the Site of Interest (SOI). An SOI is a specific stage location used to mark sample features. Z: Stage Z and T: Alpha Tilt can optionally be stored to be recalled later. The new SOI is created is the currently selected layer group.  **Note**: *This is different from the New Annotation dialog box for creating an Area of Interest, which marks an area of the stage.*
Acquire/Re-acquire	• **Acquire**: Displays if the tile has not been acquired. • **Re-acquire**: Displays if the tile has already been acquired.
Snap to stage position	Moves the clicked position on the tile layer to the current stage position.
Clear Digital Rotation	Resets the viewers digital rotation to 0 degrees.

Table 20 Viewer Tile Right-Click Menu Overview (2 of 3)

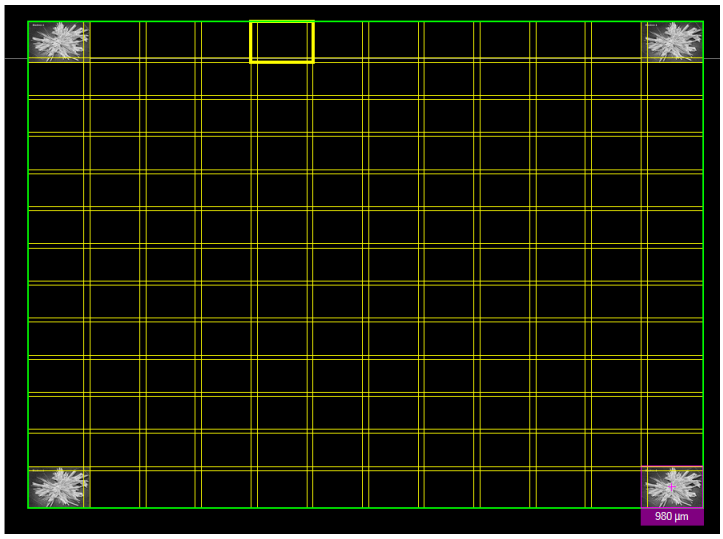
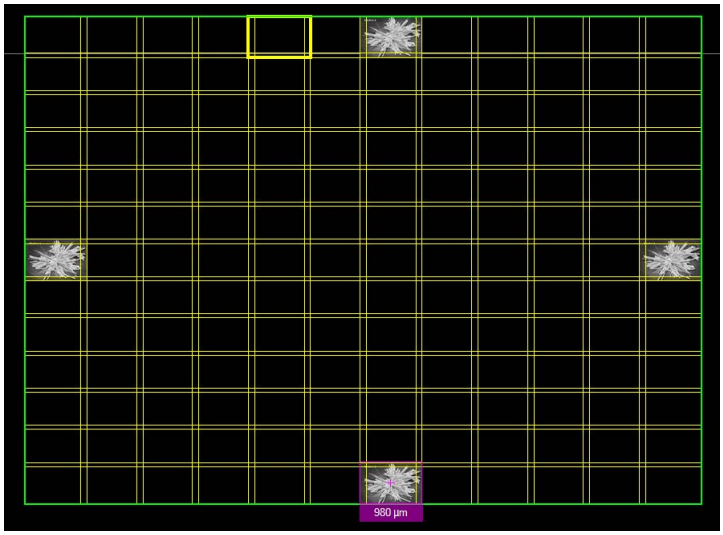
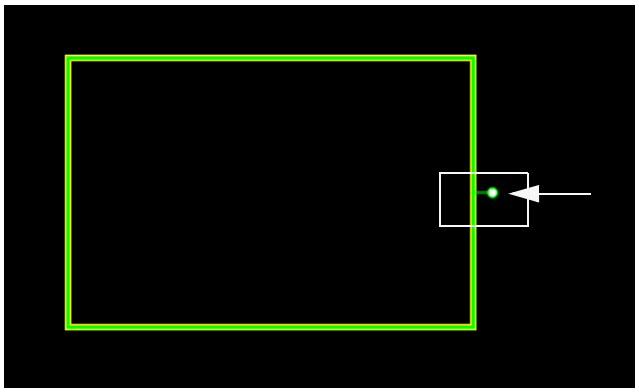
Menu Selection	Description
Add Site of Interest (Cont.)	The new SOI becomes a new Annotation layer in the Layer control. Add notes and assign a color to display in the viewer. 
Sample (SEM tiles in this example)	Displays the name of the selected tile set. The submenu has choices to: Preview Corners or Preview Edges.
Preview Corners	After running Preview Corners, preview images appear in the corners of the tile set. 

Table 20 Viewer Tile Right-Click Menu Overview (3 of 3)

Menu Selection	Description
Preview Edges	After running Preview Edges, preview images appear in center of each edge of the tile set. 
Tile X, Y	Displays the row (X) and column (Y) number for the selected tile.
Open Z-stack	Opens the Z-Stack Browser to view the focus stack of the tiles. See *"Z-Stack Browser Controls" on page 36*.
Acquire/Re-acquire	• **Acquire**: Displays if the tile has not been acquired. • **Re-acquire**: Displays if the tile has already been acquired.
Snap to Stage Position	Moves the tile to the current stage position.
Open Tile Image Folder	Opens explorer to the directory holding the tile image data.
Open Source Image	Opens the tile's source image file with the default image viewer (Windows Picture Viewer).
Preview Tile(s)	SEM/SDB: See *"Preview Tile(s)" on page 104*. TEM: See *"Viewer Tile Right-Click Menu Overview" on page 57*.
Sub-tile	SEM/SDB: See *"Sub-tile" on page 104*.
Clear Digital Rotation	Sets the digital rotation to zero if rotation had previously been applied. This only changes the displayed rotation of the images and does not rotate the microscope stage.
Auto-Rotate Viewer to FOV	TEM: See *"Viewer Tile Right-Click Menu Overview" on page 148*.
Auto-Rotate Viewer to Tile Set	TEM: See *"Viewer Tile Right-Click Menu Overview" on page 148*.

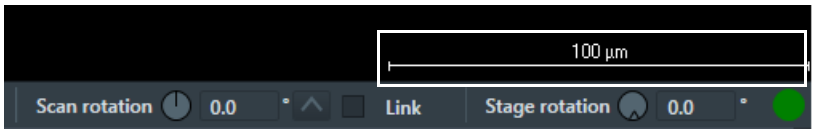
Rotation Control in the Viewer

Grab the lollipop-looking control with the mouse to easily rotate tile layers (not for TEM) and annotation layers.



Micron Bar

The micron (scale) bar scales to the magnification.



3 Basic Operations

Overview

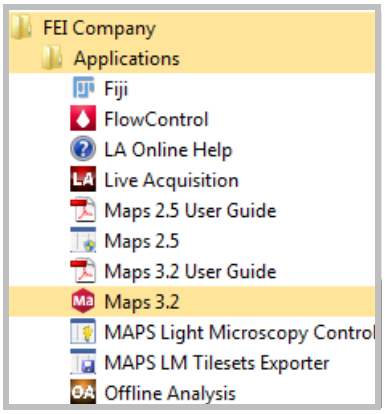
This chapter describes the basic process flow for Maps.

Topics include:

- *"Launching the Application" on page 61*
- *"Nav-Cam Prompt (SEM/SDB Only)" on page 68*
- *"Creating a Project" on page 64*
- *"Setting Options" on page 70*
- *"Nav-Cam Alignment (SEM/SDB and CorrSight Only)" on page 71*
- *"Running the Automated Holder Alignment (SEM/SDB and CorrSight Only)" on page 71*
- *"Defining a Tile Set" on page 72*
- *"Acquiring a Tile Set" on page 76*
- *"Importing Images from Other Sources" on page 77*
- *"Stage Navigation" on page 78*
- *"Stitching" on page 79*

Launching the Application

Click the desktop icon to launch Maps or use the Windows Start menu.

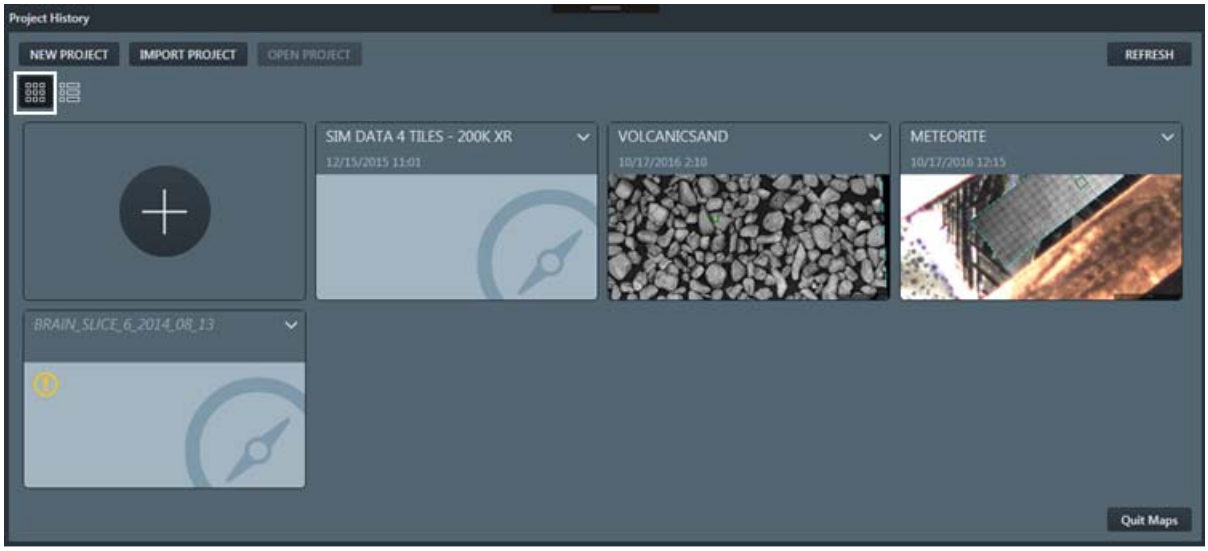


Application Window

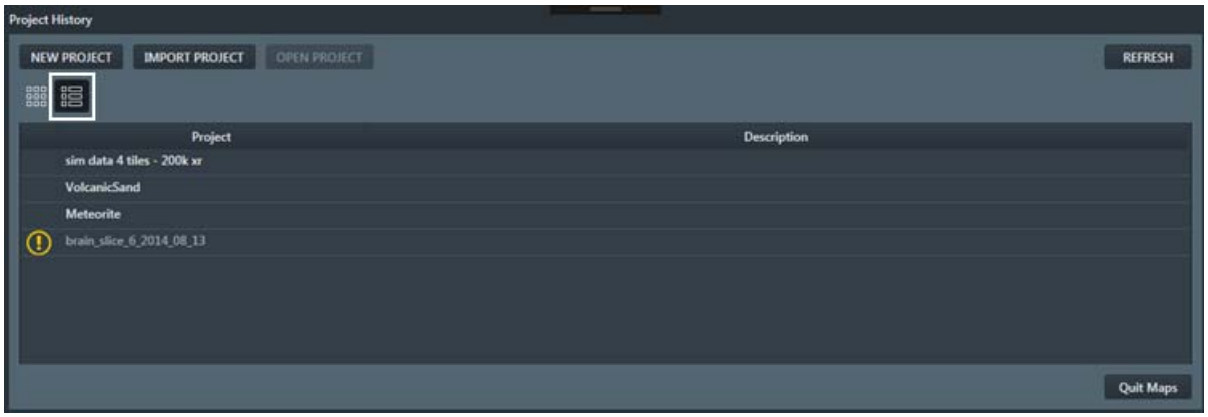
The application window displays with the Project History window open.

Select a display mode from the icons below **New Project**:

- **Thumb View**: Displays thumbnail images of project data.



- **List View**: Displays a list of project data.



New Project

If this is your first project, the list/thumb view will be empty and the **Open Project** button will be disabled. Click **New Project** and proceed to *"New Project" on page 64*.

Import a Project

To import a project that was created on another system and does not appear in the project list, click **Import Project**. The project opens and its name is added to the project history list. See *"Import Project" on page 67*.

Open a Project

To open an existing project, highlight a project name in the list and click **Open Project** and then proceed to *"Open Project" on page 66*.

Yellow Exclamation Mark in Project List

This symbol appears if the project path is not found for that data, due to a disconnected network drive, USB drive, or other drive.

Refresh

Updates the status of any disconnected projects that have been reconnected properly.

Quit Maps/Cancel

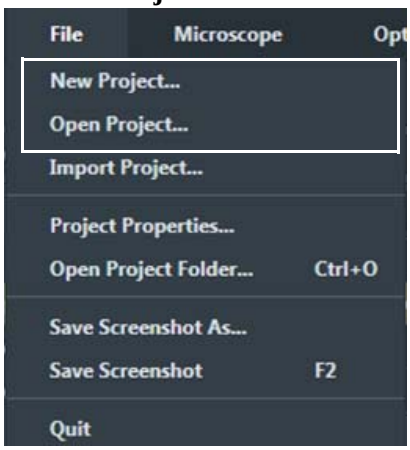
- If you have not opened a project yet, then you can directly quit Maps from the **Quit Maps** button.
- If a project is already opened, then this button is replaced by a **Cancel** button that allows you to close the Project History window.

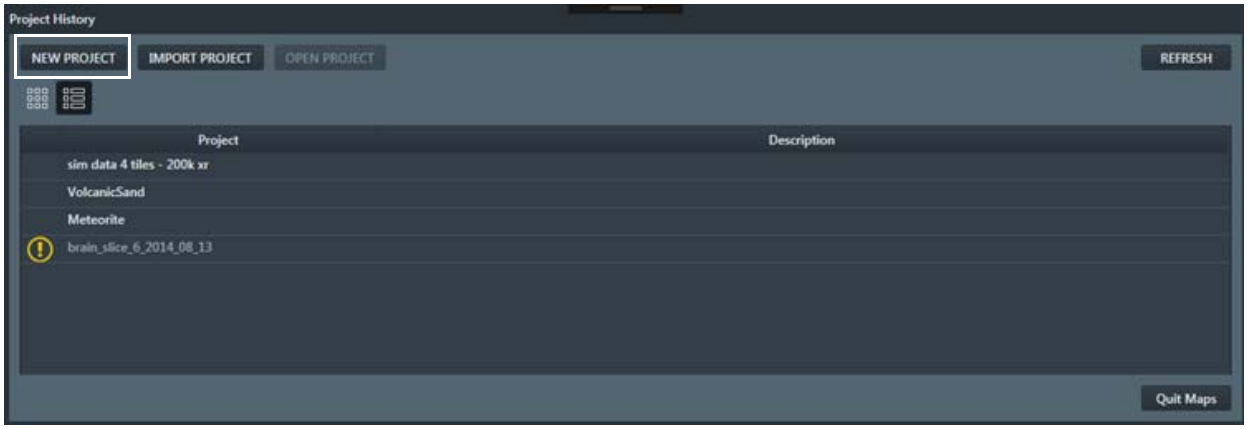
Creating a Project

Create a new project, import a project, or open an existing project using File menu selections (*page 15*) or Project History buttons.

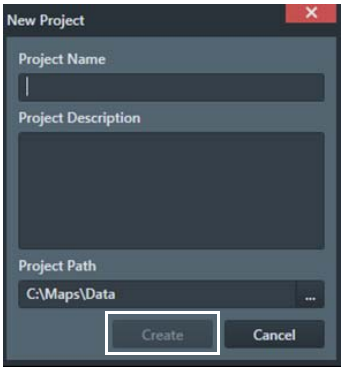
New Project

1. To create a new project, select **File > New Project** or **File > Open Project**, or click the **New Project** button from the already open Project History window.





2. In the New Project window that appears, define the new Project Name, and enter a brief Project Description. Then click **Create**.

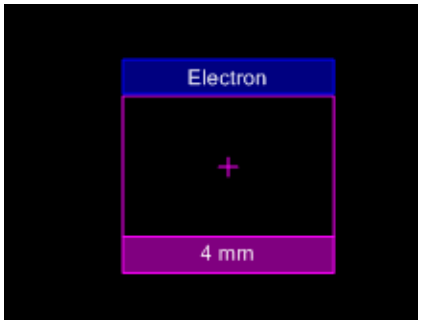


NOTE	The Project Name cannot be empty.

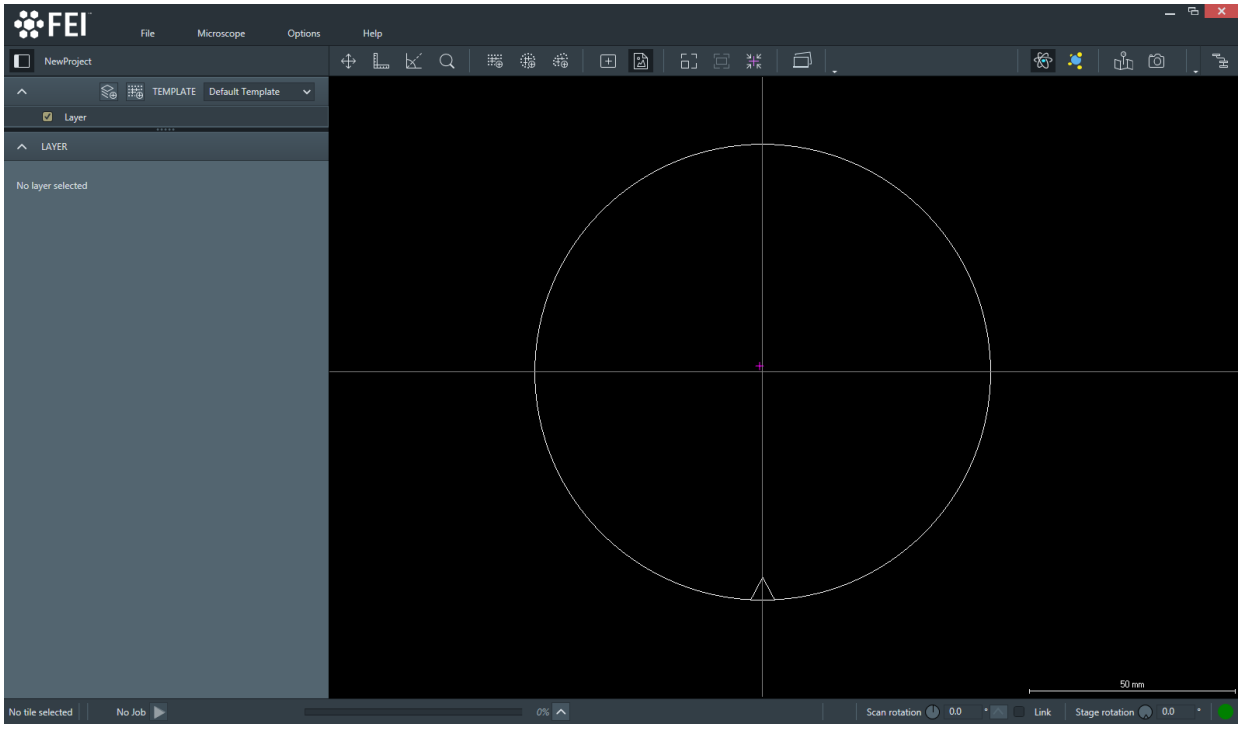
The default data directory will be used unless you browse to a new location.

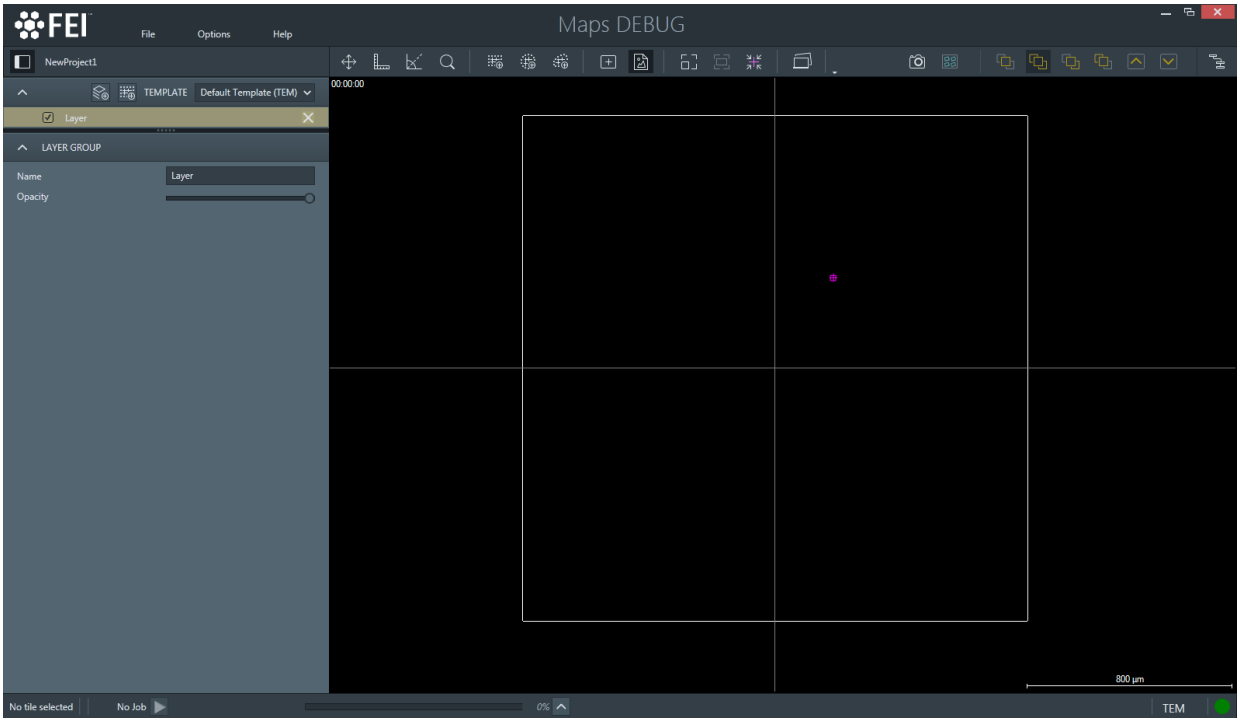
The new project name appears in the title of the Layer control panel and the Layer control shows a default Layer.

The purple rectangle represents the current field of view on the sample and the purple + is the current stage position.



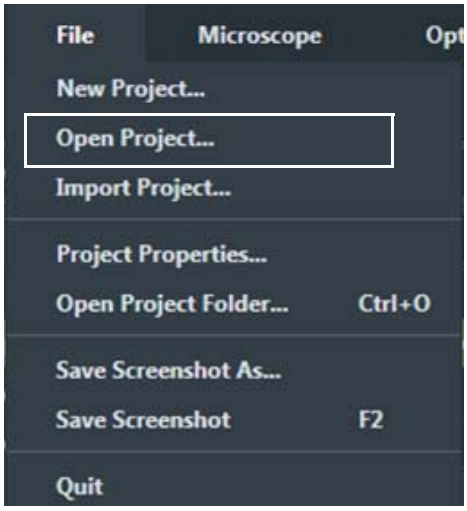
The white outline shows the stage boundaries, which can be circular or rectangular shaped depending on the instrument.

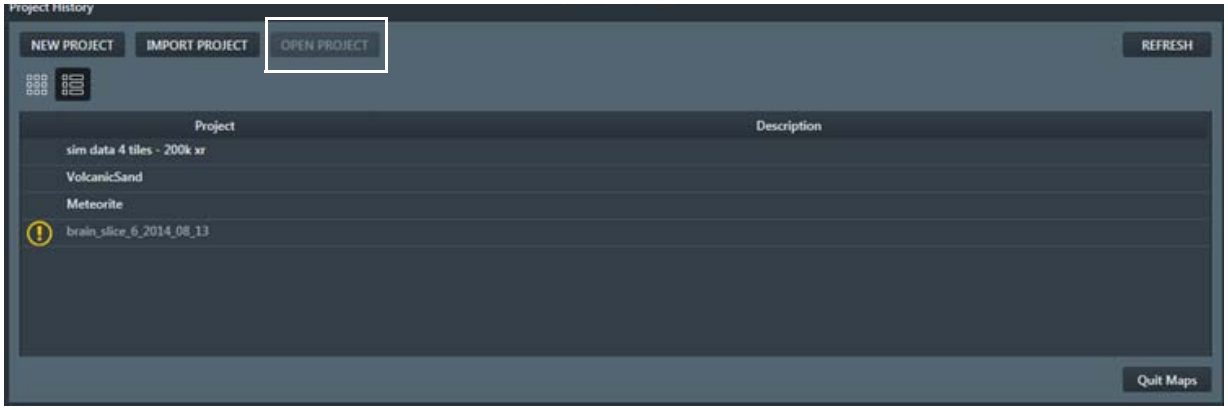




Open Project

To select an existing project, select **File > Open Project** or select a project name from the already open **Project History** window and click **Open Project**.



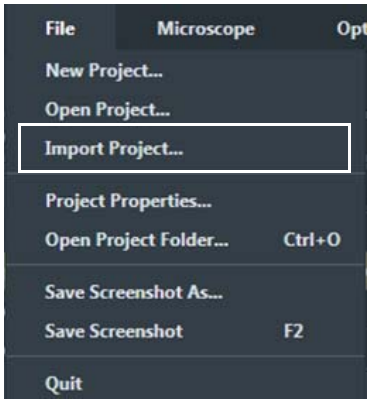


The selected project name appears in the Layer control title.

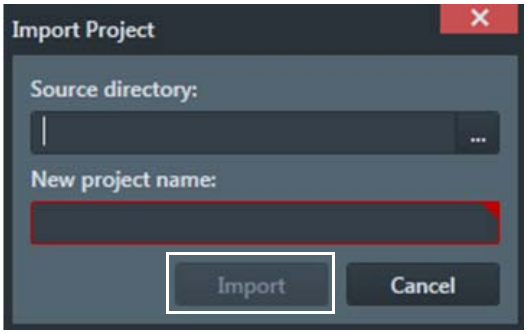
Import Project

Importing a project is useful for passing projects between computers. Once imported, you can select it as an existing project.

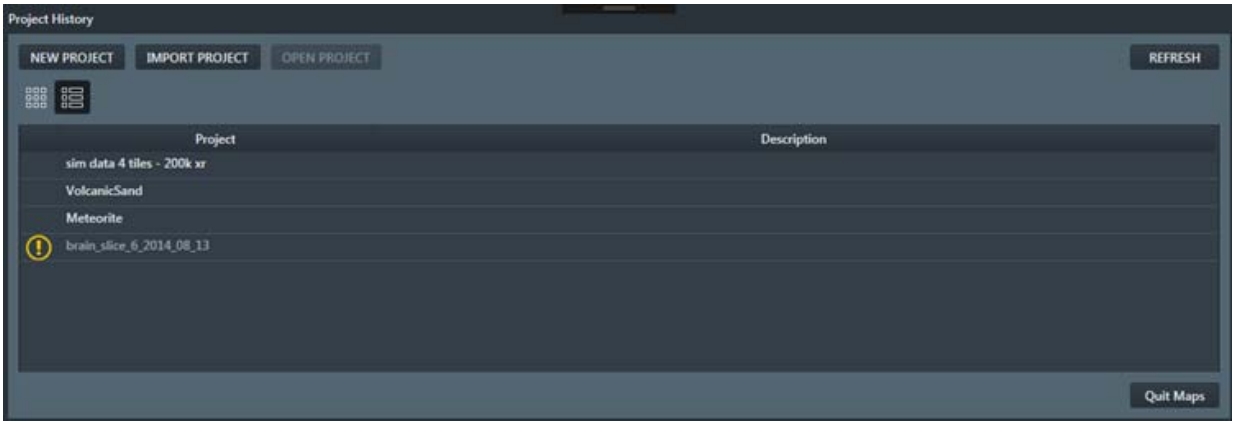
1. To import (save) an existing project, select **File > Import Project.**



2. Browse to a Source directory. Name the project to be exported and then click **Import**.

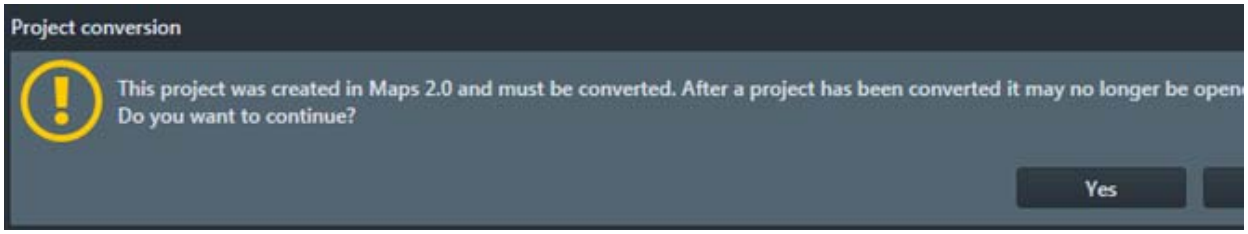


The imported project opens and the file name is added to the project history list.



Converting From Maps 1.1 or 2.0 SW

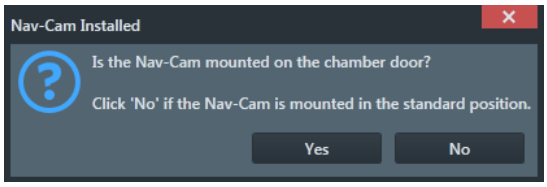
The import process is only one-way. Older software projects must be converted. When a project is imported from Maps v1.1 or Maps v2.0, a warning is displayed telling the user that the import is one-way and the project will no longer be compatible with the older copy of the software.

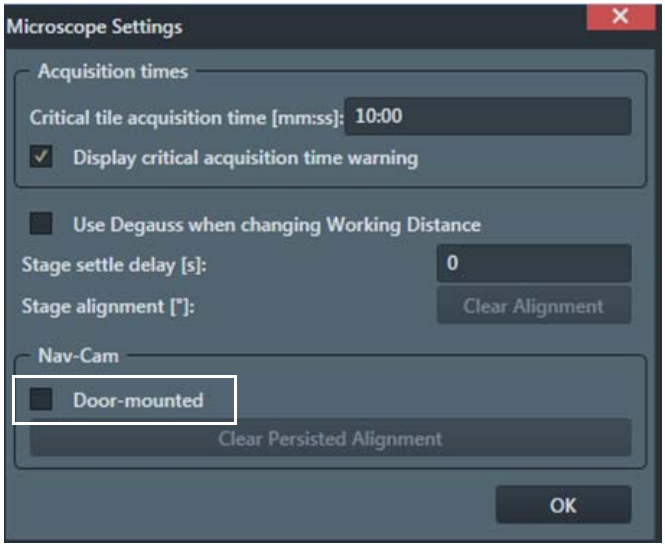


Nav-Cam Prompt (SEM/SDB Only)

NOTE	Nav-Cam is not supported for Maps on TEM systems.

When you first try to import from the Nav-Cam, the application will ask the user if it is a door-mounted Nav-Cam (see image below).





- If **No** is chosen, the **Door-mounted** check box on the Microscope Settings window is not automatically selected, but you have the option to select it manually.
- If no Nav-Cam is installed, the check box is disabled.

You will be prompted to browse to a default location for storing project data.

It is recommended that you do not use the C: drive, but you can use a supplemental data storage device such as a network drive, external USB drive, or additional internal hard drive.

Setting Options

Select new project defaults from the Options menu.

- Application Settings, below
- *"TileSet Templates" on page 70*
- *"Microscope Settings" on page 70*
- *"Snapshot Settings (SEM/SDB Only)" on page 70*
- *"SnapShot Settings (TEM Only)" on page 70*
- *"Animate Pan & Zoom" on page 71*
- *"Nav-Cam Alignment (SEM/SDB and CorrSight Only)" on page 71*
- *"Running the Automated Holder Alignment (SEM/SDB and CorrSight Only)" on page 71*

Application Settings

Select **Options > Application Settings** to display a window for setting defaults. See *"Application Settings:" on page 24* for descriptions of the controls.

TileSet Templates

Select **Options > TileSet Templates** to display a dialog that shows a collection of templates and their respective settings. These are system-specific:

- SEM/SDB: *"Tile Set Templates:" on page 90*
- CorrSight: *"Tile Set Templates:" on page 113*
- TEM: *"Tile Set Templates:" on page 136*

Microscope Settings

Select **Options > Microscope Settings** to display the Microscope Settings window.

These are system-specific:

- SEM/SDB: *"Microscope Settings:" on page 90*
- TEM: *"Microscope Settings" on page 137*

Snapshot Settings (SEM/SDB Only)

Select **Options > Snapshot Settings** to display the Microscope Settings window. See *"Snapshot Settings" on page 91*.

SnapShot Settings (TEM Only)

Select **Options > Snapshot Settings** to display the Snapshot Settings Window. See *"Snapshot Settings" on page 138*.

Animate Pan & Zoom

Select **Options** > **Animate Pan & Zoom** to allow a smoother transition of panning and zooming.

- **Pan**: To make a sweeping movement
- **Zoom**: To simulate movement rapidly away from or toward a subject using a zoom lens

NOTE	This is a slower process and takes more memory.

Nav-Cam Alignment (SEM/SDB and CorrSight Only)

- SEM/SDB: See *"Nav-Cam Alignment" on page 108*.
- CorrSight: See *"Nav-Cam Alignment" on page 129*.

Running the Automated Holder Alignment (SEM/SDB and CorrSight Only)

- SEM/SDB: See *"Automated Holder Alignment" on page 168*.
- CorrSight: See *"Automated Holder Alignment" on page 168*.

Defining a Tile Set

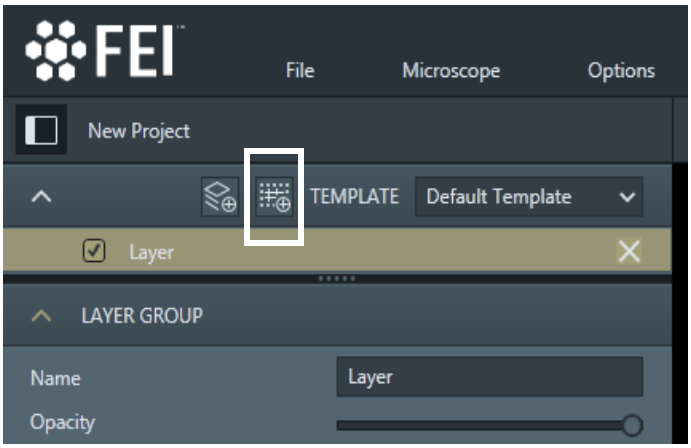
Topics include:

- Methods, below
- *"Defining a Circular Tile Set" on page 73*
- *"Defining a Freehand Tile Set" on page 74*
- *"Tile Set Display with Grid" on page 74*
- *"Configure Tiling Parameters" on page 75*

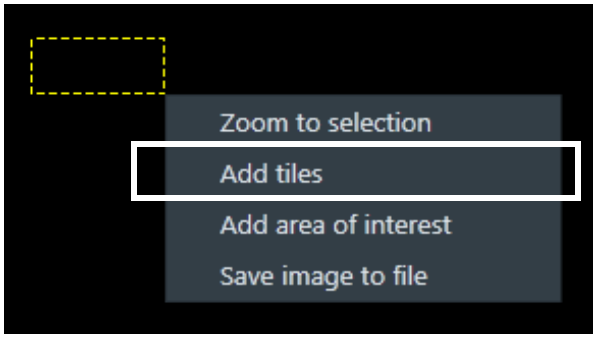
Methods

Define the tile set for your project using one of the three methods below:

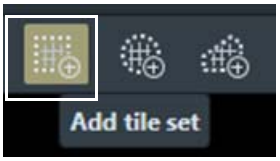
- Click the **Add Tiles** button on the Layer control.



- Or, press **Alt+left-click** and drag a rectangle in the Viewer and then select **Add Tiles** from the menu.



- Or, click the **Add Tile Set** button in the tool bar, then click and drag an area in the viewer.

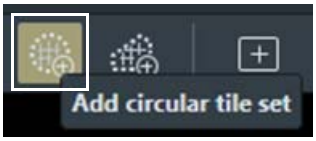


Maps creates a layer in the Layer control, depending on the type of microscope connected. You cannot create a tile set when not connected to a microscope.

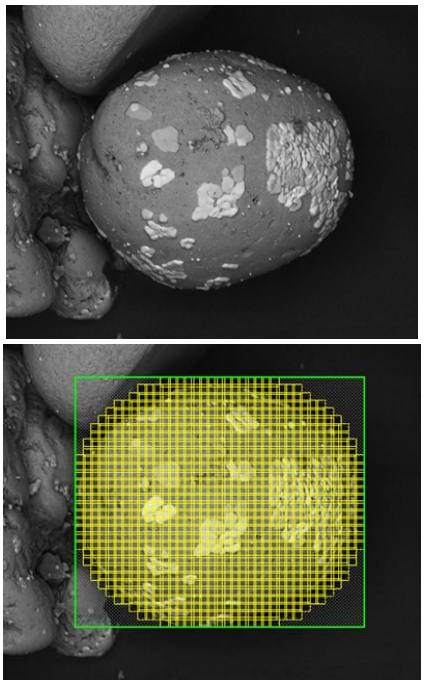
The new name is added to the list of tile sets beneath Layer in the Layer control. The Tile Set tab displays default parameters, and a grid (green square) is centered on the stage location.

Defining a Circular Tile Set

Click the **Add Circular Tile Set** button in the tool bar, then click and drag an area in the viewer to create the tile set.

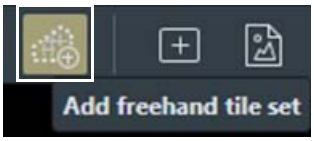


Example

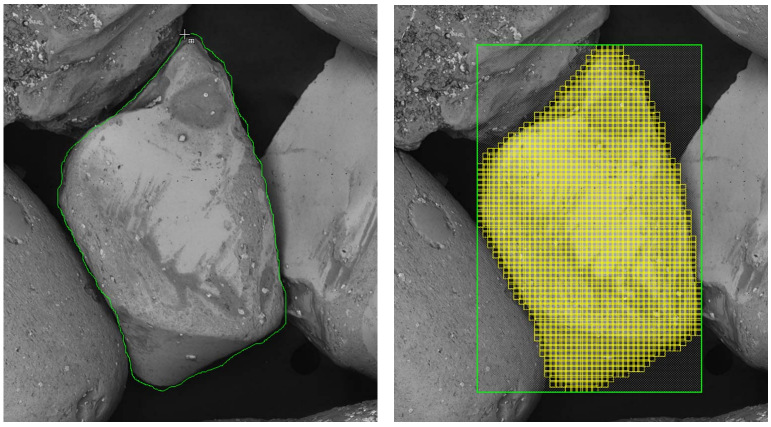


Defining a Freehand Tile Set

Click the **Add Freehand Tile Set** button in the tool bar, then click and drag an area in the viewer to create the tile set.

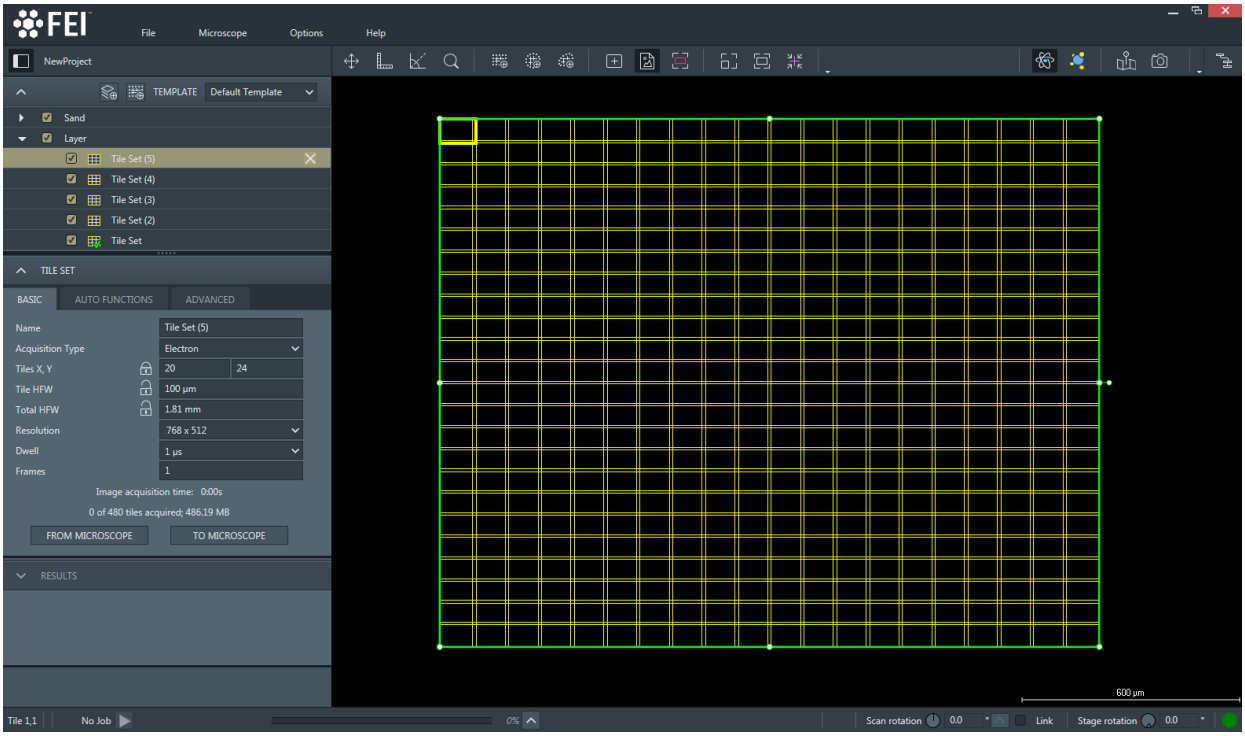


Example

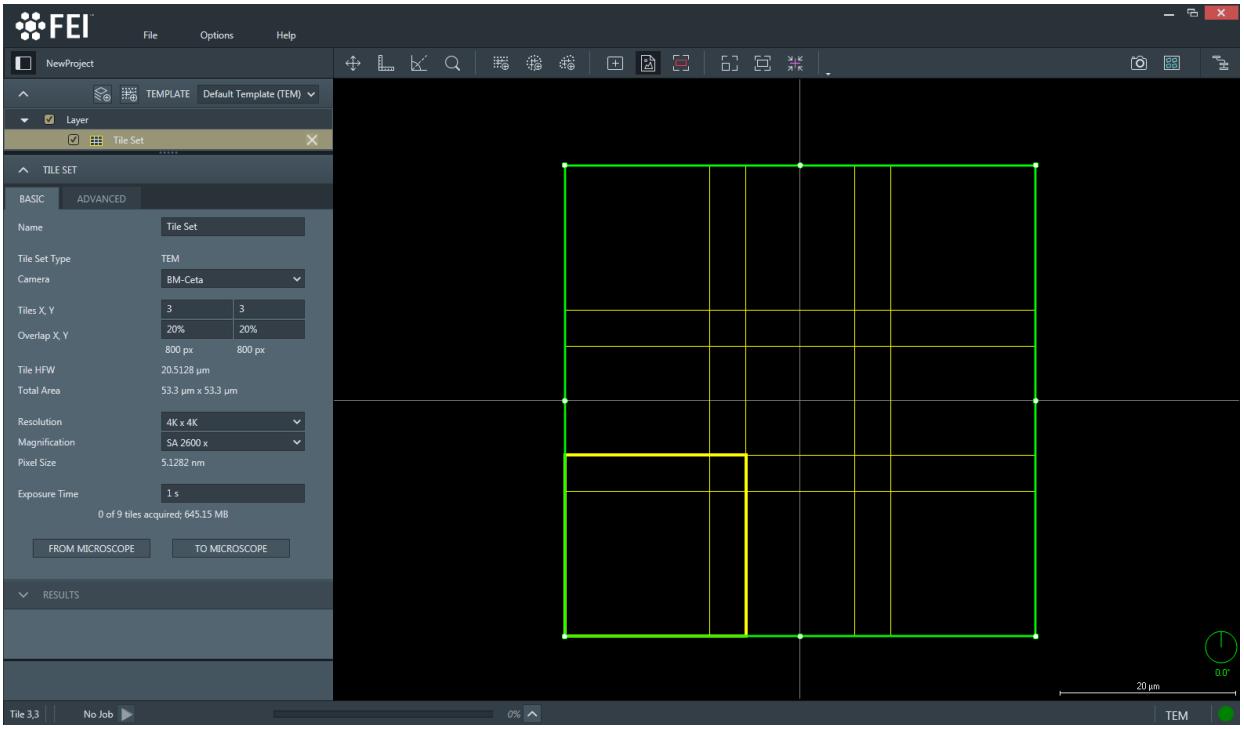


Tile Set Display with Grid

- SEM/SDB View



- TEM View



NOTE	This will look different in STEM mode.

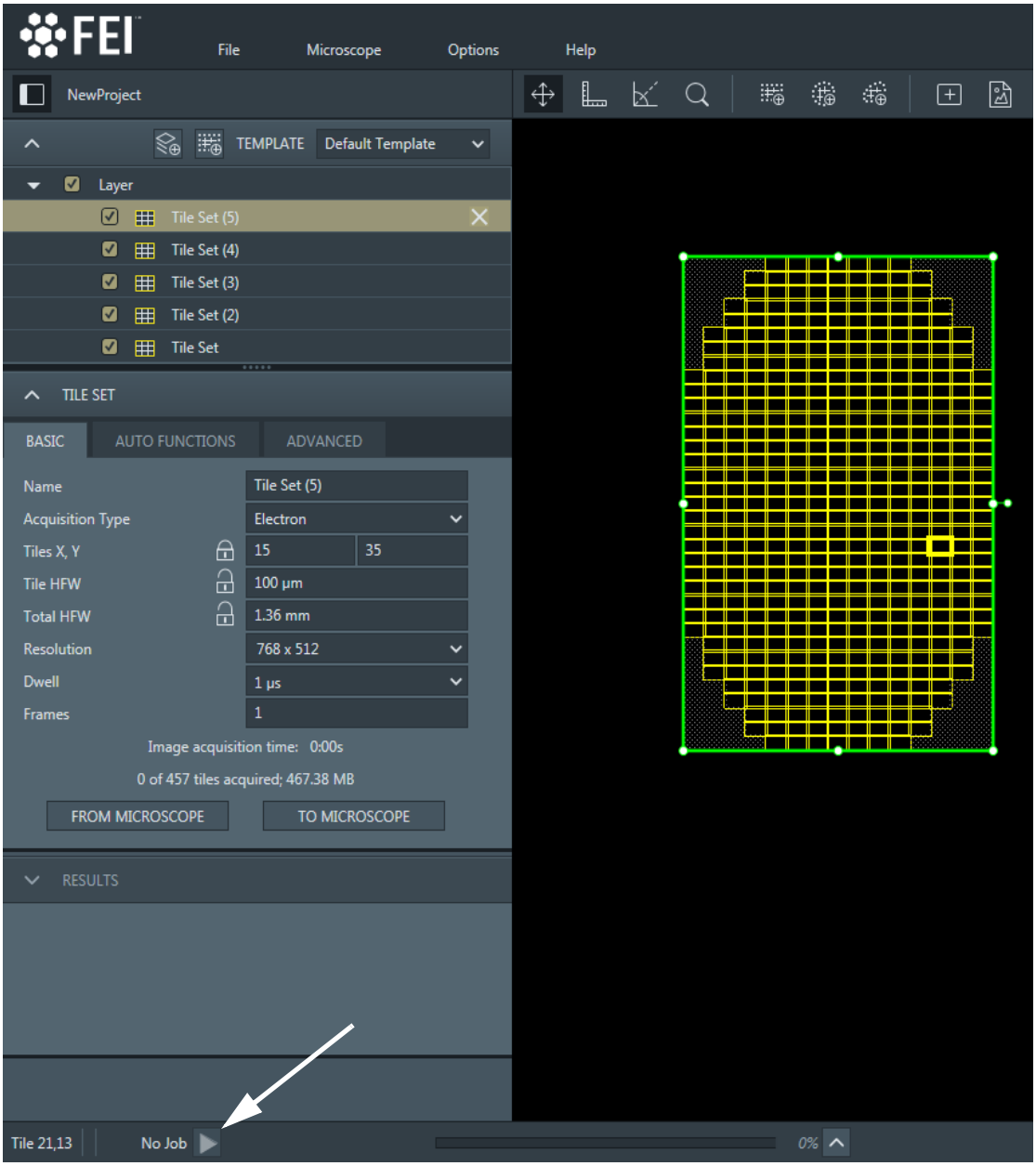
Configure Tiling Parameters

- Change the default *tiling* parameters to customize the grid for the data you will be acquiring.
- Modify the *imaging* parameters and resize the grid for the level of detail and size you need.

Acquiring a Tile Set

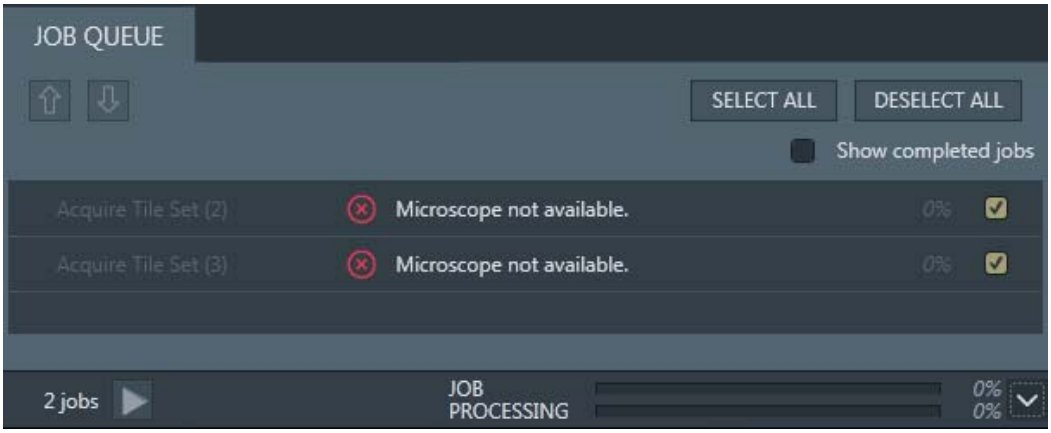
When ready to begin acquisition, click **Start Execution** located in the status bar next to **No Job**.

Tile images are registered for stitching as they are acquired and displayed in the grid. The tile sets are colored as they are registered to give immediate feedback on the stitching confidence. See *"Tile Set Grid Color Key" on page 52*.



Preconditions

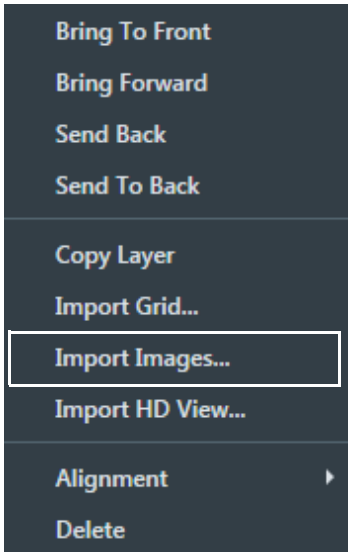
Microscope preconditions must be met to proceed with tile set acquisition. For example, if the microscope is not enabled or an acquisition parameter is invalid, then a validation error appears in relation to the tile layer and you cannot start acquisition.



Importing Images from Other Sources

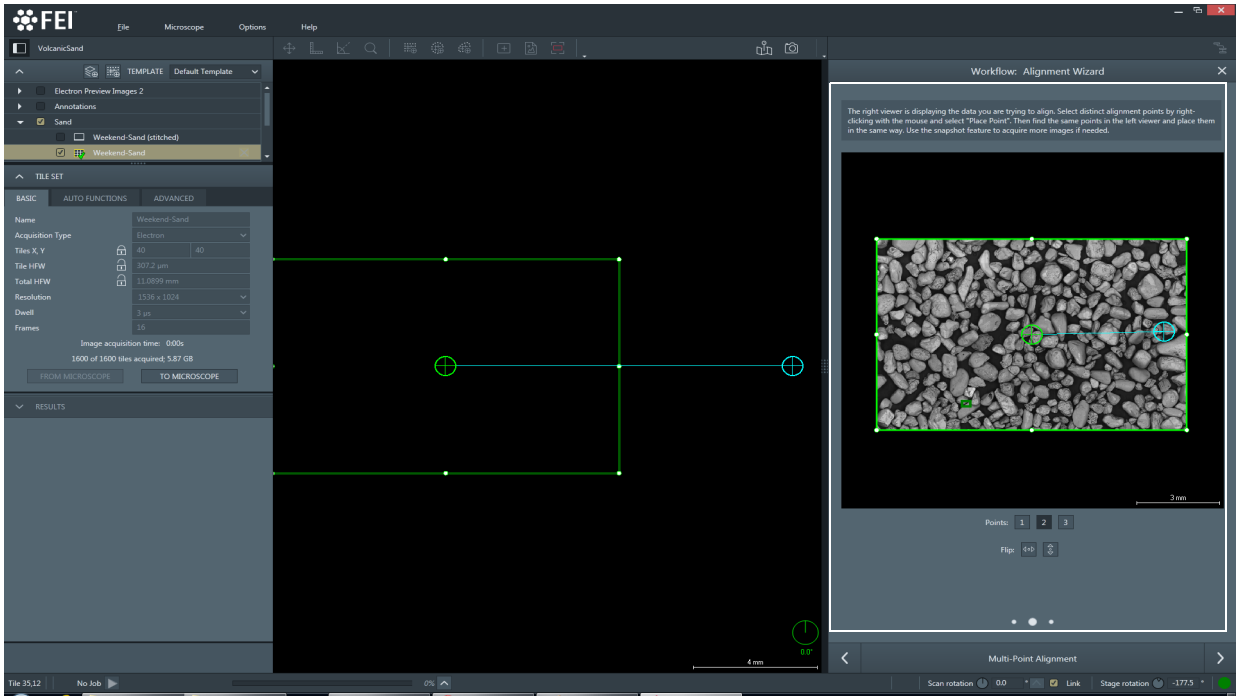
It may be useful to import an external image for navigation purposes to find the area of interest.

1. Right-click on a Layer in the Layer control and select **Import Images** on the context menu that appears.



2. In the standard **Open** window that appears, browse to a saved image and then click **Open**. Most standard image formats are supported, including but not limited to: BMP, JPG, GIF, PNG, or TIFF images.

   The imported image is displayed to the right of the main viewer while the alignment workflow is in progress. See *"Manual Alignment" on page 195*.



3. Align the two images with a simple one-, two-, or three-point alignment procedure: Move the alignment points on the imported image to match the feature on the tile set image and then click **Next**. See *"1-, 2-, or 3-Point (Coarse) Alignment" on page 196* for detailed information.

   After clicking **Next**, the viewer is restored to full size and the newly imported image is displayed aligned to the stage. The imported image name is added to the Layers control.

   Nav-Cam images (SEM/SDB and CorrSight) are automatically aligned, so this step can be skipped, although you may want to do a manual alignment for fine tweaking.

Stage Navigation

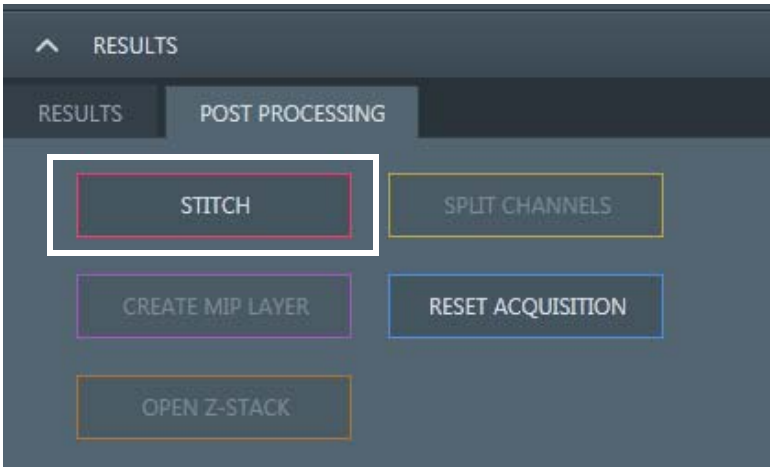
Double-click anywhere within the stage boundaries as shown by the white circle or square to move the stage to that location.

Stitching

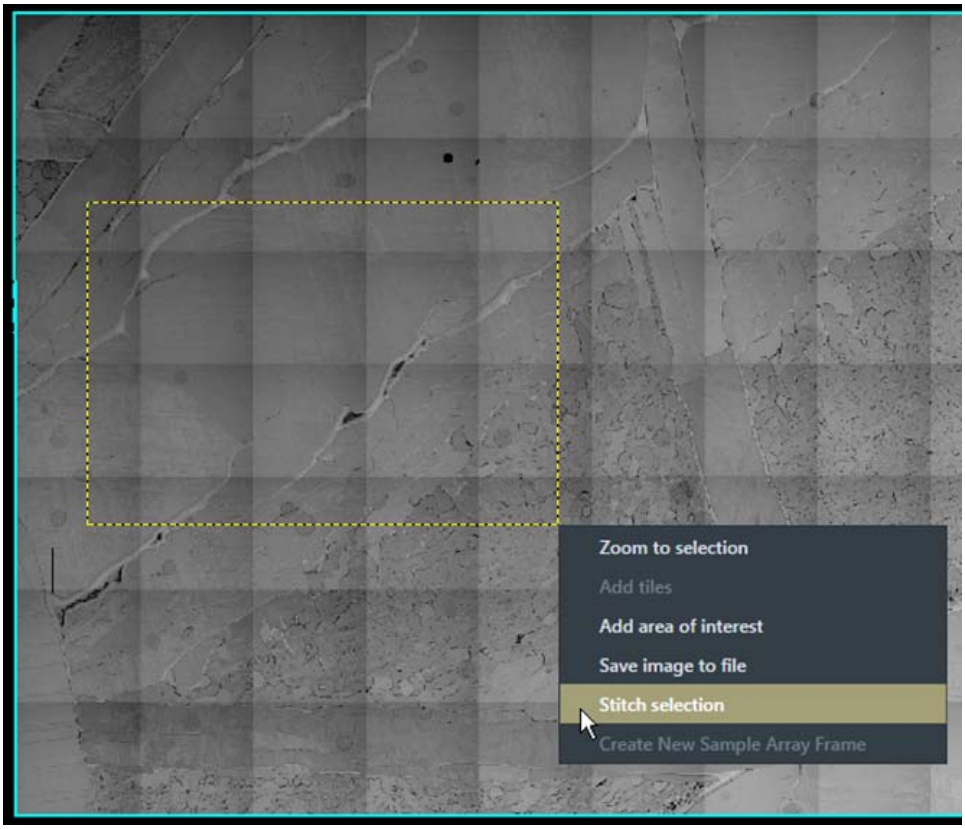
After a tile set with at least one tile has been acquired, the **Stitch** button is enabled.

Initiate Stitching

Select the acquired tile set in the Layer control and click **Stitch** in the Results Tab, Post Processing group. The manual UI immediately appears so you can confirm the tile alignments.



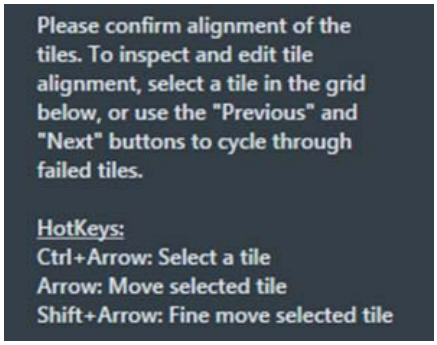
Or left-click and drag a rectangle around the tile sets to be stitched and select **Stitch Selection** in the menu.



Stitch selection will stitch all tiles inside the rectangle for the selected tile set. Tiles that are partially inside the rectangle are included as well. See *"Alt +Left-Click and Drag Selected Area" on page 52*.

Manual Alignment

Follow the guided instructions to confirm or edit the alignment of the tiles.



Stitching Manual Alignment Histogram

Displays the histogram for the Stitching Channel (see *"Stitching Channel" on page 118*).

Figure 1 Stitching Manual Alignment Histogram

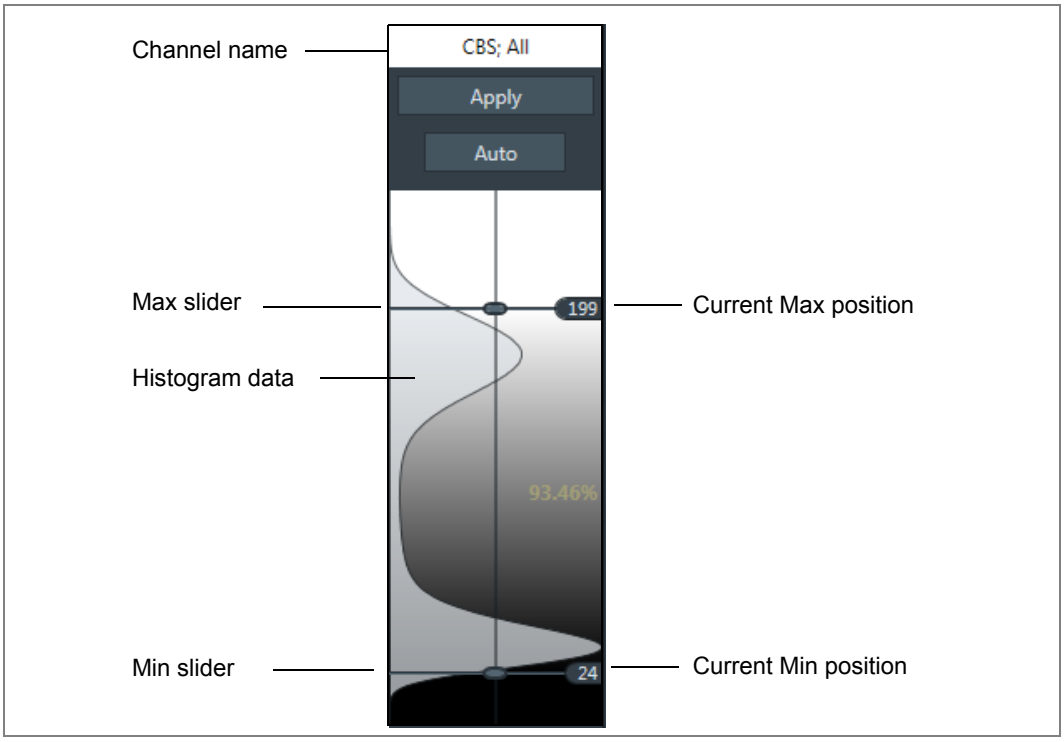


Table 1 Stitching Manual Alignment Histogram Overview

Interface Item	Description
Channel name	• **For SEM/SDB**: Displays the channel name, which is the detector name. • **For CorrSight**: Displays the individual channel name as determined by the LM software.
Apply	Applies changes to the histogram.
Auto	When selected, continuously adjusts the min and max for the histogram display.
Semi-log	When selected, changes the histogram intensity display to logarithmic.
Current Max position	Displays the current max slider position.
Histogram data	Displays the histogram data for the selected channel.
Current Min position	Displays the current min slider position.

Overlap Transparency

Overlap Transparency options are available for assigning a color for the **Center Tile** and its **Neighbor Tiles**.

Click the down arrows to select a color

Transparency is useful for seeing through the layers to line up the tiles in manual tile alignment.

Figure 2 Manual Alignment with Transparency Off

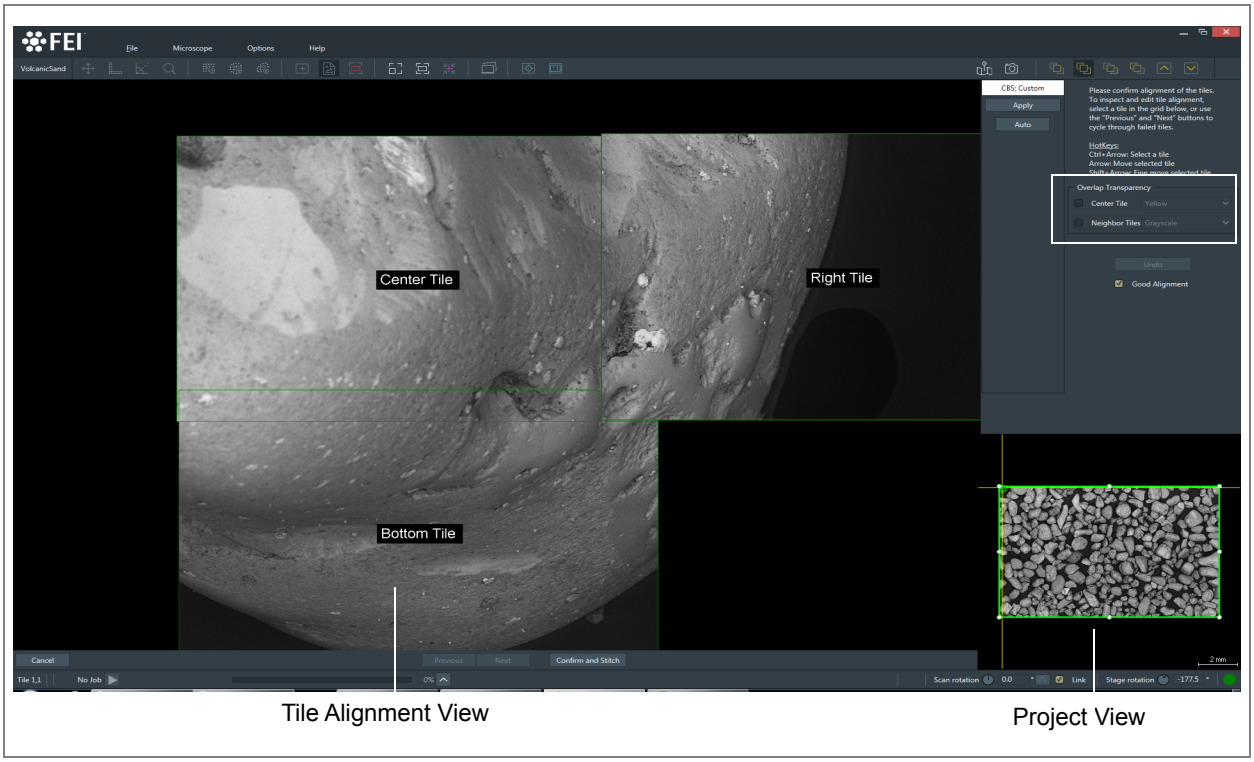
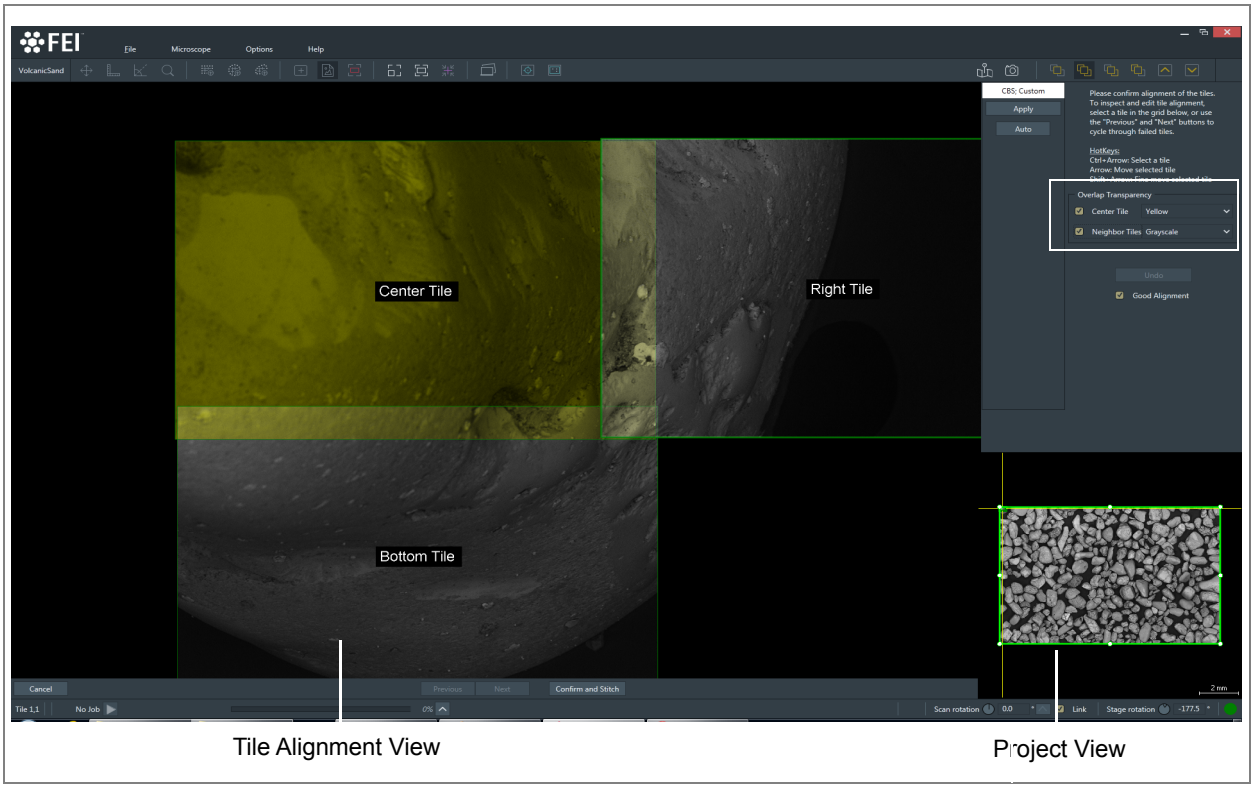


Figure 3 Manual Alignment with Transparency On



Two Views

Manual stitching makes use of two views, see *Figure 2, "Manual Alignment with Transparency Off," on page 82*:

- The **Tile Alignment View** is the larger view:

  This view displays the currently selected tile and it's top, bottom, left and right neighbors. The positions of the neighboring tiles are based on their individual registrations to the center tile. This is not the same as the final position when stitched, which will be a result of the optimization of all tile-to-tile registrations of the tile set. When manual alignment is first initiated, the first tile that failed alignment is selected and displayed here.

  The view can be panned and zoomed in the same way as the other views. The center tile is fixed, but you can click **left-mouse+drag** to move its surrounding tiles.

- The **Project View** is the smaller version of the standard viewer in the bottom right:

  This is a miniaturized version of the standard Maps project view. It can be panned, zoomed, and each tile can be selected with the mouse. When a tile is selected, it appears as the center tile in the Tile Alignment View, and it can then be manually aligned to it's neighbors.

Stitching Alignment Status Color Key

Each tile outline is colored to indicate stitching alignment status.

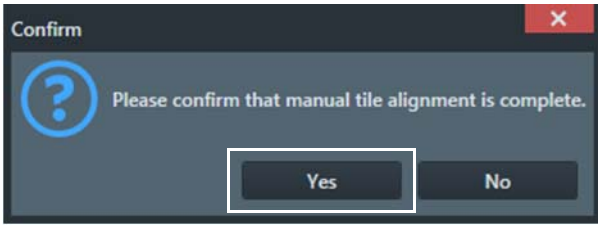
- **Green**: The automatic tile alignment succeeded.
- **Orange**: One side of the tile alignment failed, but another succeeded. These tiles do not require manual alignment.
- **Red**: The tile failed to align with either neighbor, which means that there is not enough valid registration for a tile or one of its neighboring tiles to be properly positioned in the larger picture. Often multiple tiles can be red, but only one tile needs to be manually connected to the green tiles to achieve alignment for all. After realignment, the outline turns **blue**.

Realign Failed Tiles

Use the **Next** and **Previous** buttons to jump through the list of failed tiles easily.

Confirm and Stitch

When manual alignment is finished, click **Confirm and Stitch** to begin stitching. You will be prompted to confirm and click **Yes**.



Stitching Job Options

After you click **Yes** to the Confirmation prompt, the **Stitching Job Options** window appears.

Some options are not available for all tile sets.

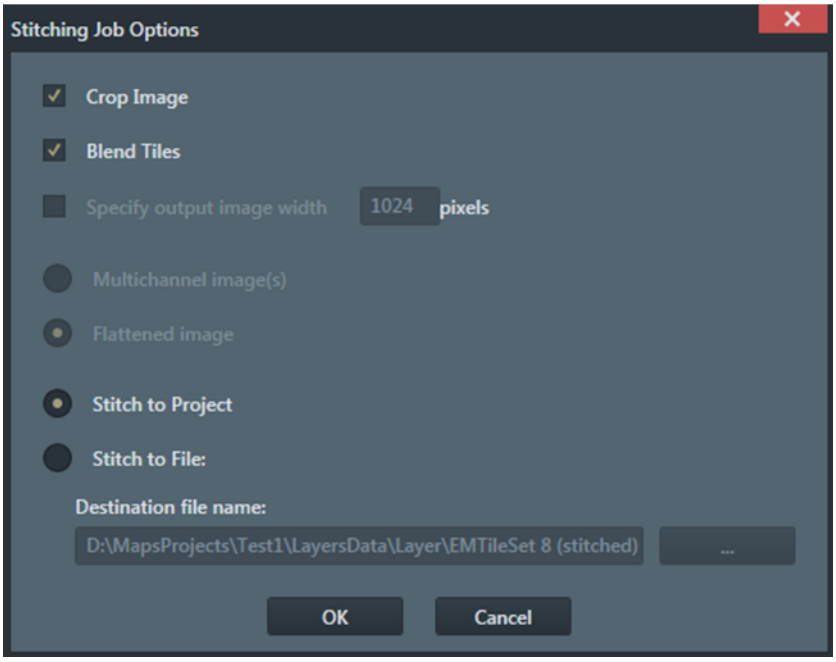
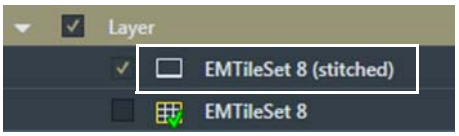


Table 2 Stitching Job Options Overview (1 of 2)

Interface Item	Description
Crop Image	Crops the outer edges of the image to make it rectangular. Otherwise, it will potentially have jagged edges where the tiles have been shifted.
Blend Tiles	Blends the overlapped regions of the tiles together to make it appear as one continuous image. In some rare cases, you may not want to blend the tiles.
Specify output image width	Determines the resolution of the stitched image.
Multichannel image(s)	*Currently enabled for Multichannel TIFF data only.* Outputs each channel to a separate image file. See *"Auto Selection" on page 86*.
Flattened image	*Currently enabled for Multichannel TIFF data only.* All channels are collapsed to a single image.

Table 2 Stitching Job Options Overview (2 of 2)

Interface Item	Description
Stitch to Project	Stitches the tile set into an HD View image and displays it in the Viewer. The stitched output will appear as a new layer in the Layer control.  When selected, the field for Destination file name is not available. Selecting **Stitch to Project** will not export the stitched output. The stitched image will only appear in the Maps project. For data export, use the **Stitch to File** feature.
Stitch to File	Stitches the tile set into an HD View or single image and exports the file to the specified folder. The output file is stored in the location and format specified: RAW, TIFF, OME.TIF (Multipage), or HDView, in the Destination File name field. The default is the current project's data location, in HD View format. ***Note:*** *You must download HD View™ from the Microsoft web site to open the HDView.htm file to see the stitched image.*
Destination File Name	Specifies the path and file name for the stitched project.
OK	Click **OK** to accept the default stitching job options and begin the stitching process.
Cancel	Closes the window.

Auto Selection

For all image types, other than TIFF, the default stitching job option is auto selected.

- **RAW and HDView**: Multichannel auto selected. Outputs each channel to a separate image file.
- **Multipage TIFF**: Outputs each channel to a separate page of a single multi-page TIFF file.

4 SEM/SDB

Overview

This chapter describes user interface elements and procedures that are specific to the SEM/SDB (Small DualBeam) systems.

Topics include:

- User Interface Elements, below
- *"Rotational Alignment" on page 105*
- *"Scan Rotation" on page 107*
- *"Stage Rotation" on page 107*
- *"Nav-Cam Alignment" on page 108*

User Interface Elements

Topics include:

- *"Microscope Menu" on page 88*
- *"Options Menu" on page 89*
- *"Tool Bar, Right Side" on page 93*
- *"Layer/Holder Definition Control" on page 94*
- *"Tile Set Tab" on page 95*
- *"Tile Set Right-Click Menu" on page 101*
- *"Nav-Cam Right-Click Menu" on page 102*

Microscope Menu

The **Microscope** menu only appears for SEM/SDB systems.

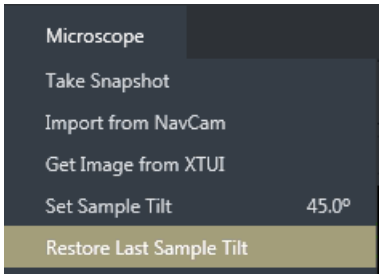
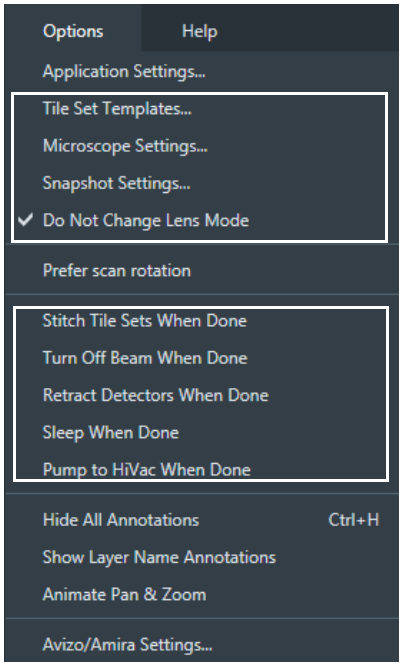


Table 1 Maps Microscope Menu Overview

Menu Selection	Description
Take Snapshot	Acquires a SEM image at the current stage position and HFW and places it on the Maps viewer as a snapshot. The HFW can be changed from the Tile right-click menu selection: *"Snapshot Settings" on page 26*.
Import from NavCam	*Disabled only while under vacuum if the door mounted Nav-Cam is installed. See "Nav-Cam Prompt (SEM/SDB Only)" on page 68.* Acquires and imports a Nav-Cam image, if the Nav-Cam is available. This top down image of the stage can be used for point and click navigation of the sample. See *"Nav-Cam Alignment" on page 108*.
Get Image from XTUI	Imports a SEM image currently displayed in the xT UI without re-acquiring the image and places it on the Maps Viewer as a snapshot.
Set Sample Tilt	Before you enable it, confirm that the sample is perpendicular to the electron beam. This preliminary step adjusts the Maps stage to keep the sample at the same distance from the beam; thus, allowing navigation and tiling at stage tilt. Once you enable this menu option, it sets the current stage tilt to *Sample Tilt*. ***NOTE**: This makes the linked focus unavailable.*
Restore Last Sample Tilt	After you make the **Set Sample Tilt** unavailable, this menu option appears. Click **Restore Last Sample Tilt** to restore the last sample tilt value.

Options Menu

- For descriptions of common selections to all systems, see *"Options" on page 22*.
- For descriptions of SEM/SDB-specific selections, see the table below.



Unique selections for SEM/SDB

Table 2 Options Menu, SEM/SDB Overview (1 of 3)

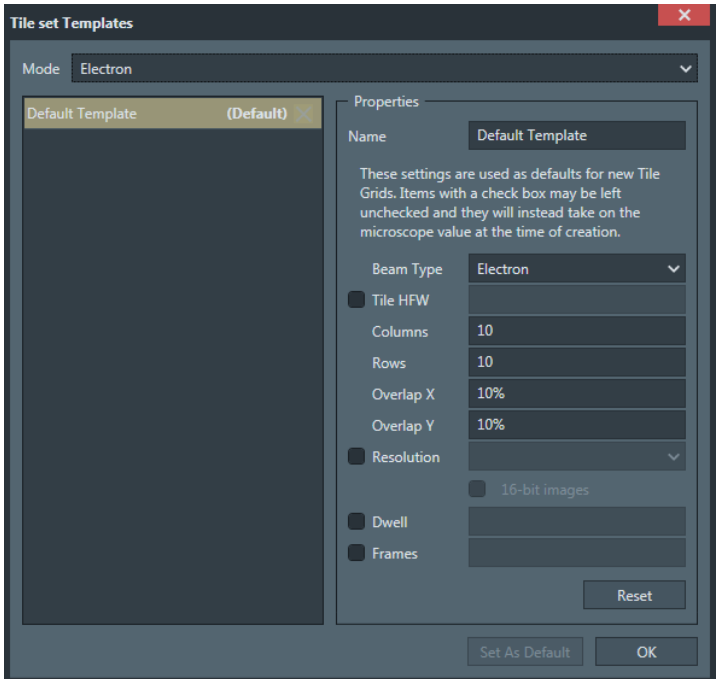
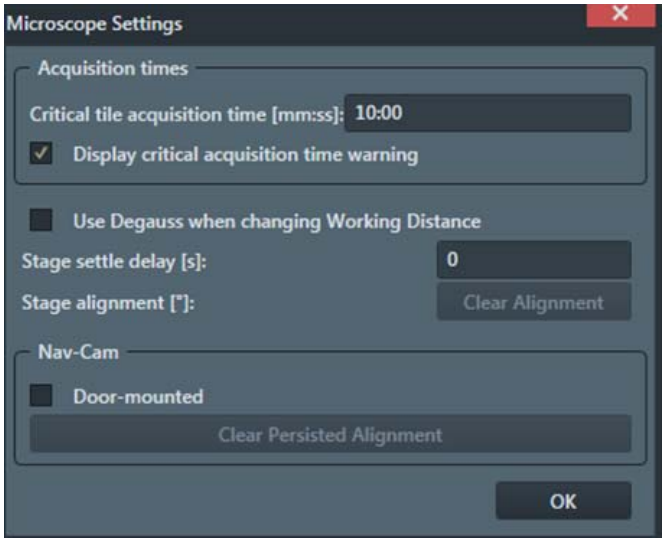
Menu Selection	Description
Tile Set Templates:	Displays a dialog that shows a collection of templates and their respective settings. 
Microscope Settings:	
Critical tile acquisition time	Maps will display a warning whenever the acquisition parameters selected for a tile set, result in a per-tile acquisition duration larger than this threshold.

Table 2 Options Menu, SEM/SDB Overview (2 of 3)

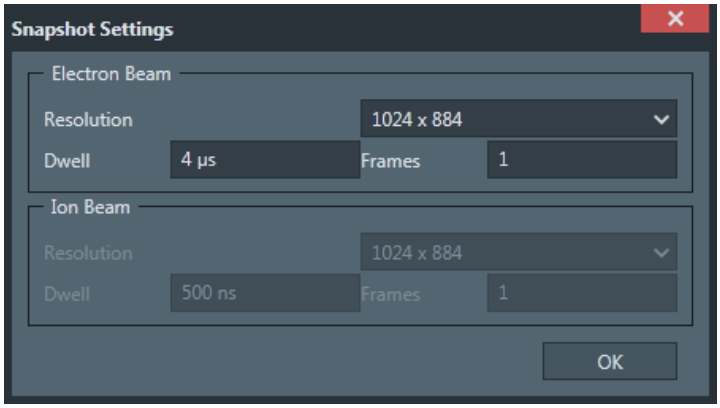
Menu Selection	Description
Display critical acquisition time warning	Select this check box to enable Maps to present the critical time warning as a dialog window that must be dismissed before proceeding. If this option is disabled, then the warning will be displayed on the Layer Definition control, but it will not require acknowledgments.
Use Degauss when changing Working Distance	Enable the check box to automatically Degauss when changing working distance during queued acquisitions.
Stage settle delay [s]	Allows setting a delay (in seconds) before acquisition to allow stage movement to settle. Without a delay, some image distortion can occur.
Stage alignment [°]	If stage alignment is in use, the **Clear** button will be enabled to clear the alignment value.
Clear Alignment	Clears the alignment value.
Nav-Cam: Door Mounted	Enable this check box if the system's Nav-Cam is mounted to the exterior of the chamber door. Leave the check box disabled if there is a Nav-Cam inside the microscope's vacuum chamber. When the Maps software is first started, you are prompted to indicate if the door-mounted Nav-Cam is installed. Therefore, this option is only used to change the setting at a later date.
Nav-Cam: Clear Persisted Alignment	Clears the saved default Nav-Cam alignment that is applied to all acquired Nav-Cam images. See*"Nav-Cam Alignment" on page 108*. To clear the Nav-Cam alignment for a single image, see *"Clear Alignment" on page 91*.
Snapshot Settings *(Disabled for SEM Offline)*	Displays the **Snapshot Settings** window and the scan settings that will be used to acquire preview images and snapshots. 

Table 2 Options Menu, SEM/SDB Overview (3 of 3)

Menu Selection	Description
Do Not Change Lens Mode	When this option is enabled, it will prevent the Maps application from switching the SEM into or out of UHR (immersion) mode. Setting this option to Disabled, allows a queued tile set to automatically activate UHR mode for image acquisition without manual user interaction in the xT UI. In most cases, you should leave this option set to its default value of Enabled.
Stitch Tile Sets When Done *(Disabled for SEM Offline)*	Automatically adds a stitching job to the end of the job queue for each completed acquisition job.
Turn Off Beam When Done *(Disabled for SEM Offline)*	Turns off the beam after any queued acquisition and stitching jobs are completed.
Retract Detectors when Done *(Disabled for SEM Offline)*	Retracts any insertable detectors after any queued acquisition and stitching jobs are completed.
Sleep When Done *(Disabled for SEM Offline)*	Puts the system into Sleep mode after any queued acquisition and stitching jobs are completed.
Pump to HiVac When Done	Returns the microscope to HiVac mode after completing a LowVac acquisition.

WARNING!

The Do Not Change Lens Mode feature exists to block the ability of Maps to perform an action with a risk of hardware damage. The SEM should never be put into UHR mode while a magnetic sample is loaded in the chamber, since this could damage equipment inside the microscope. Only make this feature unavailable if you are certain the sample is non-magnetic and you are operating in a use case that requires Maps to change lens modes without user intervention.

Tool Bar, Right Side

See *"Left Side, All Systems" on page 30* for common selections to CorrSight and TEM.

Figure 1 Tool Bar, Right Side, SEM/SDB

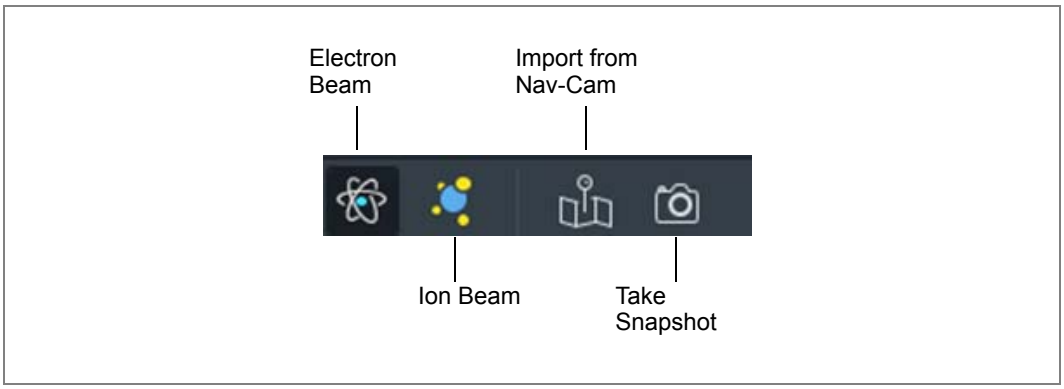


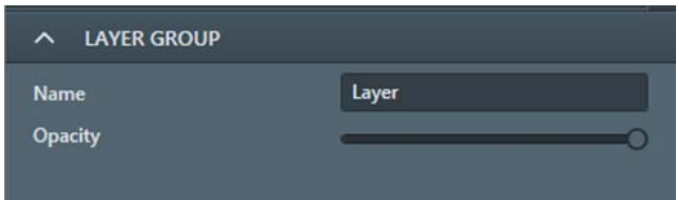
Table 3 Tool Bar Descriptions, SEM/SDB

Tool	Description
Electron Beam (SEM) *(Online version only)*	Selects the Electron beam for imaging.
Ion Beam (FIB) *(Online version only)*	Selects the Ion beam for imaging.
Import from Nav-Cam *(with Nav-Cam version only)*	*Disabled only while under vacuum if the door mounted Nav-Cam is installed. See "Nav-Cam Prompt (SEM/SDB Only)" on page 68.* Acquires and imports a Nav-Cam image, if the Nav-Cam is available. This top down image of the stage can be used for point and click navigation of the sample. See *"Nav-Cam Alignment (SEM/SDB and CorrSight Only)" on page 71*.
Take Snapshot	Acquires a SEM image at the current stage position and HFW and places it on the Maps viewer as a snapshot image. The HFW can be changed from the Tile right-click menu selection: *"Snapshot Settings" on page 26*.

Layer/Holder Definition Control

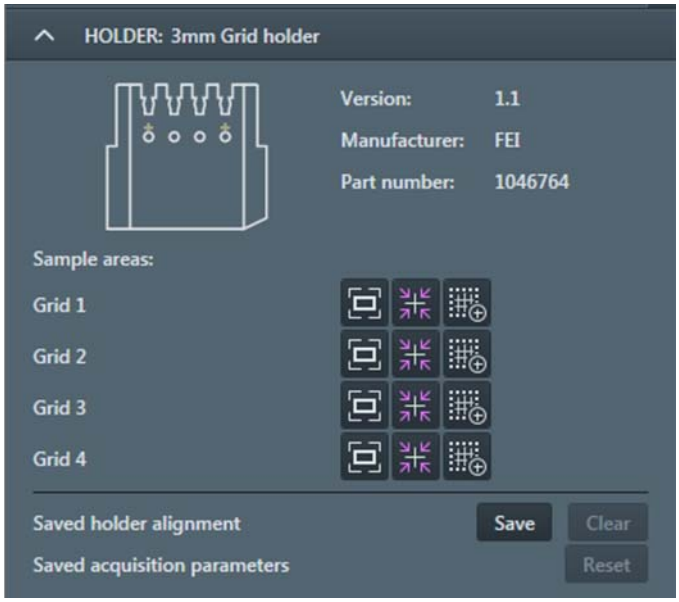
Displays controls/information for the selection in the Layer control:

- Layer Group

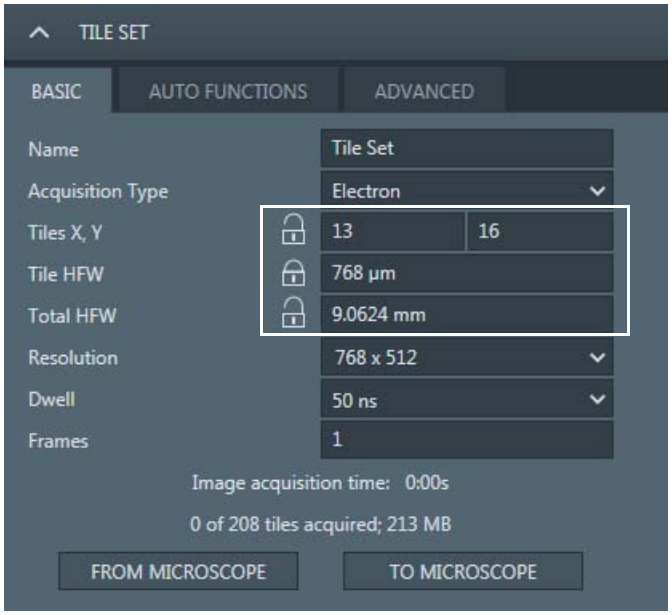


Use the **Opacity** slider to set the measure of opaqueness (how much you can see through). The less opaque, the more transparent it becomes. Transparent images are useful when overlaying one image over another

- Holder



Tile Set Tab

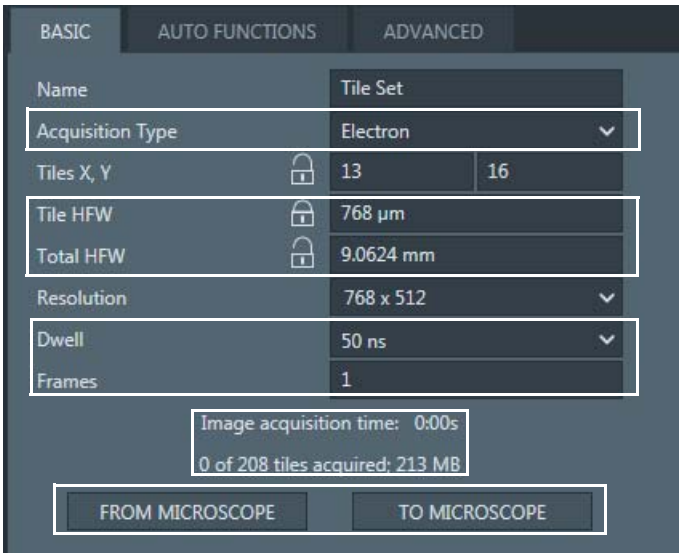


Lock methodology:
The three Tile controls are mathematically interlocked. Only one will be locked at a time. If you lock one of the controls and change another control, the third control changes to correspond with the other two control values.

Topics in this section include:

- *"Basic Tab" on page 96*
- *"Auto Functions Tab" on page 97*
- *"Advanced Tab" on page 99*

Basic Tab



Specific selections for SEM/SDB

Table 4 Tile Set Tab, Basic Tab Overview

Control	Description
Name	Displays the name of the current tile set.
Acquisition Type	Specifies the beam to be used for imaging. Choices are: Electron or Ion.
Tiles X, Y	Sets the number of tiles in the X and Y directions.
Tile HFW	Specifies the horizontal field width (HFW) for each tile.
Total HFW	Shows the total HFW for all of the tiles.
Resolution	Specifies the image resolution. Choices will be specific to each system type.
Dwell	Specifies the amount of time the beam dwells on each pixel when images are acquired.
Frames	Sets the number of image frames.
Image Acquisition Time	Displays an estimated time required to acquire the tile set.
Number of Tiles Acquired	Displays the number of tiles and the total disk space required to acquire the current tile set. A message appears if there is not enough space on disk for the acquisition.
From Microscope	Applies the current microscope setting.
To Microscope	Applies these settings to the microscope.
Acquire	Acquires this tile set.
Queue	Adds this tile set to the job queue.

Auto Functions Tab

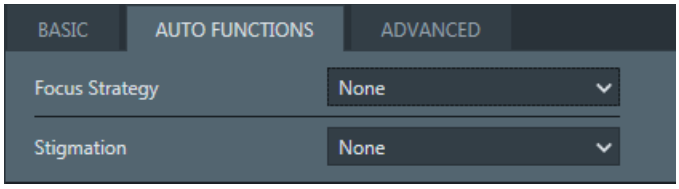


Table 5 Tile Set Tab, Auto Functions Tab Overview (1 of 2)

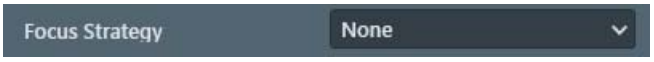
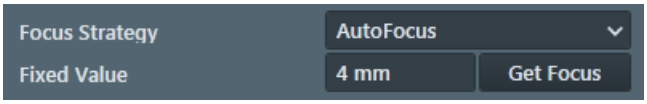
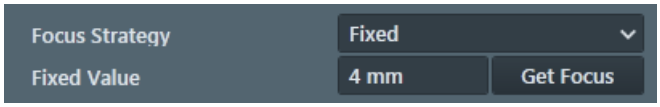
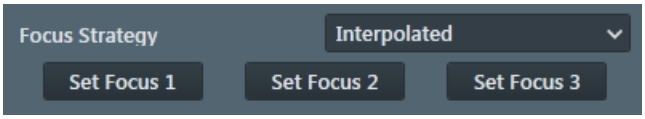
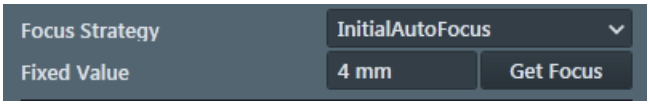
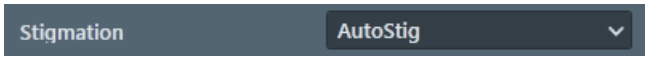
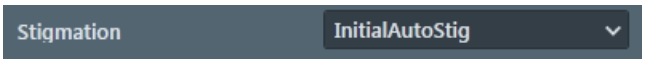
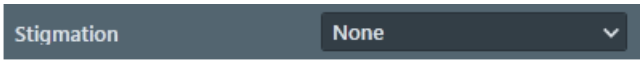
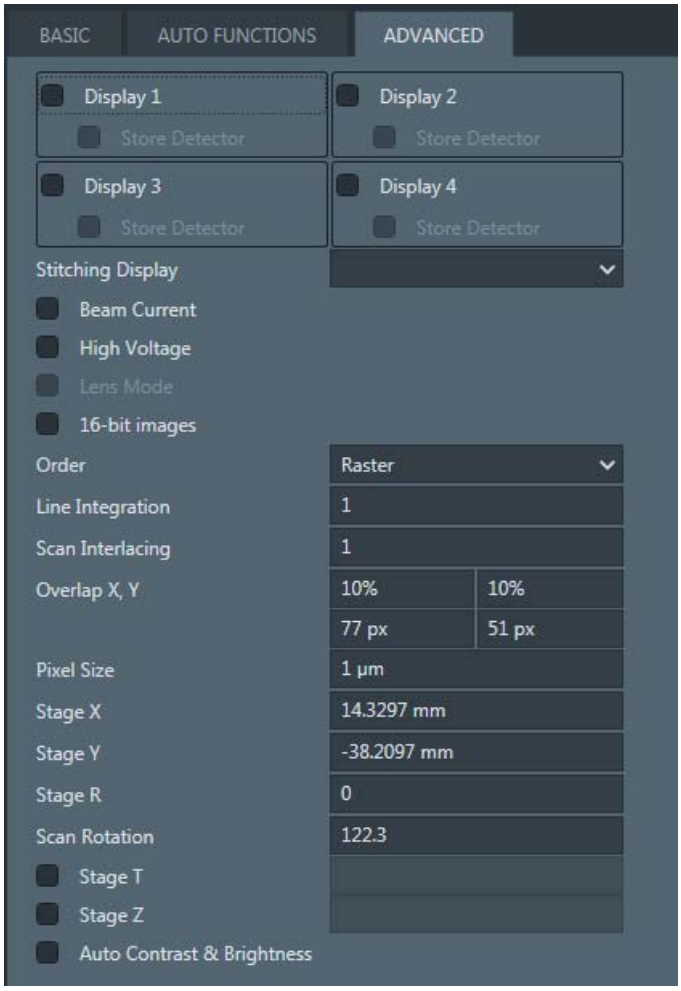
Control	Description
Focus Strategy	Specifies the focus setting. Choices are: • **None:** The application will not touch the focus at all. You are free to change the focus via the xT UI during the run.  • **Autofocus**: Runs the autofocus routine at each tile before acquisition. This can result in better focus per tile, but adds considerably more time to acquisition.  • **Fixed**: Uses the same working distance for every tile.  • **Interpolated**: User will select three focus points on the sample and a focus plane will be defined by these points. Each tile's focus will be set based on where it is located in this plane. 

Table 5 Tile Set Tab, Auto Functions Tab Overview (2 of 2)

Control	Description
Focus Strategy (cont.)	Procedure: Navigate to the first location. Manually focus the image. When image is in focus, click **Set Focus 1** button. The current working distance will be remembered for that point and the button will now be checked. Repeat for the other two **Set Focus** buttons. Once all three are checked, tiles are ready to be acquired. **Note:** *These points will then be used to define a focus plane, so that all tiles will use a WD as defined by this plane. Choosing points that are at opposite ends of the sample is important to get the best results.* To unset a focus point, click **Set Focus** for that point. **Set Focus** is an on/off toggle. • **InitialAutoFocus** or **SingleAutoFocus**: Performs a single autofocus routine at the center of the tile set and uses the resulting focus for the entire tile set. 
Stigmation	Specifies the stigmation options: • **AutoStig**: Runs the auto stig routine for every tile in the tile set.  • **Fixed**: Allows saving of the current stig from the microscope and sets this value whenever this tile set is run.  • **Initial AutoStig**: Runs the auto stig routine before running the tile set.  • **None**: No auto stig routine will be run. 

Advanced Tab

Use the controls on this tab to set advanced tile acquisition properties for each tile set.



Check box methodology:

- When the check boxes are **not selected**, the software uses the values set in the xT user interface when the tile set acquisition began.
- When the check boxes are **selected**, the software locks in the current xT user interface setting. This allows you to set specific values for different tile sets.

Table 6 Tile Set Tab, Advanced Tab Overview (1 of 2)

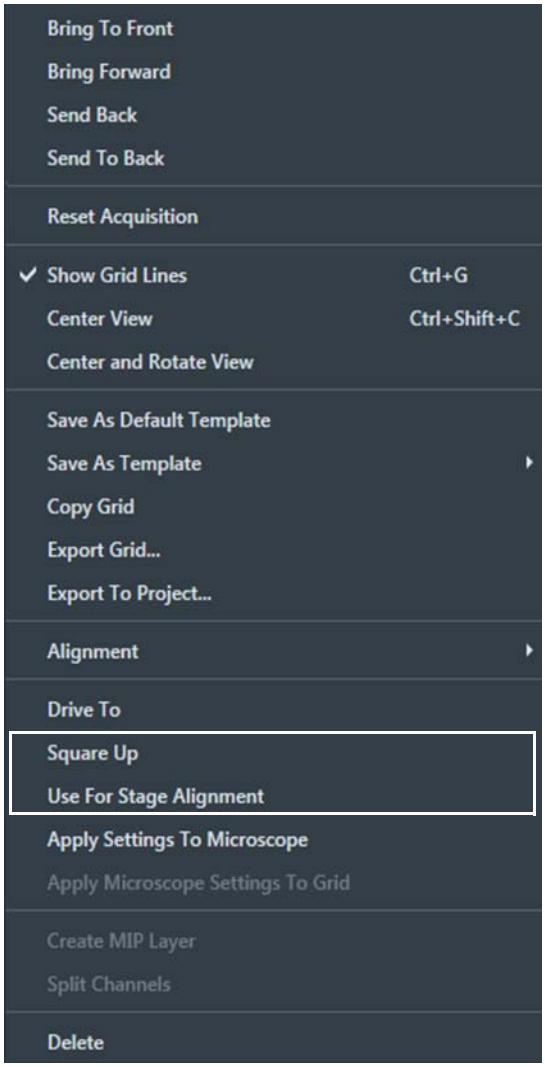
Control	Description
Quads(1-4)	Select multiple quads to acquire images from multiple display channels.
Detector	Selects the method for acquiring detector settings. You can set up different tile sets with different detectors and line them up for acquisition. When selected, a label displays the current setting.
Stitching Quad	Selects the quad to be used for stitching reference. It is best to choose the quad that will have the most image data.
Beam Current	Selects the method for acquiring the total amount of electron or ion current striking the sample. When selected, a label displays the current setting.

Table 6 Tile Set Tab, Advanced Tab Overview (2 of 2)

Control	Description
High Voltage	Selects the method for acquiring the high voltage setting. When selected, a label displays the current setting.
Lens Mode	Selects the electron beam lens mode which sets the value of either standard or immersion mode.
16-bit image	When selected, saves image as 16-bit (65K grayscale levels), else image is saved as 8-bit (256 grayscale levels).
Order	Determines the order in which the contained tiles will be acquired. Options are: • **Raster**: Left to right, then top to bottom. • **Serpentine**: Alternating left to right pattern per row, running top to bottom. • **Spira**l: Iterating over tiles in a clockwise pattern starting with the outer most tiles moving inward.
Line Integration	Specifies the number of times each raster line is scanned and integrated to combat certain types of drift.
Scan Interlacing	Specifies the number of times each frame is interlaced to combat certain types of drift.
Overlap X, Y	Displays the percentage of overlap between tiles in the tile set, as well as the pixels.
Pixel Size	Specifies the physical size of a single pixel at the current acquisition settings. This is based on Tile HFW and Resolution (from Basic settings tab).
Stage X	Sets the X stage location of the center of the tile grid.
Stage Y	Sets the Y stage location of the center of the tile grid.
Stage R	Sets the stage rotation at which the tiles will be acquired. Existing tile sets rotate with the stage. New tile grids are created at the current rotation. Select the check box to enable the text field to change the default rotation.
Scan Rotation	Allows manual adjustment of scan rotation for a tile set. Either enter a value in the edit box here or rotate the tile set in the viewer to update the scan rotation value.
Stage T	Sets the stage tilt at which the tiles will be acquired. Select the check box to enable the text field to change the default tilt.
Stage Z	Sets the stage height (Z) to be stored and used for acquisition. This allows multiple tile sets to be acquired at different heights, that is sometimes necessary for using different detectors. If left unselected, the stage height will not be changed.
Auto Contrast & Brightness	Enables the Auto Contrast & Brightness routine to be run.

Tile Set Right-Click Menu

Right-click on any tile set to access a context menu.



Unique selections for SEM/SDB

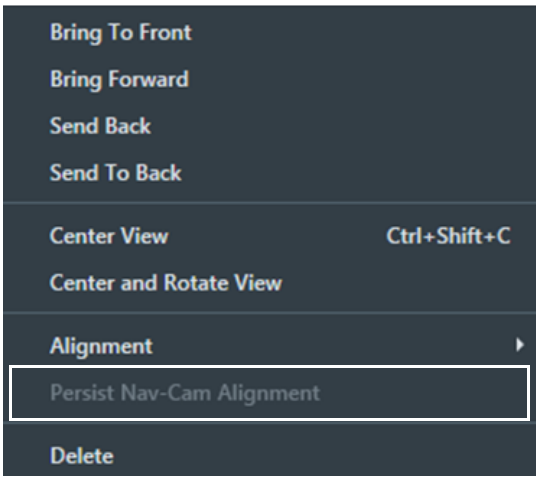
- For descriptions of common selections to all systems, see *"Tile Set Right-Click Menu" on page 46*.
- For descriptions of SEM/SDB-specific selections, see the table below.

Table 7 Tile Set Right-Click Menu Overview

Menu Selection	Description
Square Up	Rotates the stage to match the orientation of the tile set.
Use for Stage Alignment	When selected, uses the offsets calculated in the acquired tile set to correct for misalignment between the scan axes and the stage axes by applying a small scan rotation. This will allow better acquisition and alignment of future tile sets.

Nav-Cam Right-Click Menu

The Nav-Cam image is stored in the selected layer. Right-click on a Nav-Cam entry to access a context menu.



Unique selection for Nav-Cam.

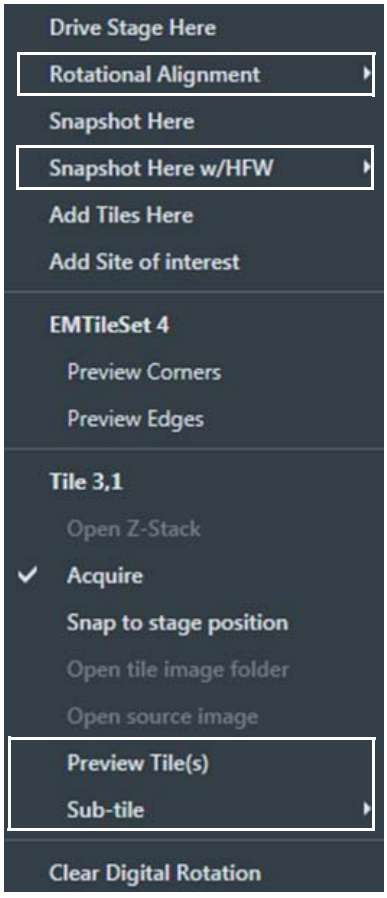
- For descriptions of common selections to all systems, see *"Annotation Right-Click Menu" on page 43*.
- For descriptions of SEM/SDB-specific selections, see the table below.

Table 8 Nav-Cam Right-Click Menu Overview

Menu Selection	Description
Persist Nav-Cam Alignment	Keeps the current Nav-Cam alignment.

Viewer Tile Right-Click Menu

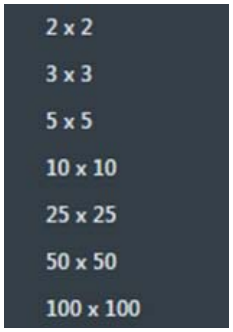
Right-click on a tile within the tile grid to access a context menu.



Specific selections for SEM/SDB

- For common selections, see *"Viewer Tile Right-Click Menu" on page 56*.
- For SEM/SDB-specific selections, see the table below.

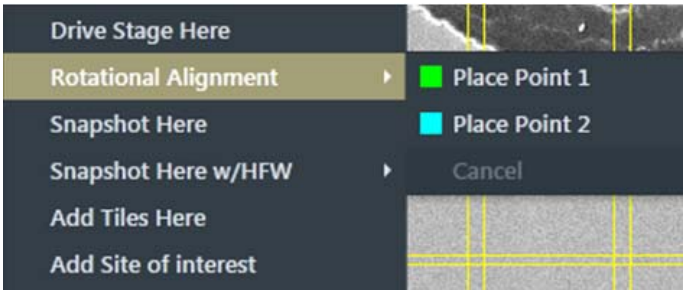
Table 9 Viewer Tile Right-Click Menu, SEM/SDB Overview

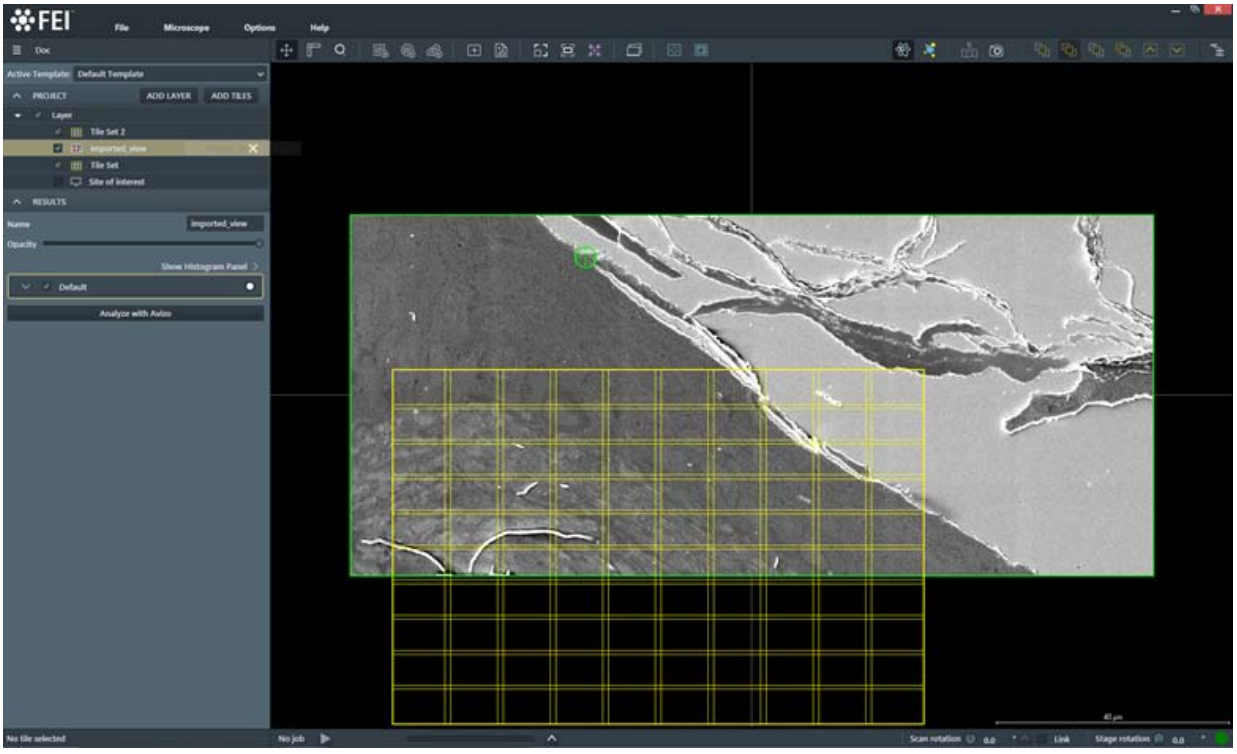
Menu Selection	Description
Rotational Alignment	Allows rotation of the stage to **Place Point 1**, **Place Point 2**, or **Cancel**. See *"“Rotational Alignment” on page 105*.
Snapshot Here with HFW	Acquires a preview SEM image at the selected stage coordinate and current HFW. Submenu choices:  This is the same functionality as using the **Take Snapshot Image** tool box button. See *“Take Snapshot” on page 93*.
Preview Tile(s)	Displays the image for that tile. Any tiles without pertinent image data can be disabled from image acquisition.
Sub-tile	Displays a submenu for selecting the number of sub-tiles to be in a mini grid within an individual tile, using the selected number of images.  The sub-tile names are automatically added to the Layers control (TileSet: (3,4)).

Rotational Alignment

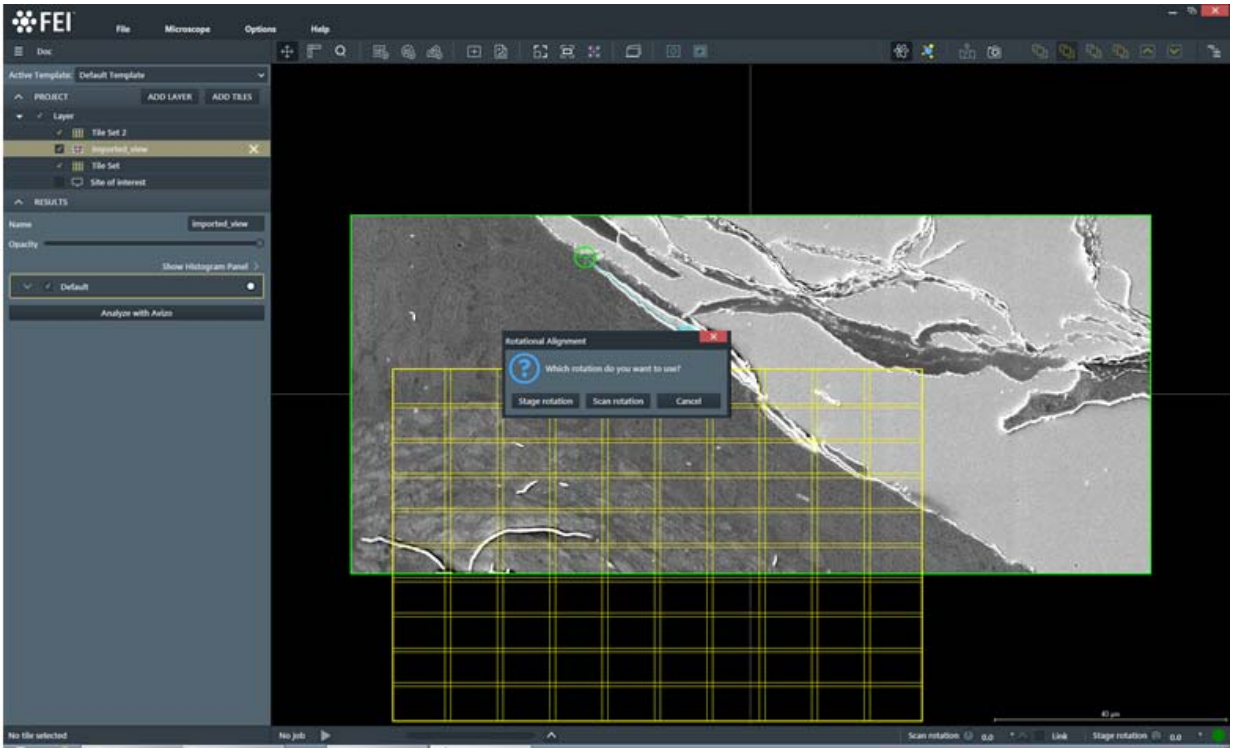
CAUTION	Take care when using this feature with detectors inserted. Also, verify the clearance of installed stage hardware manually using the xT UI before performing the rotation in Maps.

1. Right-click where point 1 is to be placed, then click Rotational Alignment, then Place Point 1.



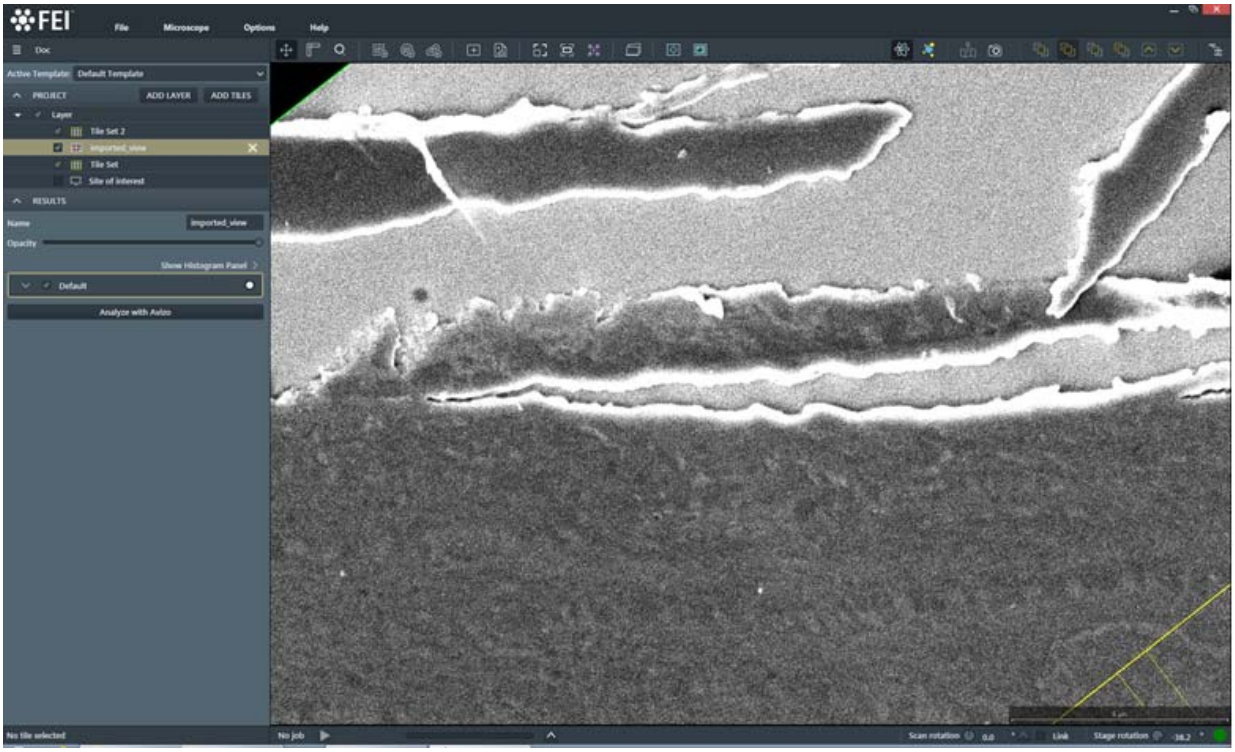


2. Right-click where point 2 is to be placed, then click Rotation Alignment, then Place Point 2. A message appears asking which rotation you want to use.



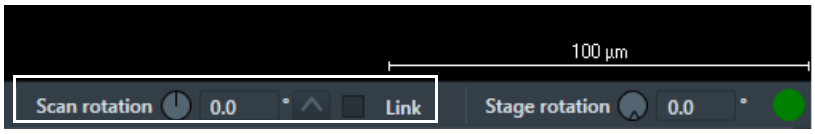
- **Stage Rotation**: Uses stage rotation to align the image.
- **Scan Rotation**: Uses scan rotation to adjust the image.

The stage rotates to make the line between Points 1 and 2 horizontal in the viewer.



Scan Rotation

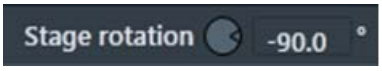
Scan rotation rotates the scan and aligns the image. It has no effect on the stage movements and is solely a scan coil function, but is used to orient the image relative to mechanical rotation and detector direction.



A non-zero scan rotation is indicated by an icon in the Status bar for the electron and ion beam independently, and its value can be shown as a tool tip. Click ^ to assign the viewer rotation to the value in the text box next to it.

Stage Rotation

Type a value into the text box and press **Enter** to rotate the stage.



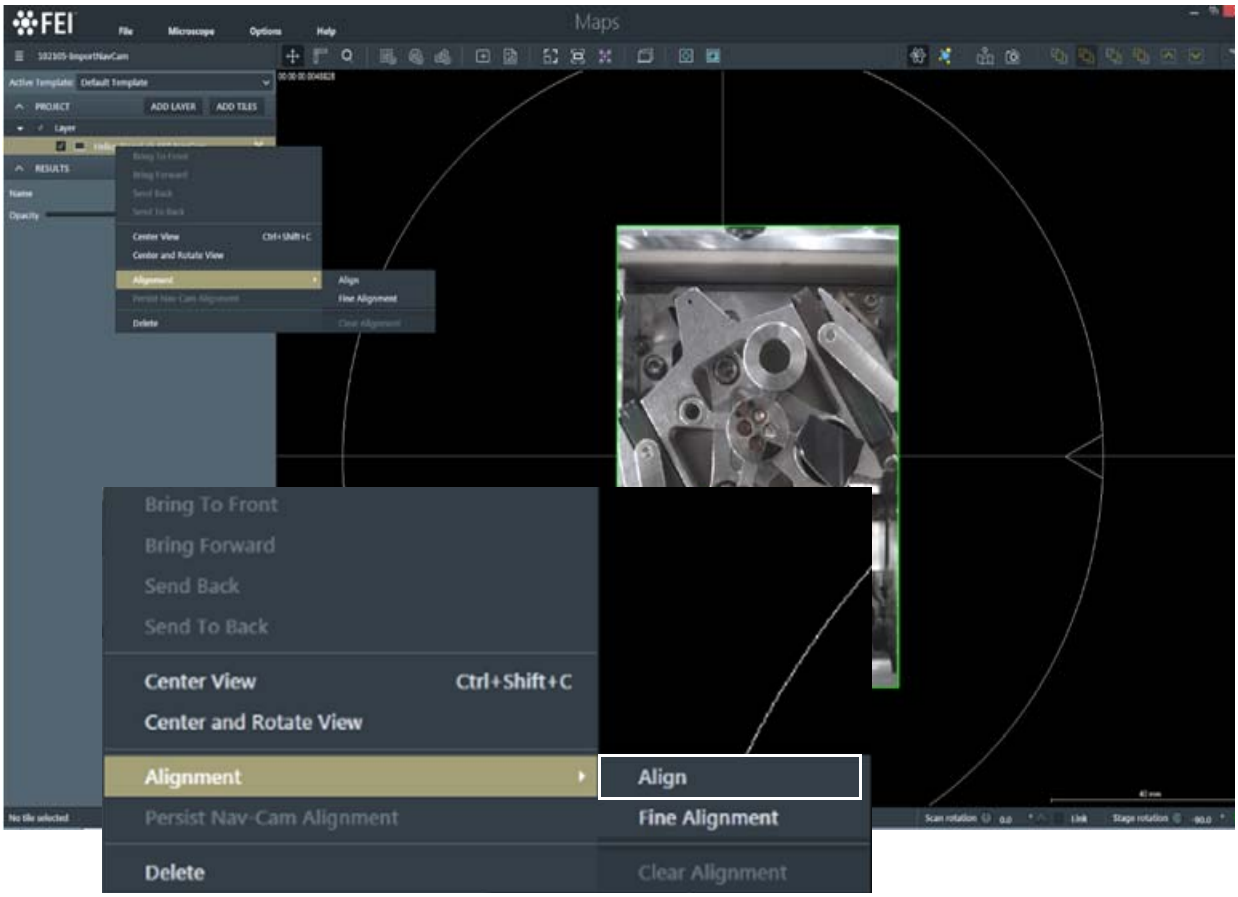
Nav-Cam Alignment

For systems that have the Nav-Cam, Maps is able to acquire Nav-Cam images and align them. Maps is also able to save the Nav-Cam Alignment to be automatically applied to all future Nav-Cam images acquired with Maps.

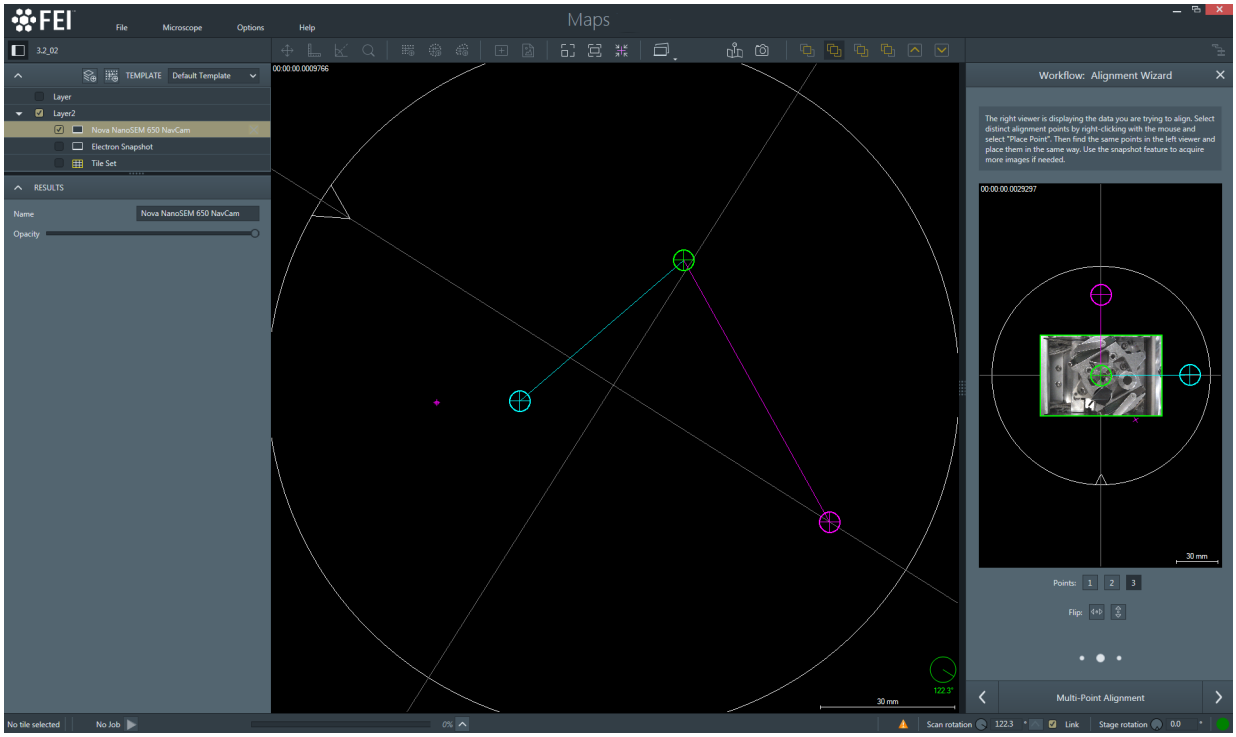
Procedure for Nav-Cam Alignment:

1. Click the **Import from Nav-Cam** tool bar icon.

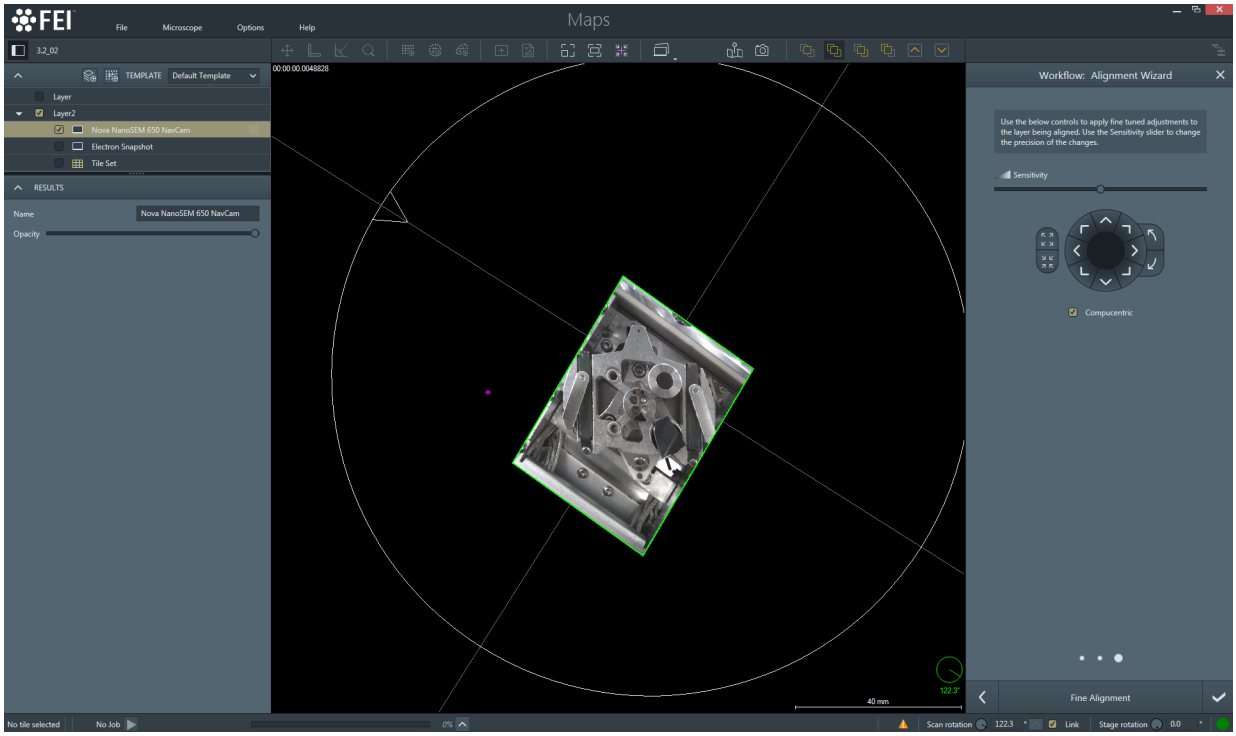
2. When the Nav-Cam image appears in Maps, right-click on its name in the Layer control and then click **Align** in the menu that appears.



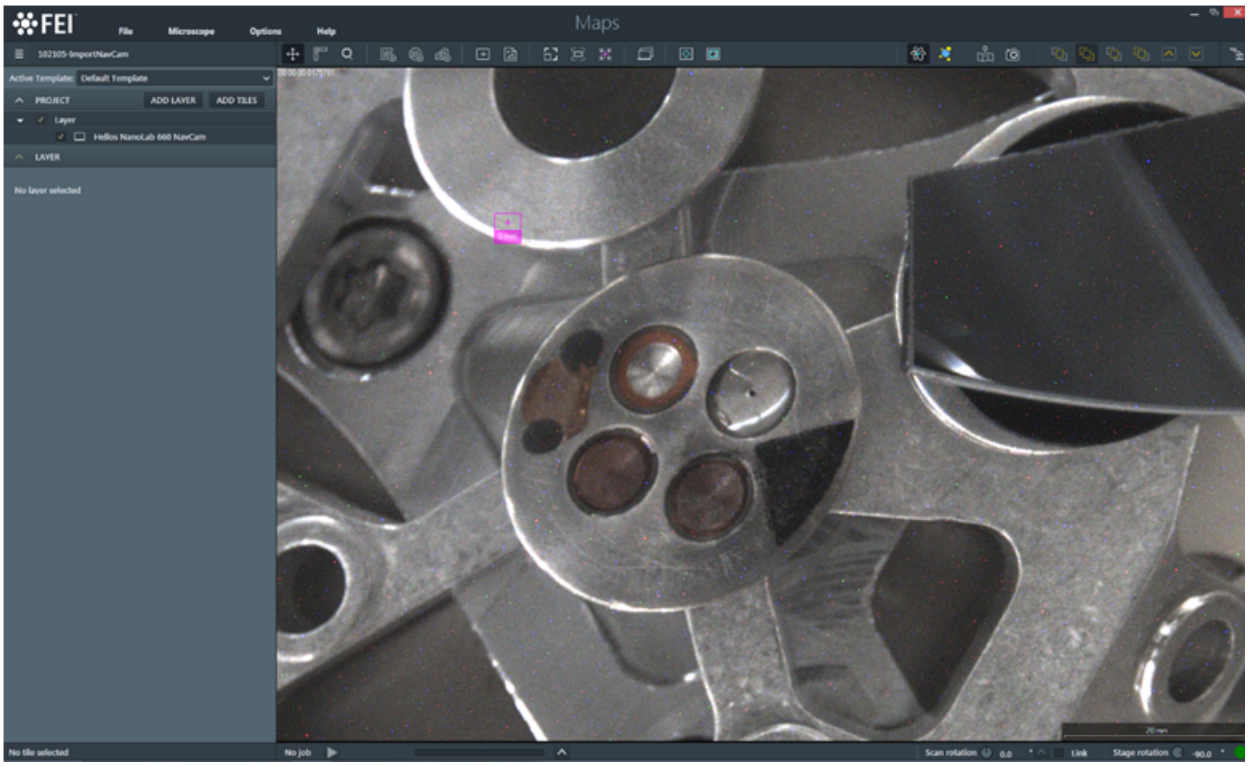
3. Follow the alignment wizard to perform the coarse point alignment (1-, 2- or 3-Point) as described in *"1-, 2-, or 3-Point (Coarse) Alignment" on page 196*.



4. Follow the alignment wizard to perform fine alignment adjustments as described in *"Fine Alignment" on page 200*.



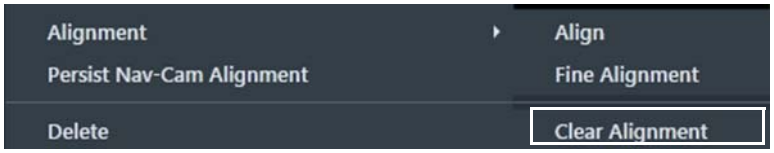
5. Test the Nav-Cam alignment by navigating to features on the sample and acquiring preview images. If the Nav-Cam image is not sufficiently aligned, perform the alignment procedure again.



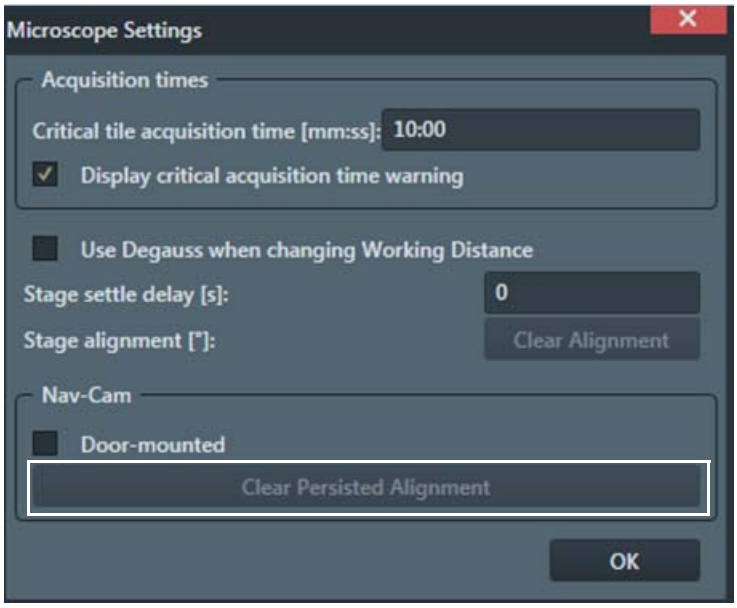
6. When the Nav-Cam image is sufficiently aligned, right-click on its name in the Layer control and select **Persist Nav-Cam Alignment** in the menu that appears.

All subsequent Nav-Cam acquisitions using Maps will use this alignment.

7. To clear the Nav-Cam alignment for an **individual** Nav-Cam image, right-click the Nav-Cam image in the Layer control and select **Alignment > Clear Alignment**.



8. To clear the saved **default** Nav-Cam alignment that is applied to **all** acquired Nav-Cam images, select **Options > Microscope Settings > Clear Persisted Alignment**.



5 CorrSight

Overview

This chapter describes user interface elements and procedures that are specific to the CorrSight systems.

For procedural information on the light microscope operation itself, refer to the documentation that came with your system.

Topics include:

- User Interface Elements, below
- *"Microscope Controls" on page 123*
- *"Live Imaging" on page 126*
- *"Live Imaging Controls" on page 127*
- *"Nav-Cam Support" on page 129*
- *"Nav-Cam Alignment" on page 129*

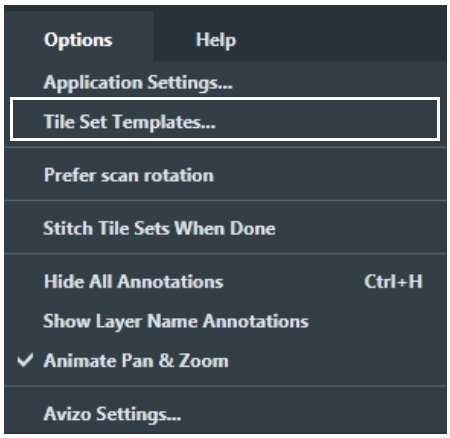
User Interface Elements

Topics include:

- *"Options Menu" on page 113*
- *"Tool Bar, Right Side" on page 114*
- *"Layer/Holder Definition Control" on page 115*
- *"Tile Set Tab" on page 116*
- *"Preview Layer, Nav-Cam Right-Click Menu" on page 121*
- *"Viewer Tile Right-Click Menu" on page 122*

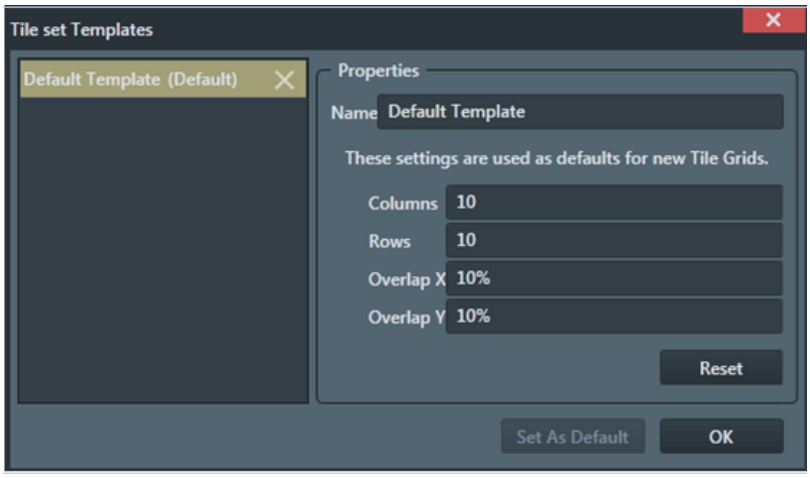
Options Menu

- For descriptions of common selections to all systems, see *"Options" on page 22*.
- For descriptions of SEM/SDB-specific selections, see the table below.



Specific selection for CorrSight

Table 1 Options Menu Overview

Menu Selection	Description
Tile Set Templates:	Displays a dialog that shows a collection of templates and their respective settings. 
Stitch Tile Sets When Done *(Disabled for CorrSight Offline)*	Automatically adds a stitching job to the end of the job queue for each completed acquisition job.

Tool Bar, Right Side

See *"Left Side, All Systems" on page 30* for common selections to SEM/SDB and TEM.

Figure 1 Tool Bar, Right Side

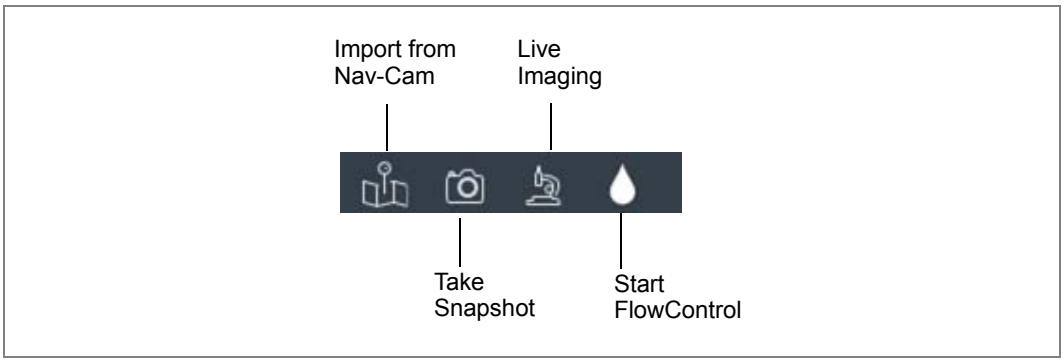


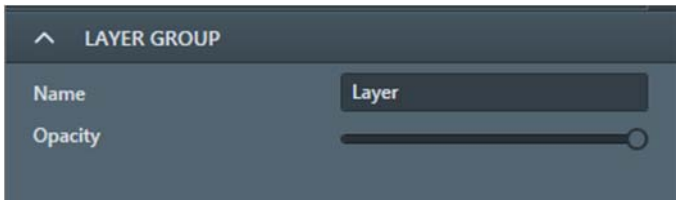
Table 2 Tool Bar Descriptions

Tool	Description
Import from Nav-Cam *(with Nav-Cam version only)*	*This is unavailable while under vacuum if the door mounted Nav-Cam is installed. See "Nav-Cam Prompt (SEM/SDB Only)" on page 68.* Acquires and imports a Nav-Cam image, if the Nav-Cam is available. This top down image of the stage can be used for point and click navigation of the sample. See *"Nav-Cam Alignment" on page 129*.
Take Snapshot	Acquires an image at the current stage position and HFW and places it on the Maps viewer as a snapshot image. The HFW can be changed from the Tile right-click menu selection: *"Snapshot Here" on page 57*.
Live Imaging	Toggles live imaging on and off. See *"Live Imaging" on page 126*.
Start FlowControl	Starts the FlowControl software used to control the pump and valves of the CorrSight live module. It allows manual and automated control of the live module by protocols defined and executed by FlowControl. The calibration of the flow rate can also be done with FlowControl.

Layer/Holder Definition Control

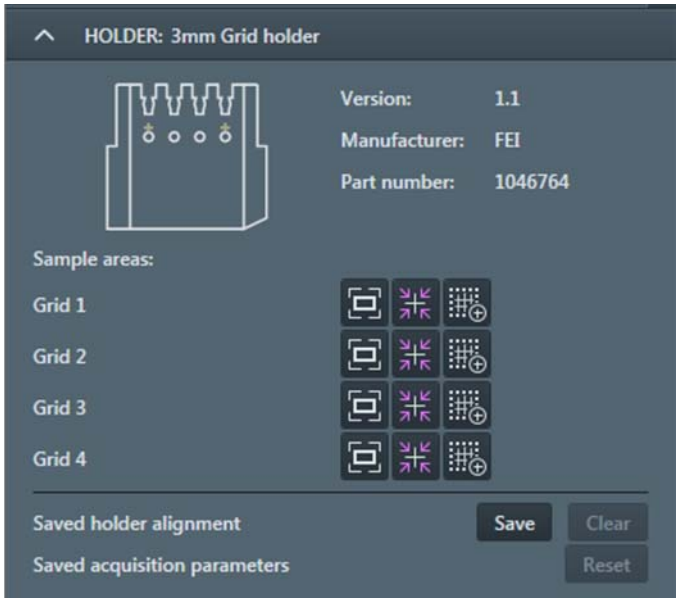
Displays controls/information for the selection in the Layer control:

- Layer Group

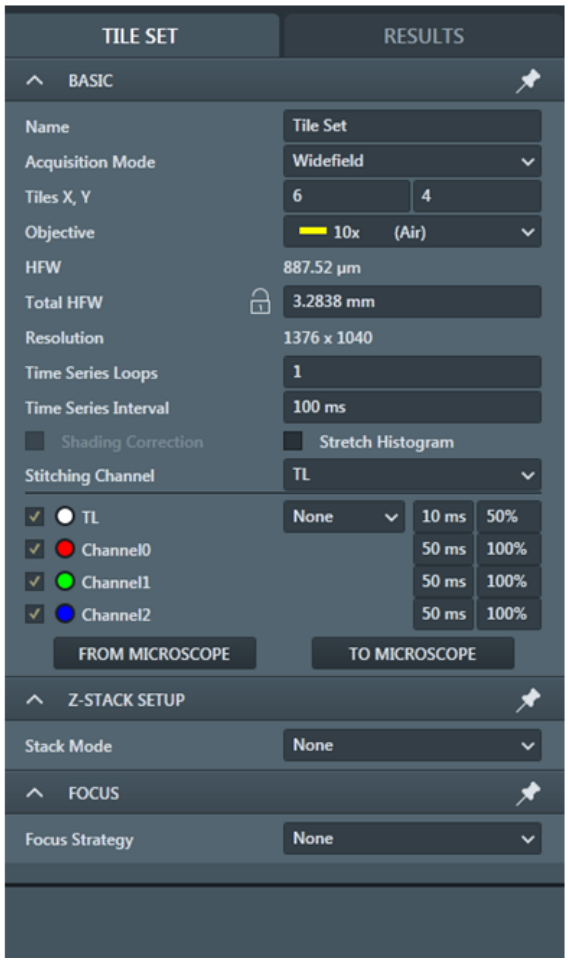


Use the **Opacity** slider to set the measure of opaqueness (how much you can see through). The less opaque, the more transparent it becomes. Transparent images are useful when overlaying one image over another

- Holder



Tile Set Tab



Topics in this section include:

- *"Basic Group" on page 117*
- *"Z-Stack Setup Group" on page 119*
- *"Focus Group" on page 120*

Basic Group

Specific selections for CorrSight

Table 3 Tile Set Tab, Basic Group Overview (1 of 2)

Control	Description
Name	Displays the name of the current tile set.
Acquisition Mode	Specifies the Acquisition mode (hardware specific): • Widefield • Structured Illumination • Spinning Disk **Note:** *Some acquisition modes are not available on all systems.*
Tiles X, Y	Sets the number of tiles in the X and Y directions.
Objective	Displays the objective lens selections: 4X, 10X, and 40X, and the immersion type: Air, Oil, Glycerol, Water, or Special installed on your microscope. *The display is configured in the Live Acquisition software.* The depressed blue indicator button is the selected objective lens. **Note:** *Take care when changing between objective lenses with different immersion types.*
HFW	Specifies the horizontal field width (HFW) for each tile.
Total HFW	Shows the total HFW for all of the tiles.
Resolution	Specifies the image resolution. Choices will be specific to each system type.

Table 3 Tile Set Tab, Basic Group Overview (2 of 2)

Control	Description
Time Series Loops	Specifies the number of times to acquire a tile set at a specified time interval to collect images of a sample that is changing with time.
Time Series Interval	Specifies the time interval for the specified number of times to acquire a tile set to collect images of a sample that is changing with time.
Shading Correction	Uses a blank reference image to correct (remove) shading effects in all images acquired inside the tile set. ***Note:*** *When the shading Correction feature is enabled on a tile set, the corrected (post-processed) images are saved with the Maps project and the original (pre-processed images) are discarded. If your use case requires preserving the information in the original (uncorrected) image for later analysis, then make sure that shading correction is disabled for your critical tile set.* ***Note:*** *Before this feature can be used in the Maps application, the reference must be acquired and stored using the Live Acquisition application.*
Stretch Histogram	Automatically adjusts the min/max pixel intensities as images are acquired.
Stitching Channel	*The channel names and colors are configured in the Live Acquisition software.*  At least one channel must be selected. The Exposure Time (ms) and Intensity (%) display for each selected channel. • **Exposure Time**: The time (in milliseconds) that the sample is illuminated for imaging. • **Intensity**: The percentage of maximum (full) illumination. As soon as you create a tile set, all enabled channels are applied when the tile set is acquired.
From Microscope	Applies the current microscope settings to this tile set.
To Microscope	Applies these settings to the microscope.

Z-Stack Setup Group

A Z-stack is a series of multiple images taken with varying focus. Each tile in a grid is a separate stack for a Z-stack acquisition.

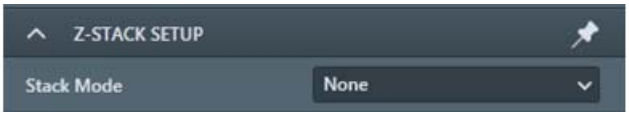


Table 4 Tile Set Tab, Z-Stack Setup Overview

Control	Description
Stack Mode	Defines the Z-stack as: • **None:** No Z-stack defined • **Relative:** To the defined midpoint of the stack  • **Absolute:** To the top and bottom of the stack 

Focus Group

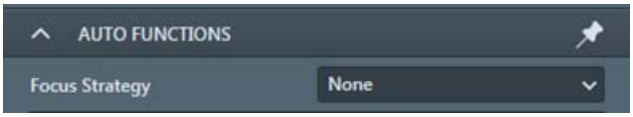
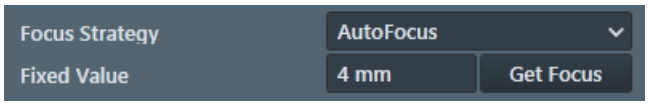
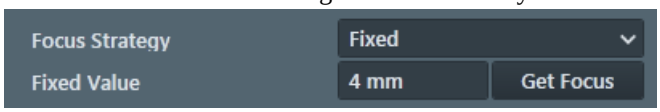
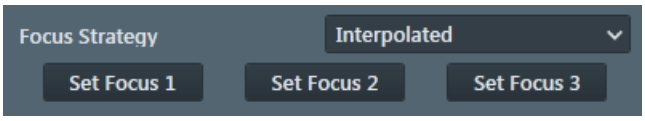
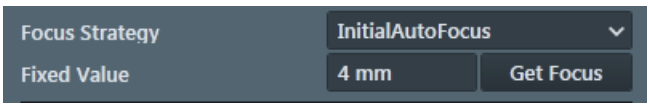
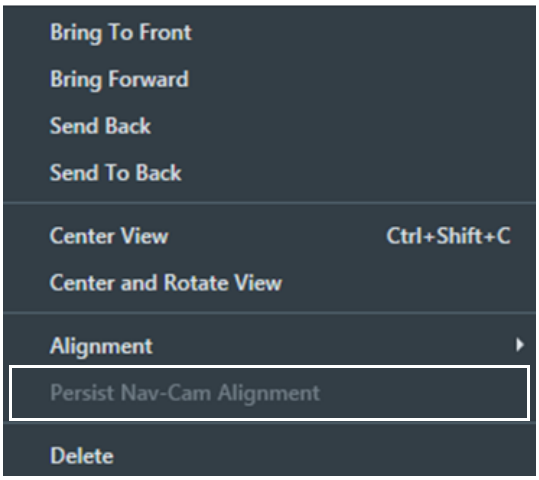


Table 5 Tile Set Tab, Focus Group Overview

Control	Description
Focus Strategy	Specifies the focus setting. Choices are: • **None:** The application will not touch the focus at all. You are free to change the focus via the xT UI during the run.  • **Autofocus**: Runs the autofocus routine at each tile before acquisition. This can result in better focus per tile, but adds considerably more time to acquisition.  • **Fixed**: Uses the same working distance for every tile.  • **Interpolated**: User will select three focus points on the sample and a focus plane will be defined by these points. Each tile's focus will be set based on where it is located in this plane.  Procedure: Navigate to the first location. Manually focus the image. When image is in focus, click **Set Focus 1** button. The current working distance will be remembered for that point and the button will now be checked. Repeat for the other two **Set Focus** buttons. Once all three are checked, tiles are ready to be acquired. **Note:** *These points will then be used to define a focus plane, so that all tiles will use a WD as defined by this plane. Choosing points that are at opposite ends of the sample is important to get the best results.* To unset a focus point, click **Set Focus** for that point. **Set Focus** is an on/off toggle.
Focus Strategy (cont.)	• **InitialAutoFocus** or **SingleAutoFocus**: Performs a single autofocus routine at the center of the tile set and uses the resulting focus for the entire tile set. 

Preview Layer, Nav-Cam Right-Click Menu

The Nav-Cam image is stored in the selected layer. Right-click on a Nav-Cam entry to access a context menu.



Unique selection for Nav-Cam.

- For descriptions of common selections to all systems, see *"Annotation Right-Click Menu" on page 43*.
- For descriptions of CorrSight-specific selections, see the table below.

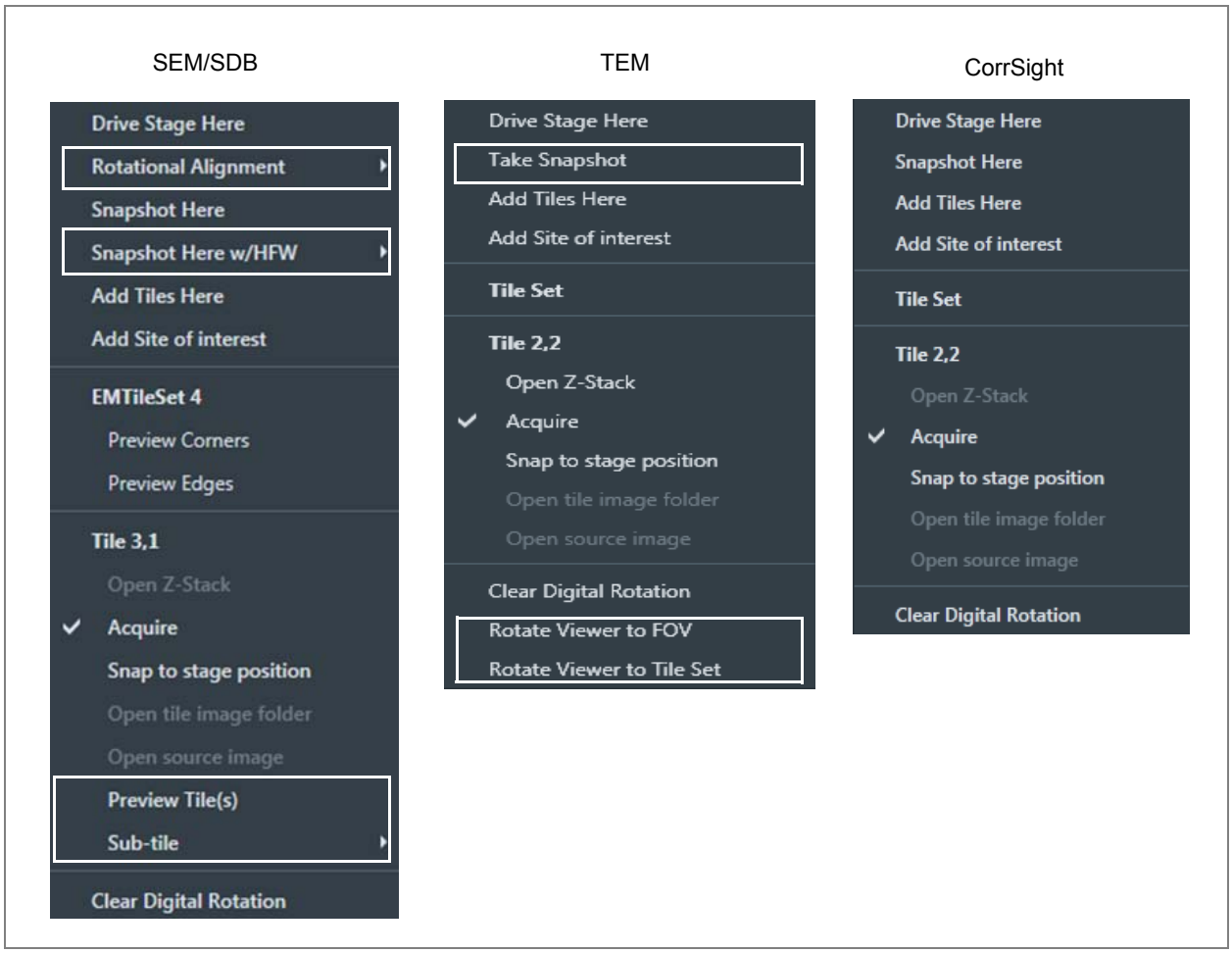
Table 6 Preview Layer, Nav-Cam Right-Click Menu Overview

Menu Selection	Description
Persist Nav-Cam Alignment	Keeps the current Nav-Cam alignment.

Viewer Tile Right-Click Menu

Right-click on a tile within the tile grid to access a context menu.

Figure 2 Viewer Tile Right-Click Menu, All Systems



Microscope Controls

Figure 3 Microscope Controls

Table 7 Microscope Controls Overview (1 of 2)

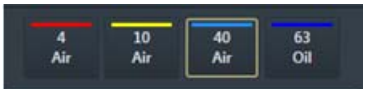
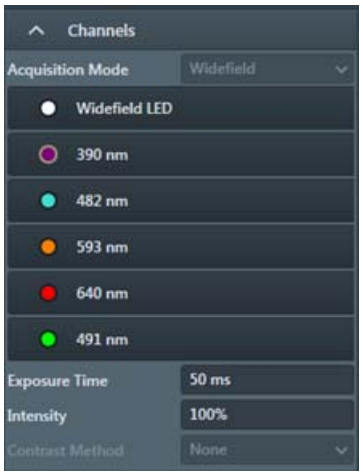
Interface Item	Description
Objective Lens Selection	 Displays the objective lens selections: 4X, 10X, and 40X, and the immersion type: Air, Oil, Glycerol, Water, or Special installed on your microscope. *The display is configured in the Live Acquisition software.* The outlined button is the selected objective lens. **Note:** *Take care when changing between objective lenses with different immersion types.*
Channels:	
Acquisition Mode	 Specifies the Acquisition mode (hardware specific): • Widefield • Structured Illumination • Spinning Disk **Note:** *Some acquisition modes are not available on all systems.*
Channel Selection	The channel names and colors are configured in the Live Acquisition software. The active channel is highlighted when live imaging. At least one channel must be selected. As soon as you create a tile set, all enabled channels are applied when the tile set is acquired.
Exposure Time	Time the camera sensor is exposed to the light source.
Intensity	Intensity of the light source.
Contrast Method	Transmitted light contrast is a software supported method to improve the contrast of transmission images for CorrSight instruments.

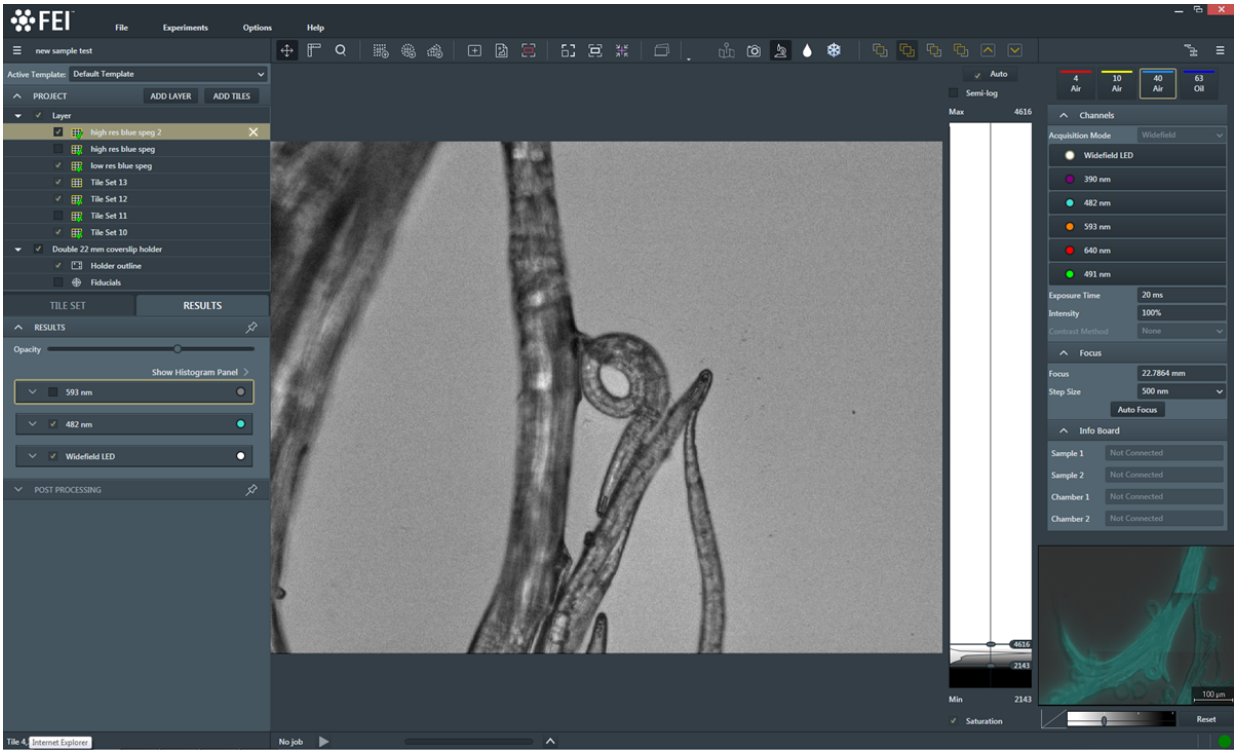
Table 7 Microscope Controls Overview (2 of 2)

Interface Item	Description
Focus:	
Focus	Specifies the current focus position.
Step Size	Specifies the step size of the auto focus.
Auto Focus	Uses the step size for live imaging Auto Focus.
Info Board	For microscopes with the a Cryogenic cooling system. 

Live Imaging

Click the **Live Imaging** icon in the tool bar to start live imaging.

Live Image Window



Double-click on a feature to center that point on the stage and within the Live Image window.

Use the mouse scroll wheel or right-click and drag to change focus.

Live Imaging Controls

Figure 4 Live Imaging Controls

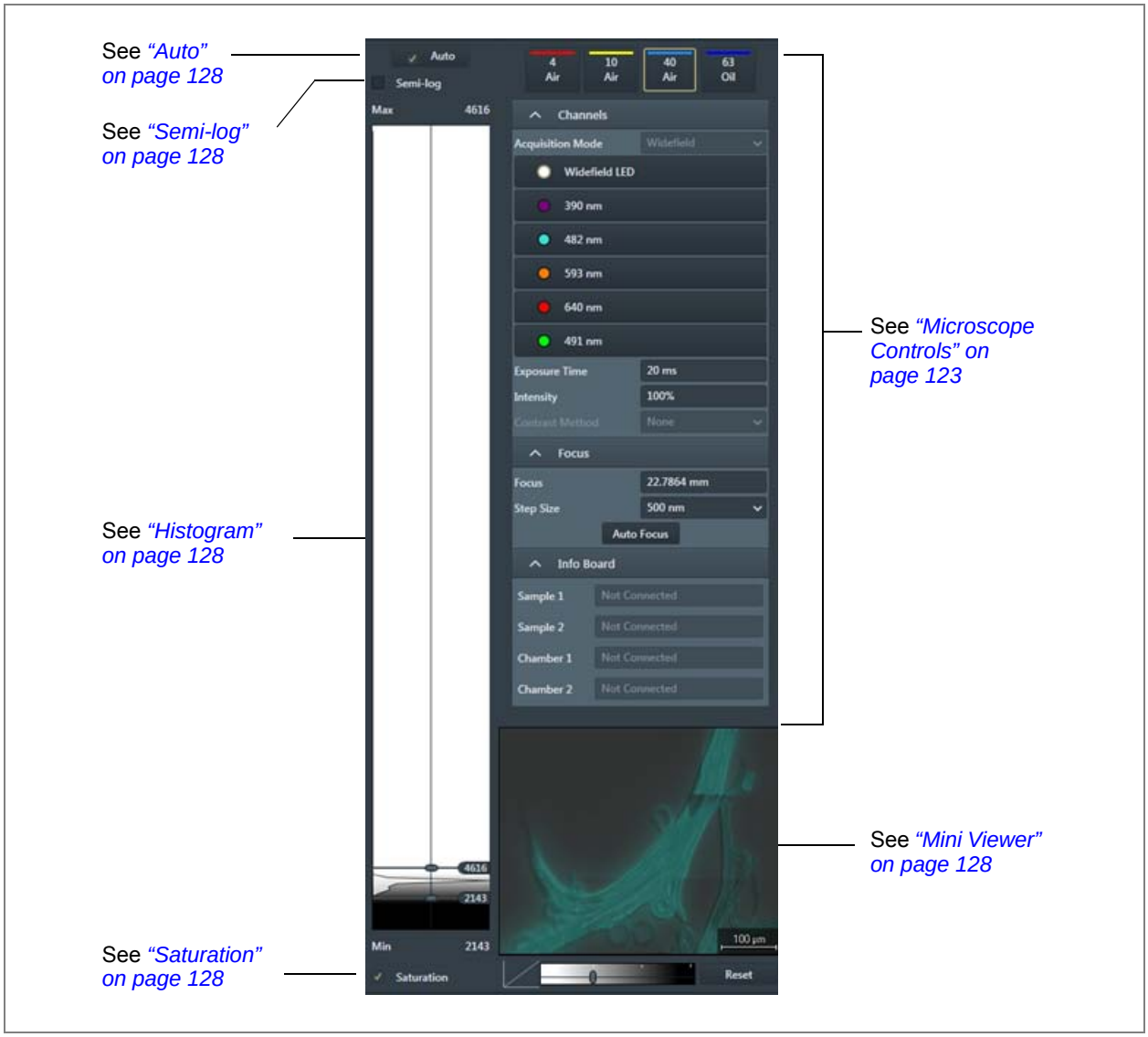


Table 8 Live Imaging Controls Overview (1 of 2)

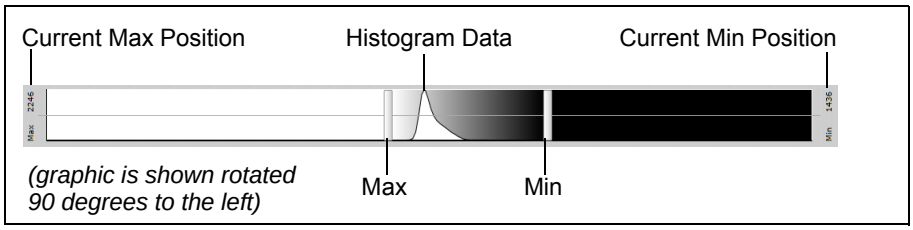
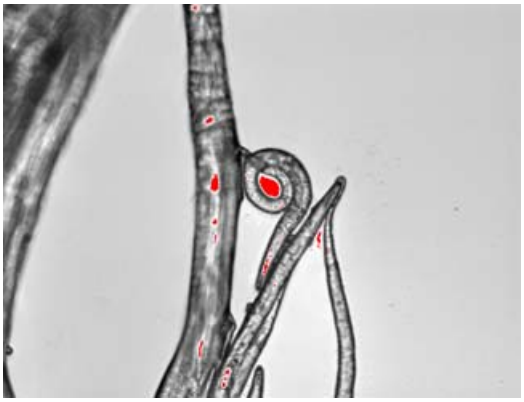
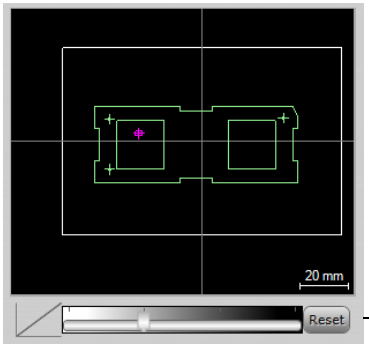
Interface Item	Description
Auto	When selected, continuously adjusts the min and max for the histogram display.
Semi-log	When selected, changes the histogram intensity display to logarithmic.
Histogram	 • Any data above the max line is white. • Any data below the min line is black. • In between max and min are the graylevels.
Saturation	When selected, the saturated pixels are colored red. 
Mini Viewer	The Mini Viewer has all of the functionality of the main viewer. 
Gamma Control Slider	Adjusts the gamma for the live image.

Table 8 Live Imaging Controls Overview (2 of 2)

Interface Item	Description
Reset	Returns to the default gamma setting.

Nav-Cam Support

Maps supports the use of importing a Nav-Cam image while on a CorrSight via the same tool bar button found when running Maps on xT.

See Tool Bar *"Tool Bar, Right Side" on page 114*.

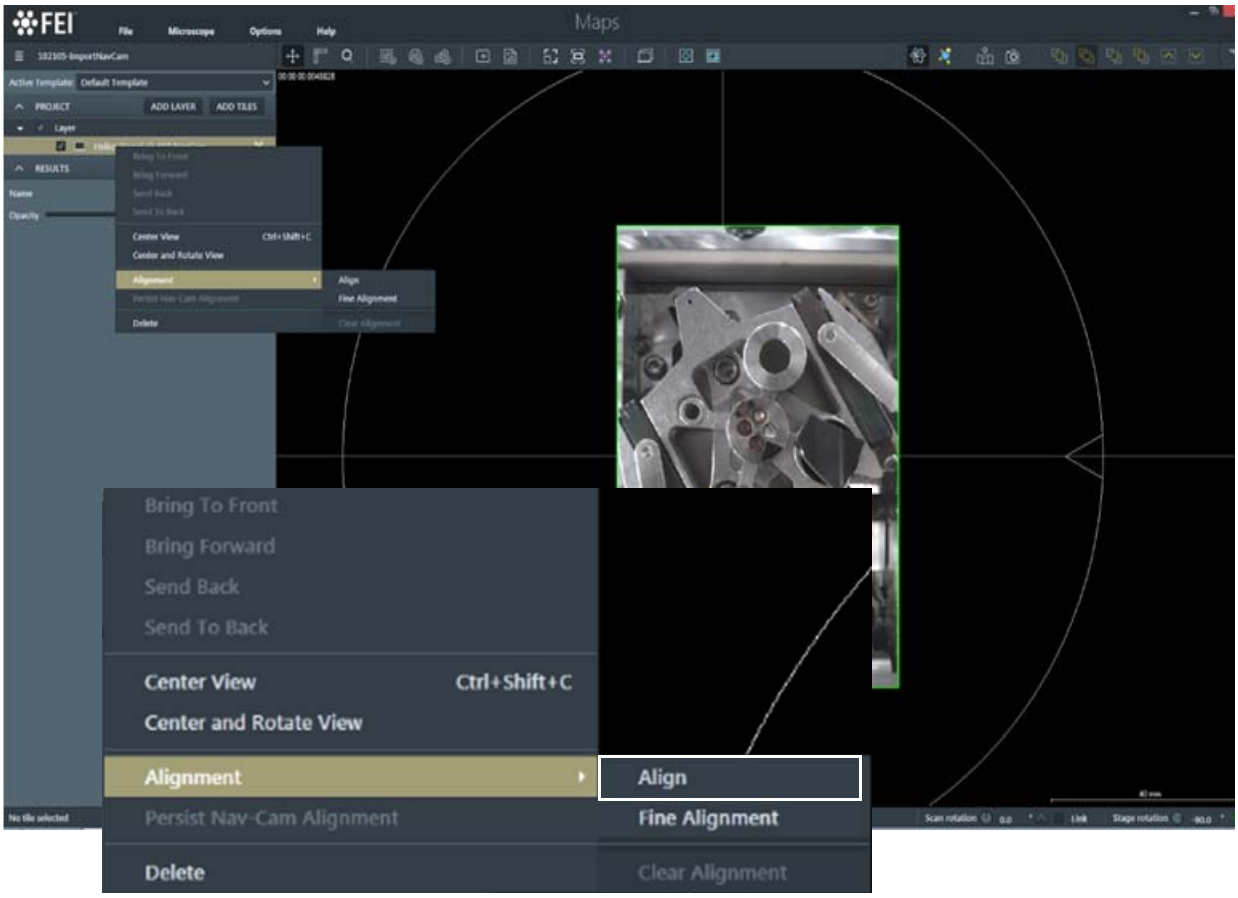
Nav-Cam Alignment

For systems that have the Nav-Cam, Maps is able to acquire Nav-Cam images and align them. Maps is also able to save the Nav-Cam Alignment to be automatically applied to all future Nav-Cam images acquired with Maps.

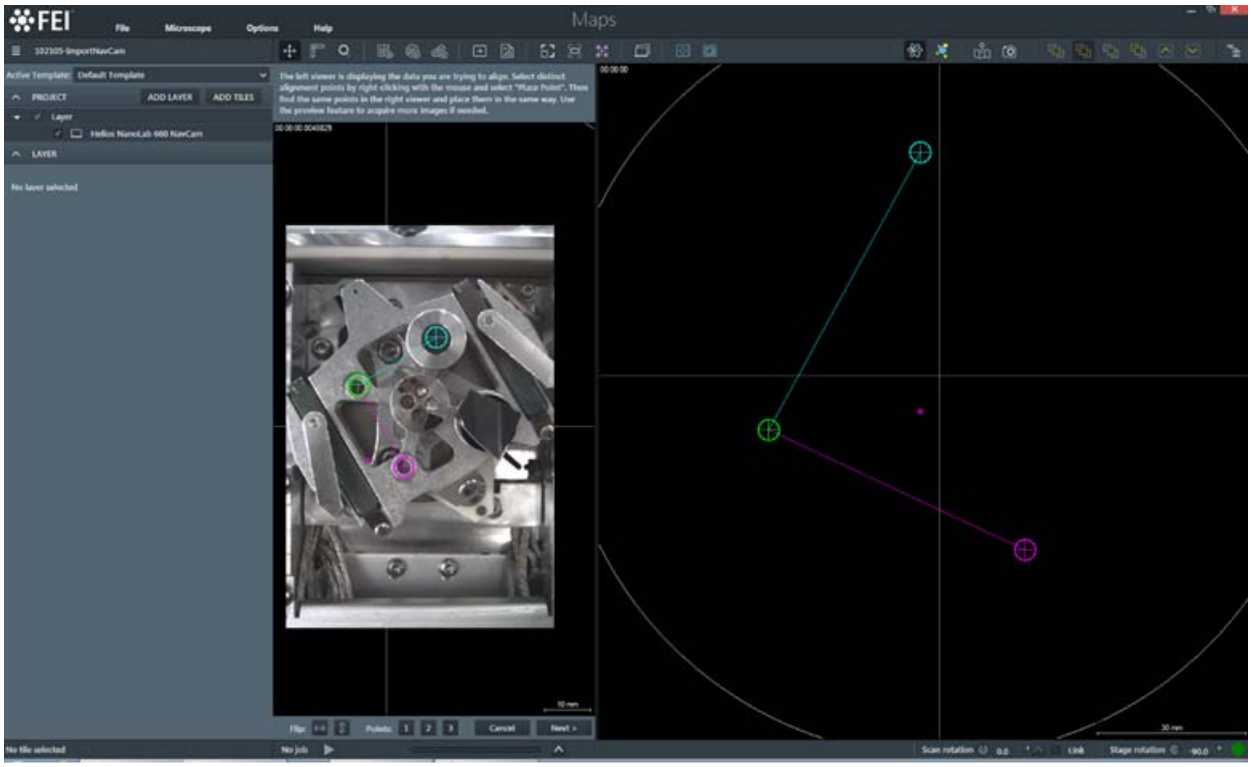
Procedure for Nav-Cam Alignment:

1. Click the **Import from Nav-Cam** tool bar icon.

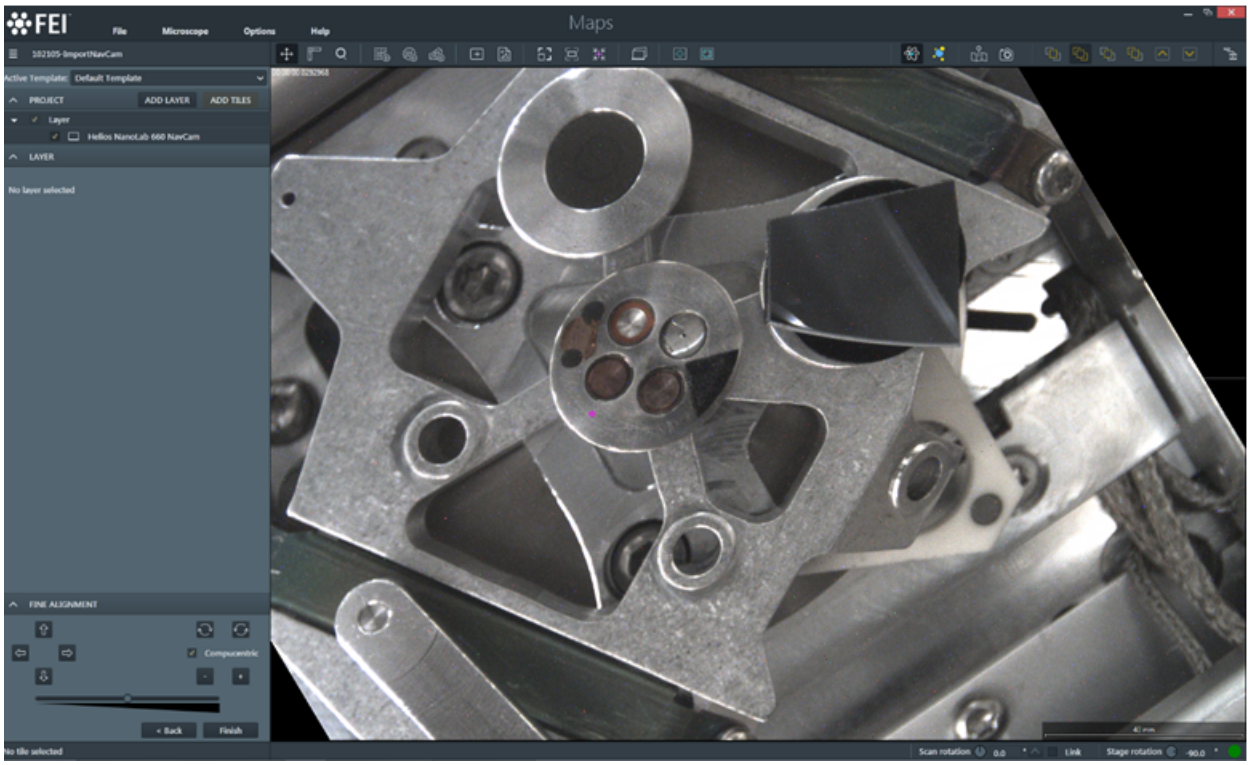
2. When the Nav-Cam image appears in Maps, right-click on its name in the Layer control and click **Align** in the menu that appears.



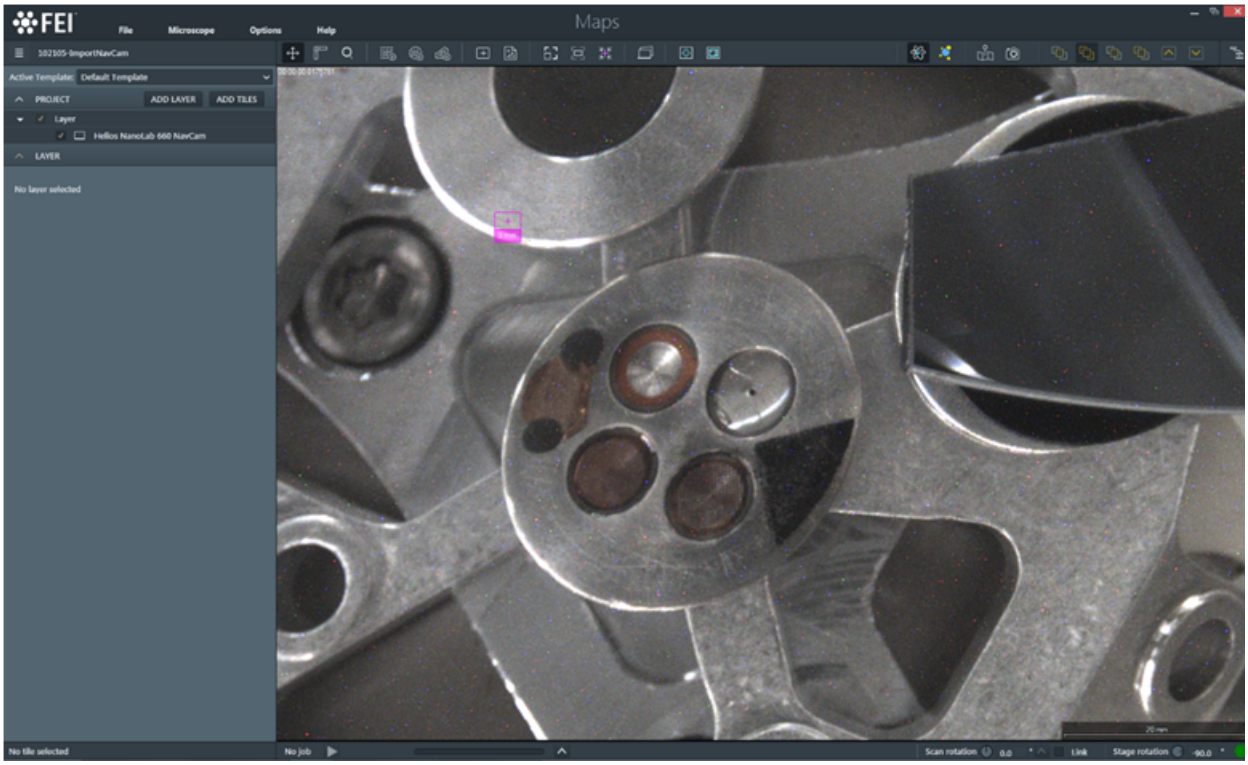
3. Perform a 2 or 3 point alignment as described in *"1-, 2-, or 3-Point (Coarse) Alignment" on page 196*.



4. Perform fine alignment adjustments, if needed, as described in *"Fine Alignment" on page 200*.



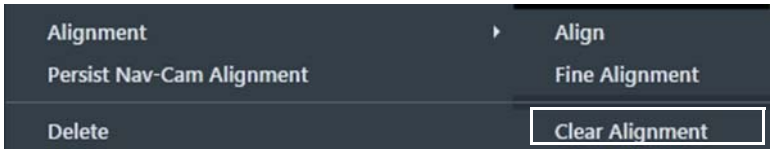
5. Test the Nav-Cam alignment by navigating to features on the sample and acquiring preview images. If the Nav-Cam image is not sufficiently aligned, perform the alignment procedure again.



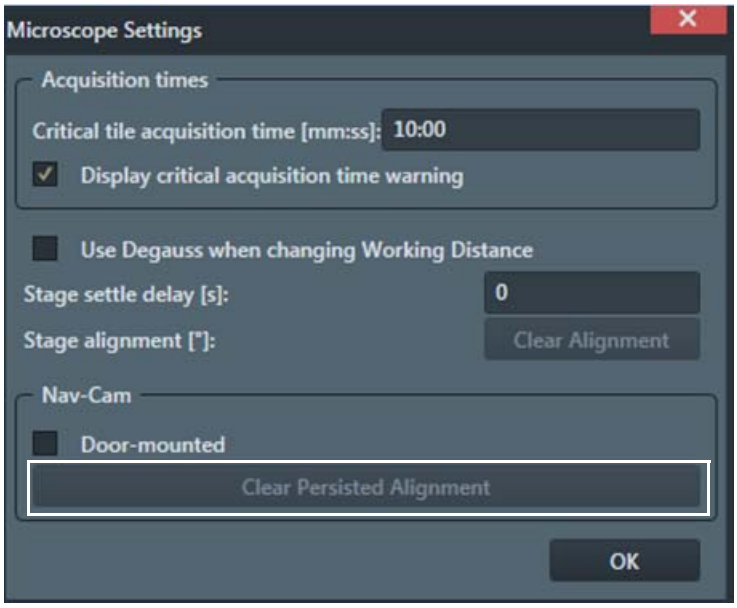
6. When the Nav-Cam image is sufficiently aligned, right-click on its name in the Layer control and select **Persist Nav-Cam Alignment** in the menu that appears.

All subsequent Nav-Cam acquisitions using Maps will use this alignment.

7. To clear the Nav-Cam alignment for an *individual* Nav-Cam image, right-click the Nav-Cam image in the Layer control and select **Alignment > Clear Alignment**.



8. To clear the saved default Nav-Cam alignment that is applied to *all* acquired Nav-Cam images, select **Options > Microscope Settings > Clear Persisted Alignment**.



6 TEM

Overview

This chapter describes user interface elements and procedures that are specific to the TEM and STEM modes on TEM systems.

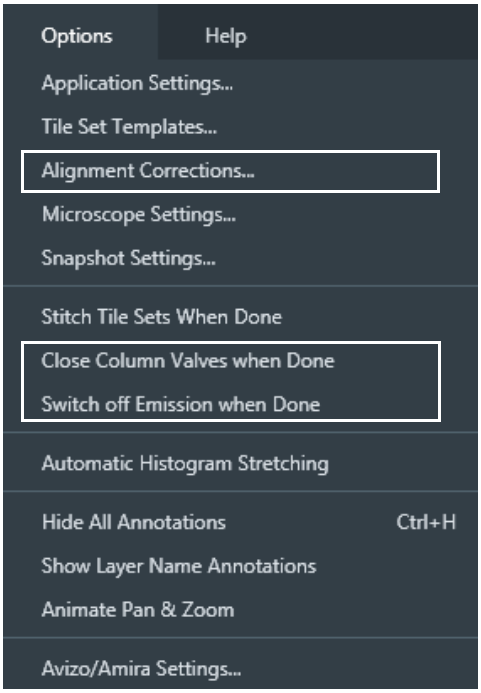
User Interface Elements

Topics include:

- Options Menu, below
- *"Tool Bar, Right Side" on page 139*
- *"Tile Set Tab, TEM Mode" on page 140*
- *"Tile Set Tab, STEM Mode" on page 144*
- *"Viewer Tile Right-Click Menu" on page 148*
- *"Magnification Alignment" on page 149*

Options Menu

- For descriptions of common selections to all systems, see *"Options" on page 22*.
- For descriptions of TEM-specific selections, see the table below.



Specific selections for TEM

Table 1 Options Menu Overview (1 of 3)

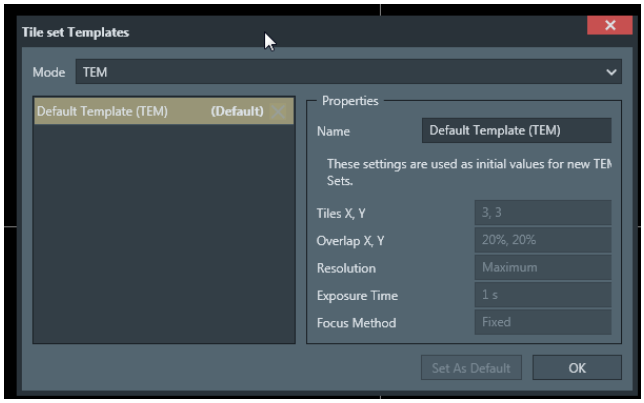
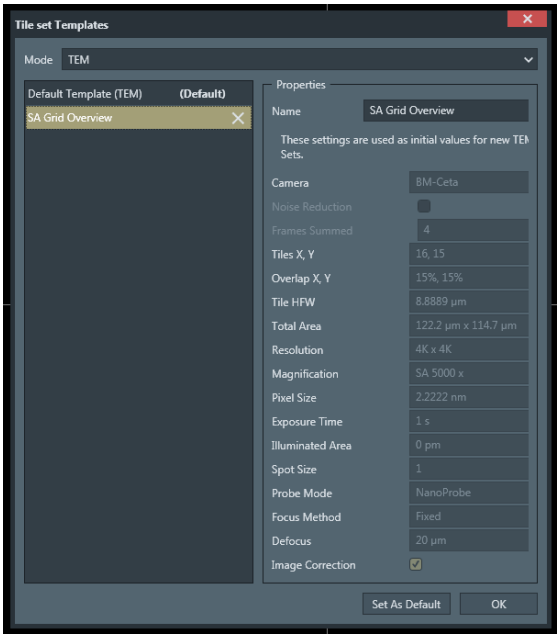
Menu Selection	Description
Tile Set Templates:	Displays a dialog that shows a collection of Tile Set templates and their respective settings. These templates contain user-defined presets for commonly used acquisition settings. The TEM mode has a default template and a standard template. Note that TEM and STEM templates differ slightly with their exact properties. **Default Template**: This template contains a subset of acquisition settings, filling the rest of the acquisition settings from the current settings on the microscope when the Tile Set is created. This facilitates the more interactive mode when the instrument UI is used for defining optical settings.  Default Template **Standard Template**: This template is editable, so you can utilize more settings than the default template  Standard Template

Table 1 Options Menu Overview (2 of 3)

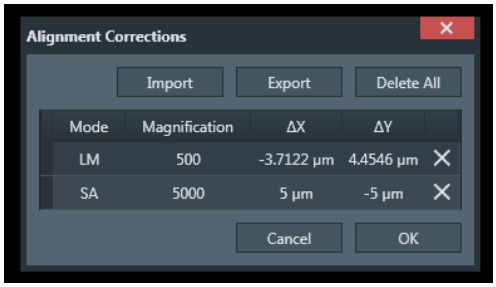
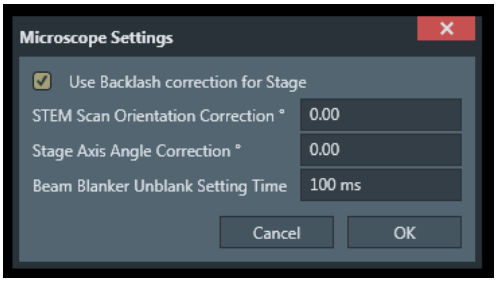
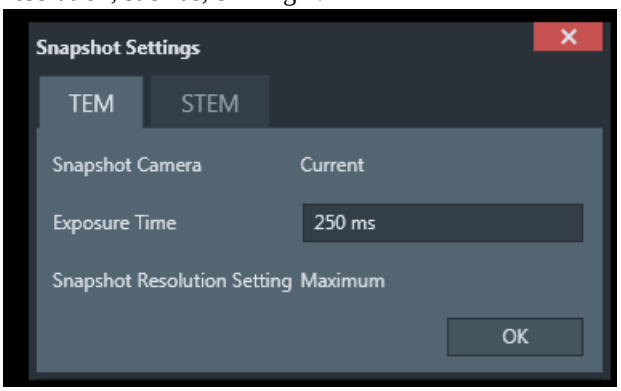
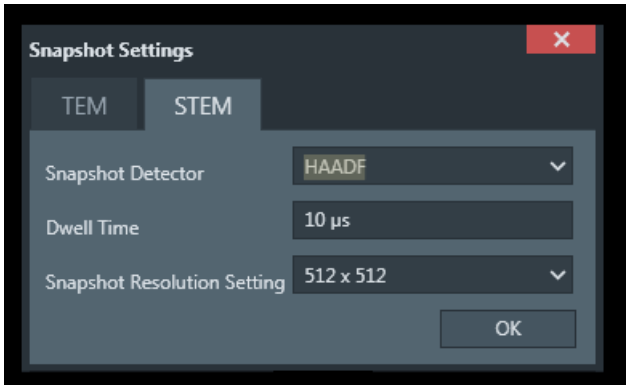
Menu Selection	Description
Alignment Corrections	Displays the corrections that currently apply for metrology analysis. 
Microscope Settings	 • **Use Backlash correction for Stage check box:** When enabled (default), stage moves are more accurate as the moves will be performed in a way to reduce backlash and wind-up effects. Usually there is no need to turn this correction off and this switch is mostly used for diagnostics. One might consider to temporarily turn the correction off to speed up low-magnification overviews (at the cost of accuracy) as the corrected move takes a bit longer. • **STEM Scan Orientation Correction:** This is a small correction angle for compensating imperfections in the calibration of the STEM scan rotation. The default value for this setting is 0. Only adjust this value if there is a significant systematic misalignment between tiles in STEM mode. • **Stage Axis Angle Correction**: This is a small correction angle that allows for compensating for mechanical imperfections, if present, in the orthogonality between the X and Y axis of the stage. The default value for this setting is 0. Only adjust this value if there is a recurring, significant misalignment between tiles in TEM and STEM modes. Contact your application engineer for a measurement procedure or consult an application note. • **Beam Blanker Unblank Setting Time:** After blanking or unblanking the beam, it takes a short time for the optics to settle. The default values are sufficient for most systems, but on Tecnai systems without a fast beam blanker, a value of 1–3 seconds is recommended. An insufficient setting time manifests itself only in STEM mode, resulting in tile images where the top part starts out black and unstable.

Table 1 Options Menu Overview (3 of 3)

Menu Selection	Description
Snapshot Settings	**TEM Mode**: Selects the Exposure Time that will be used for the acquisition of snapshot images. Snapshots will always be acquired with the maximum camera resolution, such as, binning 1.  TEM view **STEM Mode**: Displays the Detector, Dwell Time, and Resolution Settings that will be used for acquisitions of snapshot images when in STEM mode.  STEM view
Close Column Valves When Done	Closes the column valves after any queued acquisition and stitching jobs are completed.
Switch off Emission when Done	Applies only to systems with thermionic guns (such as, Talos LaB6). When enabled, turns of the gun emission after queued acquisition to preserve the filament. Note that re-enabling emission must be performed outside *MAPS* via the TEM User Interface.

Tool Bar, Right Side

See *"Left Side, All Systems" on page 30* for common selections to CorrSight and SEM/SDB.

Figure 1 Tool Bar, Right Side, TEM

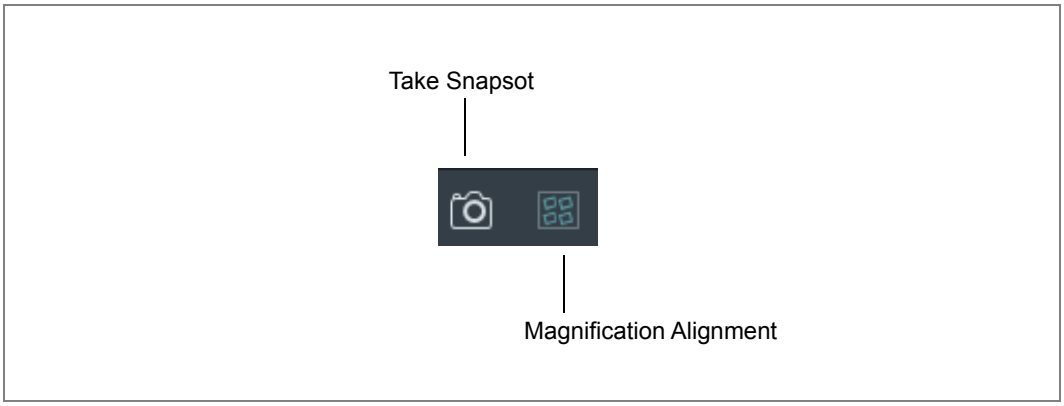


Table 2 Tool Bar Descriptions, TEM

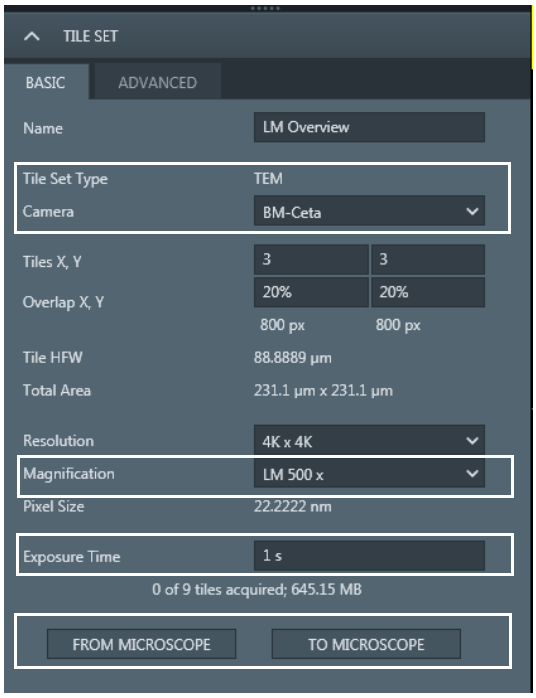
Tool	Description
Take Snapshot	Acquires a TEM image at the current stage position and magnification and places it on the Maps viewer as a snapshot image. See *"Snapshot Settings" on page 138*
Magnification Alignment	Aligns tile sets from one magnification to another to correct small shifts. See *"Magnification Alignment" on page 149*.

Tile Set Tab, TEM Mode

Topics in this section include:

- Basic Group, TEM Mode, below
- *"Advanced Group, TEM Mode" on page 142*

Basic Group, TEM Mode



Specific selections for TEM mode

Table 3 Tile Set Tab, TEM Mode, Basic Group Overview (1 of 2)

Control	Description
Name	Displays the name of the current tile set.
Tile Set Type	Indicates that the acquisition mode for this tile set is TEM or STEM.
Camera	Selects the camera that will be used for the acquisition of tile images. Unsupported cameras are also listed and indicated in the camera dropdown list. These unsupported cameras may work with Maps, but compatibility is not guaranteed.
Tiles X, Y	Sets the number of tiles in the X and Y directions.
Overlap X, Y	Displays the percentage of overlap between tiles in the tile set as well as the pixels.
Tile HFW	For TEM, this is not editable. It is determined by the magnification, camera, and other optical settings.
Total Area	Displays the total HFW for all of the tiles.

Table 3 Tile Set Tab, TEM Mode, Basic Group Overview (2 of 2)

Control	Description
Resolution	Specifies the image resolution. Choices will be specific to each system type.
Magnification	Selects the magnification that will be used for the acquisition of the tile images
Pixel Size	Displays the physical size of a single pixel at the current acquisition settings. This is based on Tile HFW and Resolution.
Exposure Time	Specifies the exposure time on the camera for each tile acquisition.
Number of Tiles Acquired	Displays the number of tiles and the total disk space required to acquire the current tile set. A message appears if there is not enough space on disk for the acquisition.
From Microscope	Reads the current optical settings from the microscope and stores them in the acquisition settings of the selected tile set.
To Microscope	Sets the optical parameters of the acquisition settings of the selected tile set to the microscope.

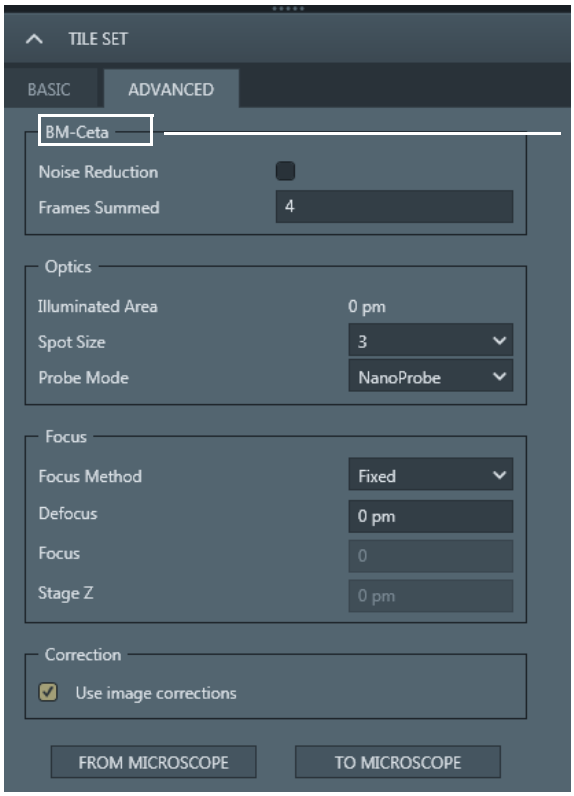
NOTE

From Microscope/To Microscope controls:

- Camera settings are not read from or written to the microscope; camera settings only apply during acquisition.
- Not all the optical acquisition settings can be edited directly. Some optical acquisition settings require clicking **From Microscope** to update the values.

Advanced Group, TEM Mode

The advanced parameters are initially set at the creation of the tile set and filled with the settings of the selected template or with the current settings on the system if the default template is used. After creation of the tile set, click **From Microscope** to update the settings to the current state on the instrument. Some settings can also be typed in directly.



These camera settings are specific to the selected camera. The BM-Ceta camera is shown in this example and it has two settings: Noise Reduction and Frames Summed.

Table 4 Tile Set Tab, TEM Mode, Advanced Group Overview (1 of 2)

Control	Description
Noise Reduction	Specifies the Noise Reduction setting associated with the BM-Ceta camera. See the *Ceta 16M Application Instructions* for details.
Frames Summed	Specifies the Frames Summed setting associated with the BM-Ceta camera. See the *Ceta 16M Application Instructions* for details.
Illuminated Area	Specifies the beam intensity that will be used during the tile set acquisition.
Spot Size	Specifies the spot size that will be used during the tile set acquisition.
Probe Mode	Specifies the probe mode such as the NanoProbe or MicroProbe that will be used during the tile set acquisition.

Table 4 Tile Set Tab, TEM Mode, Advanced Group Overview (2 of 2)

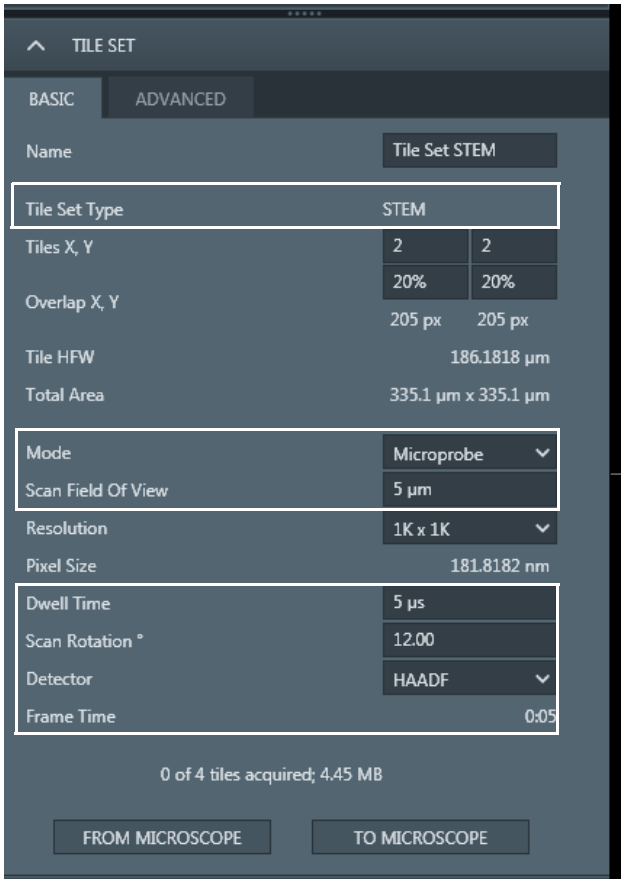
Control	Description
Focus Method	Specifies if the tile set should be acquired with specific focus settings or none at all. There the following two options: • **None**: The focus on the microscope will be left *as is*. • **Fixed**: The tile set will be acquired at a fixed focus value to be stored in the tile set with the **From Microscope** button plus an optional relative Defocus for contrast formation.
Defocus	When Focus Method is set to **Fixed**, specifies the amount of relative defocus that will be applied when acquiring the tiles. ***Note:*** *Sets the focus to the microscope, not the defocus. The defocus is only applied during acquisition as a relative value on top of the Focus setting that was stored with the tile set.*
Focus	When the Focus Method is set to **Fixed**, this is the lens focus setting that will be applied when acquiring the tile set. To update this value, click **From Microscope**.
Stage Z	When Focus Method is set to **Fixed**, specifies the Z coordinate at which the tile set will be acquired. Click the check box to retrieve the current value from the instrument.
Use image corrections	When this setting is enabled, a small image correction is applied to the acquired tile images resulting in improved tile set accuracy. Using this setting slightly affects the raw data recorded by Maps. If this result is undesired, turn off the image correction. This results is less accurate navigation. ***Note****: When the correction is enabled, the tiles are slightly smaller. Also, with the correction enabled you can notice a small difference between the purple field-of-view rectangle and outline of the tiles.*
From Microscope	Reads the current optical settings from the microscope and stores them in the acquisition settings of the selected tile set.
To Microscope	Sets the optical parameters of the acquisition settings of the selected tile set to the microscope.

Tile Set Tab, STEM Mode

Topics in this section include:

- Basic Group, STEM Mode, below
- *"Advanced Group, STEM Mode" on page 146*

Basic Group, STEM Mode



Specific selections for STEM mode

Table 5 Tile Set Tab, STEM Mode, Basic Group Overview (1 of 2)

Control	Description
Name	Displays the name of the current tile set.
Tile Set Type	Displays the microscope mode for the tile set, which is either TEM (*"Tile Set Tab, TEM Mode" on page 140*) or STEM
Tiles X, Y	Sets the number of tiles in the X and Y directions.
Overlap X, Y	Displays the percentage of overlap between tiles in the tile set as well as the pixels.

Table 5 Tile Set Tab, STEM Mode, Basic Group Overview (2 of 2)

Control	Description
Tile HFW	For STEM, this is not editable. It is determined by the magnification, camera, and other optical settings.
Total Area	Displays the total HFW for all of the tiles.
Mode	Selects the probe mode of the beam: Microprobe, Nanoprobe, or LM. Note that in order to use LM STEM, it is strongly recommended to first enable LM STEM via the TEM User Interface.
Scan Field Of View	Defines the size of the scan area and magnification for the acquisition of each tile.
Resolution	Specifies the image resolution. Choices will be specific to each system type.
Pixel Size	Displays the physical size of a single pixel at the current acquisition settings. This is based on Tile HFW and Resolution.
Dwell Time	Specifies the amount of time the beam dwells on each pixel when images are acquired.
Scan Rotation	Defines the orientation of the scanning, which determines the orientation of the image. The tile set orientation is adjusted accordingly. Typical use of this property is to line up the acquired images to a feature on the sample.
Detector	Selects the detector that will be used for the acquisition of tile images.
Frame Time	The estimated time each tile acquisition will take.
Number of Tiles Acquired	Displays the number of tiles and the total disk space required to acquire the current tile set. A message appears if there is not enough space on disk for the acquisition.
From Microscope	Reads the current optical settings from the microscope and stores them in the acquisition settings of the selected tile set.
To Microscope	Sets the optical parameters of the acquisition settings of the selected tile set to the microscope.

NOTE

From Microscope/To Microscope controls:

- Camera settings are not read from or written to the microscope; camera settings only apply during acquisition.
- Not all the optical acquisition settings can be edited directly. Some optical acquisition settings require clicking **From Microscope** to update the values.

Advanced Group, STEM Mode

Use the controls on this group to set advanced tile acquisition properties for each STEM tile set. The advanced parameters are initially set at the creation of the tile set and filled with the settings of the selected template or with the current settings on the instrument if the default template is used. After creation of the tile set, click From Microscope to update the settings to the current state on the microscope. Some settings can also be entered directly.

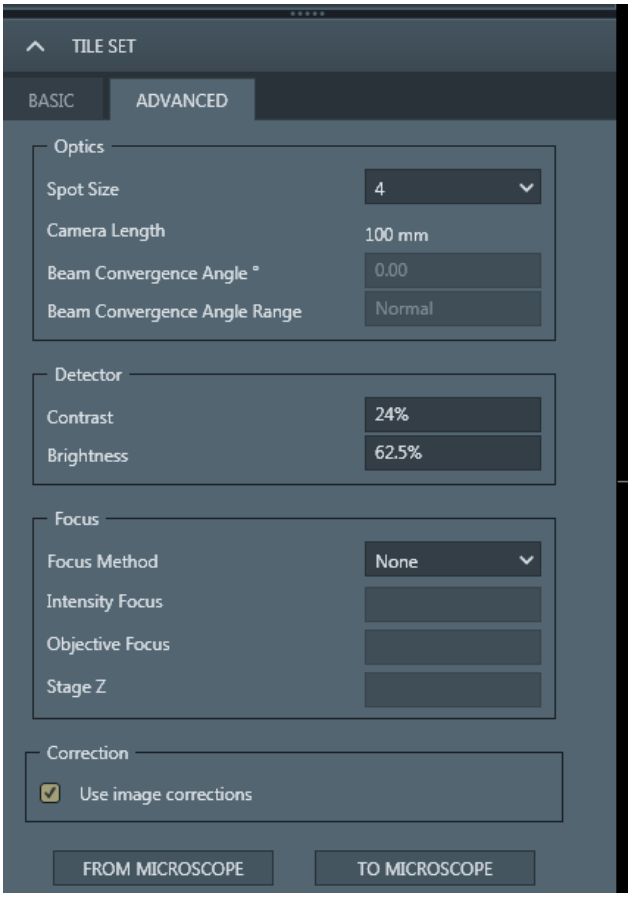


Table 6 Tile Set Tab, STEM Mode, Advanced Group Overview (1 of 2)

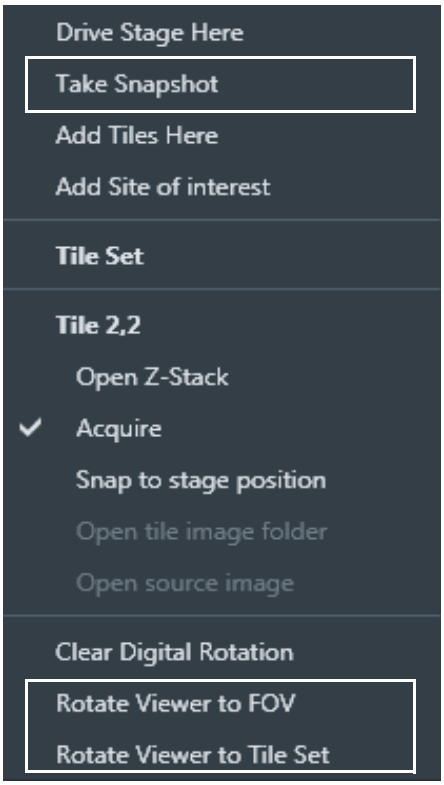
Control	Description
Spot Size	Specifies the spot size that will be used during the tile set acquisition.
Camera Length	Specifies the STEM camera length, which is typically used to tune the contrast of the images.
Beam Convergence Angle°	Specifies the convergence of the beam used during STEM acquisition.

Table 6 Tile Set Tab, STEM Mode, Advanced Group Overview (2 of 2)

Control	Description
Beam Convergence Angle Range	The convergence range specifies whether or not large angles are allowed for the convergence angle. Note that large angular range implies an additional cross over.
Contrast	Specifies the contrast setting of the detector, which represents the dynamic range of the detected signal (that is, it defines the amplification of the detector signal).
Brightness	Specifies the brightness setting of the detector, which represents the null level of the detected signal (that is, it defines the offset of the detector signal).
Focus Method	Specifies if the tile set should be acquired with specific focus settings or none at all. There the following two options: • **None**: The focus on the microscope will be left *as is*. • **Fixed**: The tile set will be acquired at a fixed focus value to be stored in the tile set with the **From Microscope** button.
Intensity Focus	When the Focus Method is set to **Fixed**, specifies the beam intensity at which the tile set will be acquired. This lens focus setting will be set during tile set acquisition. To update this value, click **From Microscope**.
Objective Focus	When the Focus Method is set to **Fixed**, specifies the objective focus at which the tile set will be acquired. This lens focus setting will be set during tile set acquisition. To update this value, click **From Microscope**.
Stage Z	When Focused Method is set to **Fixed**, specifies the z coordinate at which the tile set will be acquired.
Use image corrections	When this setting is enabled, a small image correction is applied to the acquired tile images resulting in improved tile set accuracy. Using this setting slightly affects the raw data recorded by Maps. If this result is undesired, turn off the image correction (that is, at cost of slightly less accurate navigation). ***Note**: When the correction is enabled, the tiles are slightly smaller. Also, with the correction enabled you can notice a small difference between the purple field-of-view rectangle and outline of the tiles.*
From Microscope	Reads the current optical settings from the microscope and stores them in the acquisition settings of the selected tile set.
To Microscope	Sets the optical parameters of the acquisition settings of the selected tile set to the microscope.

Viewer Tile Right-Click Menu

Right-click on a tile within the tile grid to access a context menu.



Specific selections for TEM

- For common selections, see *"Viewer Tile Right-Click Menu" on page 56*.
- For TEM-specific selections, see the table below.

Table 7 Viewer Tile Right-Click Menu Overview

Menu Selection	Description
Take Snapshot	Takes a single snapshot at the current microscope location.
Rotate Viewer to FOV	Digitally rotates the viewer to line up the display to the orientation of the current field-of-view (as indicated by the purple rectangle).
Rotate Viewer to Tile Set	Digitally rotates the viewer so the current tile set is aligned with the screen. See *Figure 20, "Viewer Tile Right-Click Menu Overview," on page 57*.

Magnification Alignment

Introduction

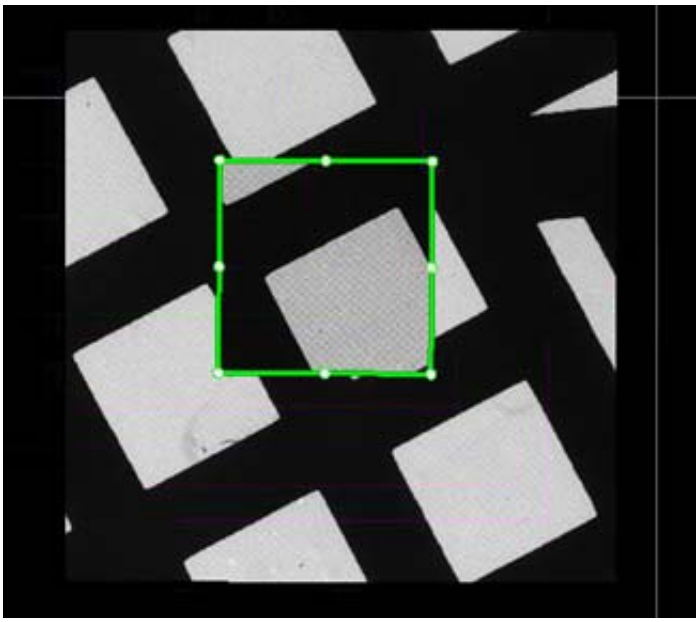
On TEM systems, you will observe imperfections in the tile set alignment from one magnification to another. These imperfections result in small shifts when changing the magnifications. You must always properly calibrate and align the system, but small shifts may still remain since changes in the optics can lead to additional shift. To compensate for the small shifts that may remain, you can use the drag-and-drop functionality of Maps to align tile sets.

Magnification Alignment versus Manual Alignment

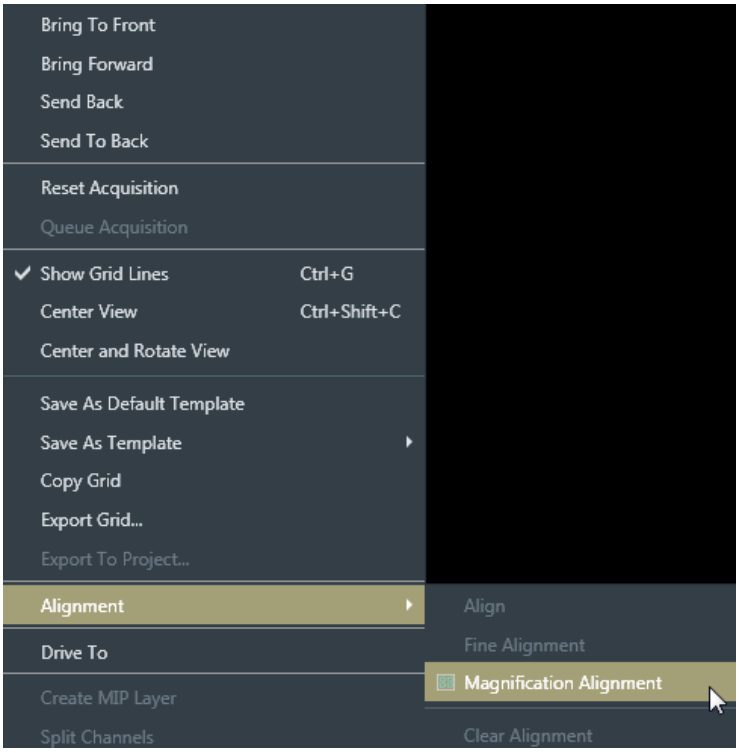
There are two alignment mechanisms available in Maps: Magnification Alignment and manual alignment. The Magnification Alignment moves the data in the Maps canvas and stores this alignment for subsequent acquisitions (at the same magnification). The manual alignment described in *"Manual Alignment" on page 195* only aligns the data within the Maps canvas. Any subsequent acquisition will not have access to information about the alignment.

Aligning a Tile Set and Its Magnification

1. Click the acquired tile set that is slightly misaligned. For example, the higher magnification tile set within the green border is misaligned with respect to the lower magnification.



2. Right-click on the selected tile set and then click **Alignment > Magnification Alignment.**

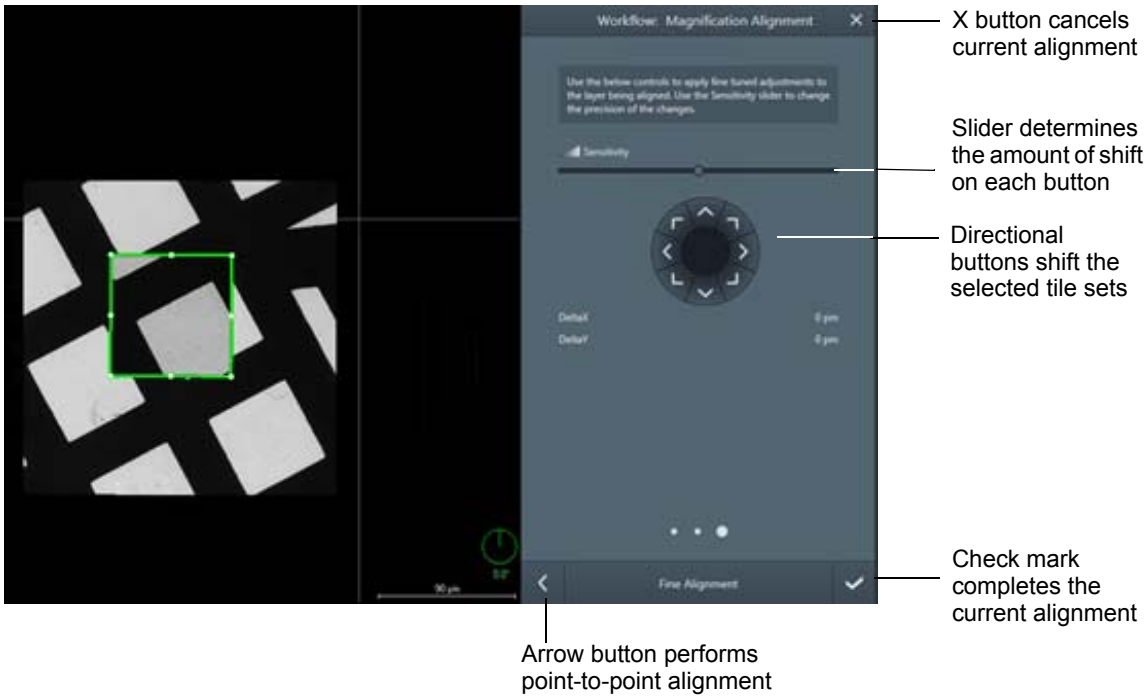


3. Or, click either of the following buttons on the right side of the tool bar:

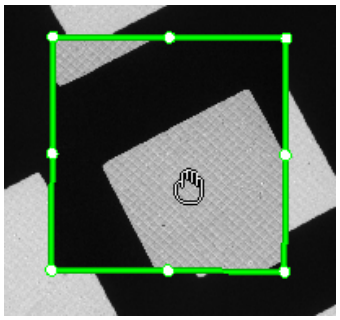
   ■ **Magnification Alignment**

Workflow - **see** ***"Workflow" on page 196***

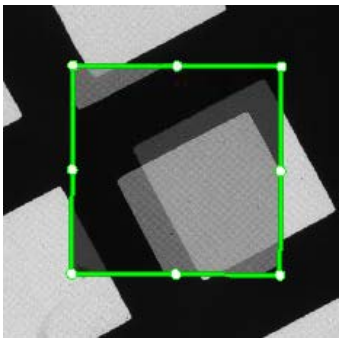
4. The Workflow Magnification Alignment panel appears.



5. Reposition the tile set using the directional buttons in the Workflow Magnification Alignment panel.



You can reposition the tile set by clicking and holding the left-mouse button while hovering over the tile set.



6. Click the **check mark** within the alignment workflow panel to complete the current alignment, or click the **X** button to cancel it.

The results of the alignment are twofold:

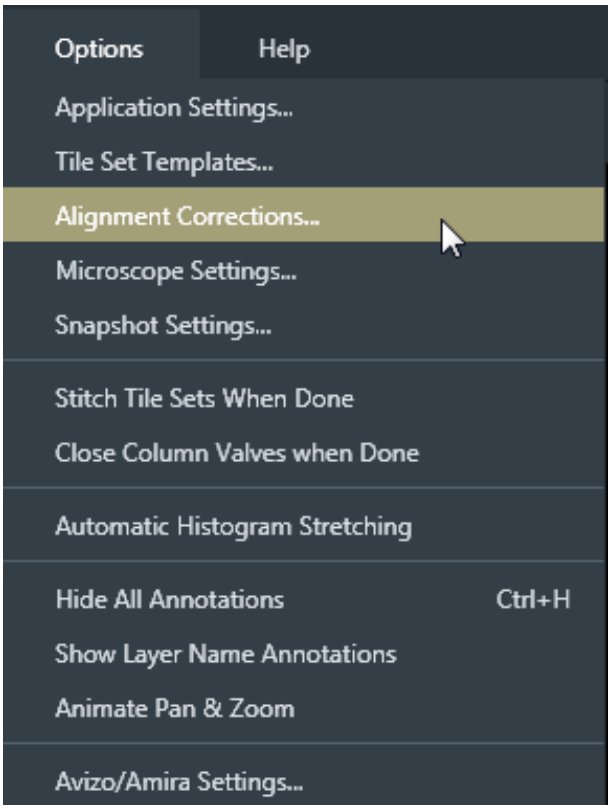
- The tile set with the existing data is realigned within the Maps canvas.
- An *alignment correction* record is stored for the magnification associated with the tile set and all subsequent acquisitions will be aligned. See *"Alignment Corrections Table" on page 153*.

Alignment Tips

- You can refine the alignment at any point in time, even when job execution is paused during tile set acquisition.
- A magnification alignment automatically applies to higher magnifications, so not all magnifications need realignment. See *"Propagation of Alignment Corrections" on page 154*.
- You can align multiple tile sets simultaneously by selecting a number of tile sets before starting the alignment.
- The alignment corrections are stored on disk and remain active for subsequent sessions. See *"Alignment Corrections Table" on page 153*.
- You can view which alignment corrections are present. See *"Alignment Corrections Table" on page 153*.
- If alignment corrections apply, you should see the field-of-view rectangle shift when changing the magnification on the system as Maps tracks the current field-of-view. Tracking includes the alignment corrections.

Alignment Corrections Table

When a tile set is aligned, Maps stores a record of the magnification associated with the tile set. Click **Options > Alignment Corrections** to view the record in the Alignment Corrections table.



The Alignment Corrections table is stored, so subsequent sessions also benefit from the alignments. Note that the corrections are not part of the project; they are part of the Maps global settings.

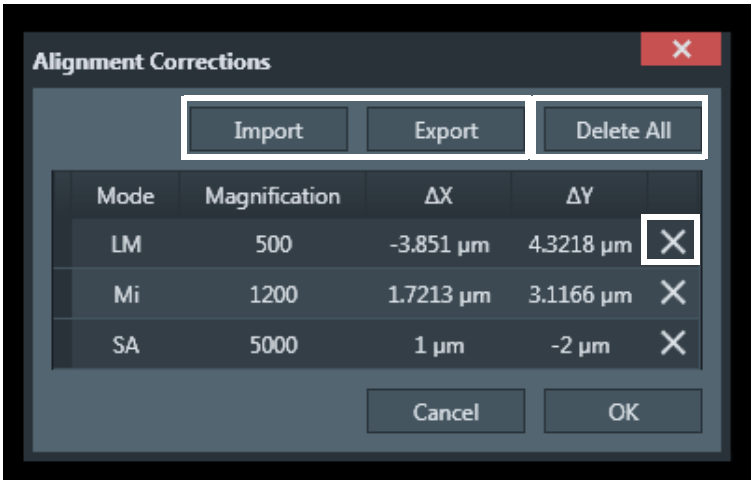


Table 8 Alignment Corrections Table Overview

Interface Item	Description
Import	Imports the alignment corrections.
Export	Exports the alignment corrections.
Delete All	Removes all alignment corrections.

Propagation of Alignment Corrections

The magnification alignments automatically propagate to higher magnifications that do not have an entry in the Alignment Correction table. However, individual magnifications can be aligned at any time, overruling the propagated alignment. With this approach, only a minimum number of alignments is required and it allows for refinement when needed. An additional benefit of this approach is that it naturally follows the typical Maps workflow from low-to-high magnification, allowing you to realign during the workflow when needed.

Alignment corrections for the low magnification mode (LM) do not automatically propagate to the high magnification modes (Mi/Sa/Mh), because on TEM systems these modes are mostly uncorrelated. The concept of the propagation is demonstrated in the example described below.

Example: Assuming you have an Alignment Corrections table as shown below and the system supports the magnifications as listed in *Table 9*, the following occurs:

The first magnifications have no alignment correction; the LM 500x magnification has an alignment correction, which propagates to the next magnifications LM 1000x and LM 1800x, but not to the high magnification modes; the first high magnifications do not have an entry in the alignment corrections table, hence they have no correction; the SA 5000x is the first high magnification with a correction, which propagates only to the next magnification because SA 20000x has its own entry in the alignment correction tables, which overrules the propagated correction of SA 5000x. The correction of SA 20000x propagates to all the next magnifications.

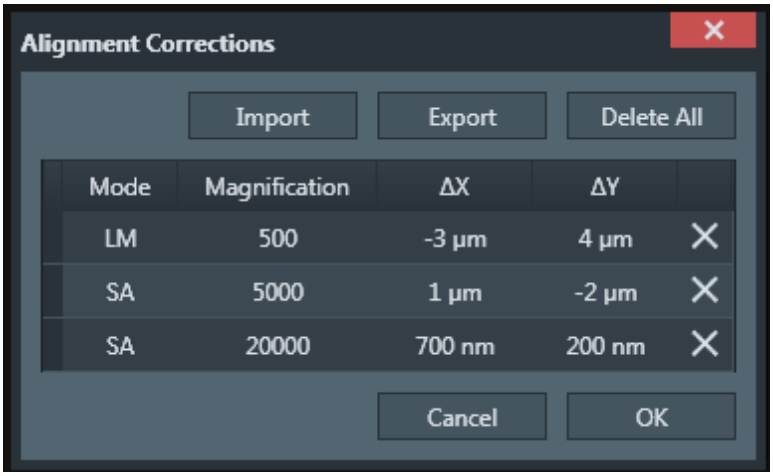
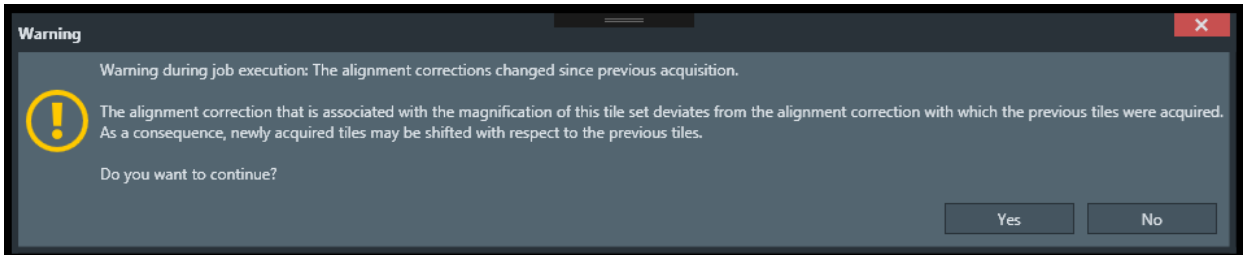


Table 9 Example of Propagation

	Mode	Magnification	Correction	
Low Magnifications	LM	50x	*none*	1
	LM	100x	*none*	
	LM	500x	*(-3 um, 4 um)*	2
	LM	1000x	*(-3 um, 4 um)*	
	LM	1800x	*(-3 um, 4 um)*	
High Magnifications	Mi	2000x	*none*	3
	Mi	2500x	*none*	
	SA	3000x	*none*	
	SA	5000x	*(1 um, -2 um)*	4
	SA	12000x	*(1 um, -2 um)*	
	SA	20000x	*(0.7 um, 0.2 um)*	5
	SA	50000x	*(0.7 um, 0.2 um)*	
	Mh	100000x	*(0.7 um, 0.2 um)*	
	Mh	200000x	*(0.7 um, 0.2 um)*	

Working with Propagation of Alignment Corrections

- The magnification alignments are for Maps only. They determine how Maps controls the stage and how acquired images are positioned in the Maps canvas without affecting the optics of the microscope. This mechanism can safely coexist with other applications that use different means for realignment.
- The Magnification Alignment is related to Align Layer (see *"Layer Right-Click Menu" on page 44*) and Global Alignment functions (see *"Global Alignment" on page 32*), but serves a different purpose. The latter is intended to realign existing data to the coordinate system of the current system that is typically used for imported data. The Magnification Alignment targets alignments on the system itself.
- The magnification alignment is a mechanism to compensate for imperfections in the optical alignments; therefore, it is global in nature and applies to all positions. Small position-dependent shifts may remain due to other factors.
- If the magnification alignment for a specific magnification has changed and an existing tile set that was acquired before the alignment change is reacquired, the image shift (that is, because the alignment state changed). If this situation is detected, then the system displays the following warning:



7 Sample Holders

Overview

This chapter describes the sample holders that are available for Maps.

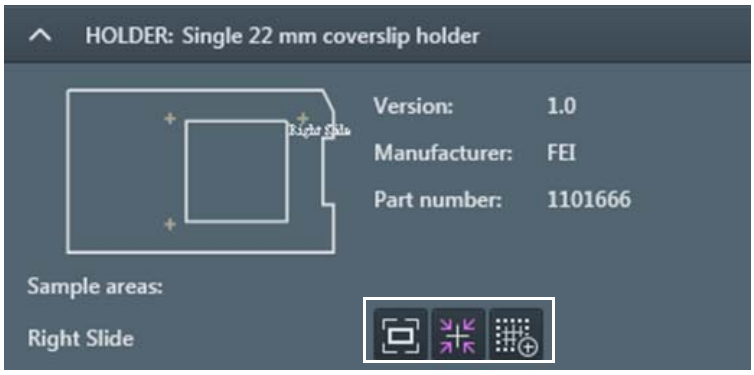
Topics include:

- *"Holder Tab" on page 157*
- *"Holders Supported for Automated Holder Alignment" on page 158*
- *"Holders Supported for Graphical Overlay" on page 161*

Holder Tab

When a holder is selected and applied to the project:

- The outline displays in red on the viewer if automated holder alignment is supported. If automated alignment is not supported, then the outline displays in green.
- The name, drawing, version, and so on are displayed on the Holder tab.
- A new Layer is added to the Layer Control. This is where you choose to show or hide the Holder outline and Fiducials. The Fiducials only show after image acquisition. The default is to not show them. Enable the check box to view your fiducial images.
- Sample area quick access buttons appear.



- **Left**: Centers the view on the sample area

- **Middle**: Drives the stage to the center of the sample area

- **Right**: Creates a new tile set that covers the sample area

Holders Supported for Automated Holder Alignment

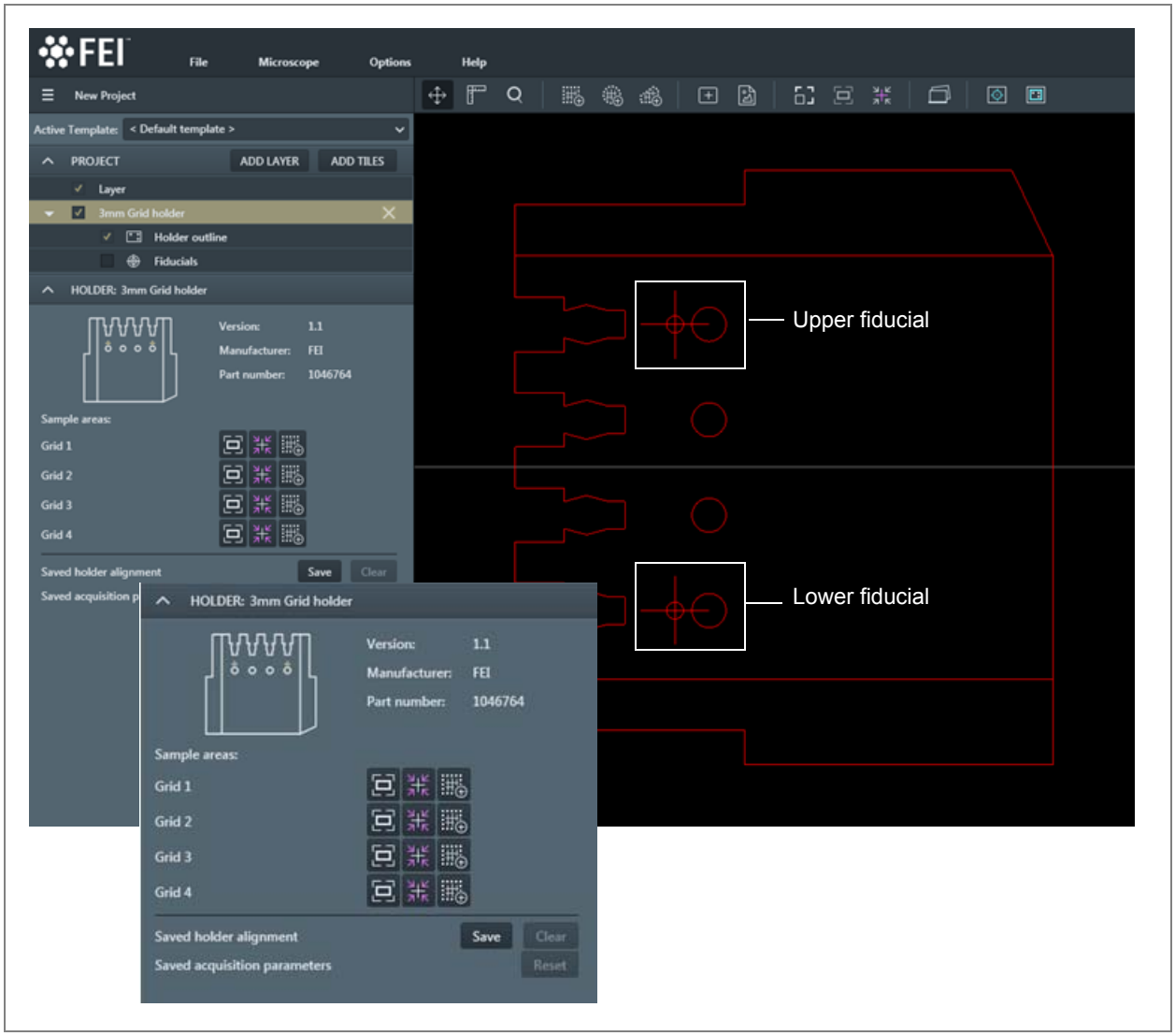
When selected, the holder outline displays in red on the viewer, if automated holder alignment is supported. See *"Automated Holder Alignment" on page 168*.

- *"3mm Grid Holder" on page 158*
- *"Multiwell Slide Holder" on page 164*
- *"Double 22 mm Coverslip Holder" on page 160*

3mm Grid Holder

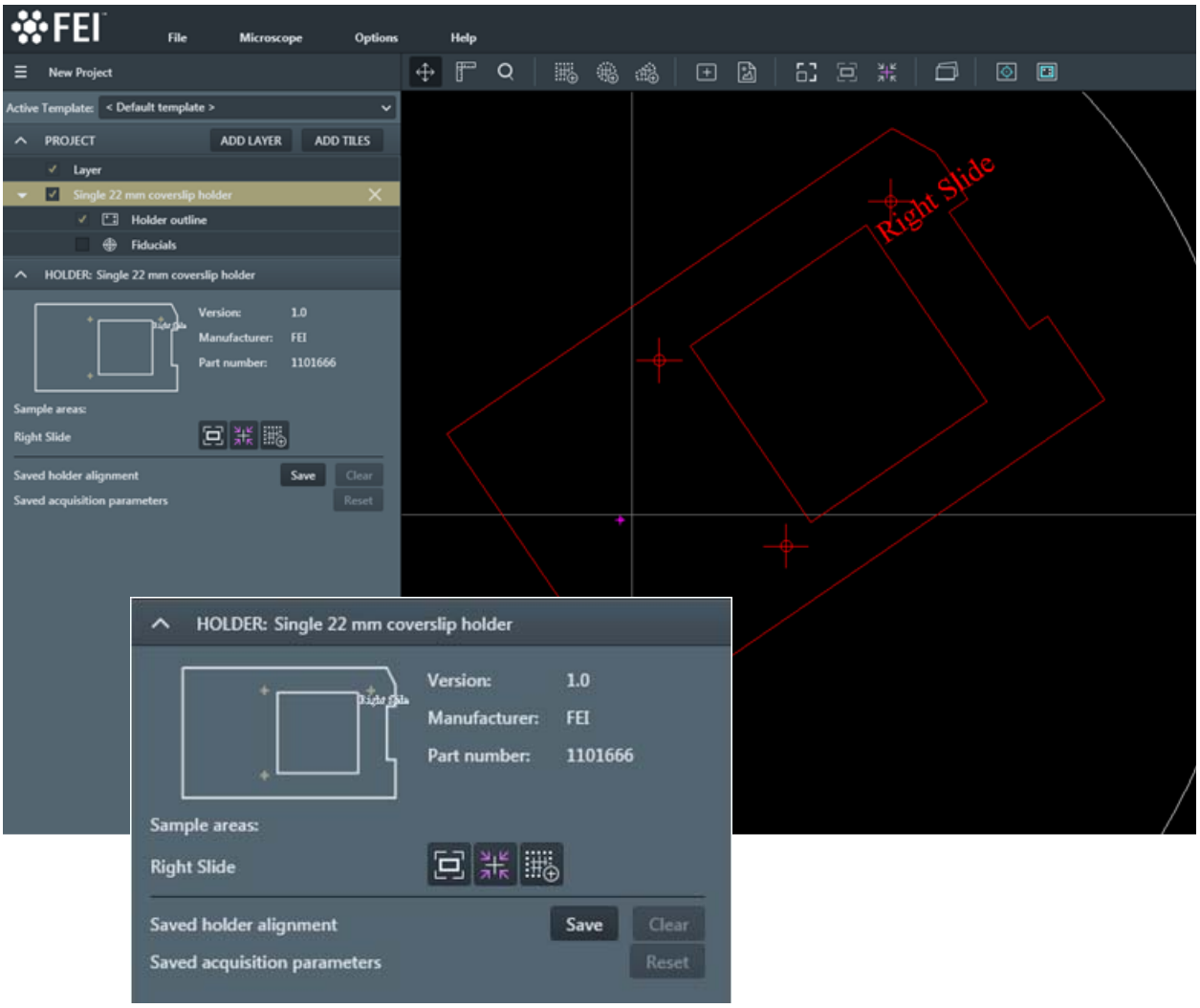
This is a holder for 3 mm diameter grids that can be loaded inside a transmission electron microscope (TEM).

Figure 1 3mm Grid Holder with Upper and Lower Fiducials Marked



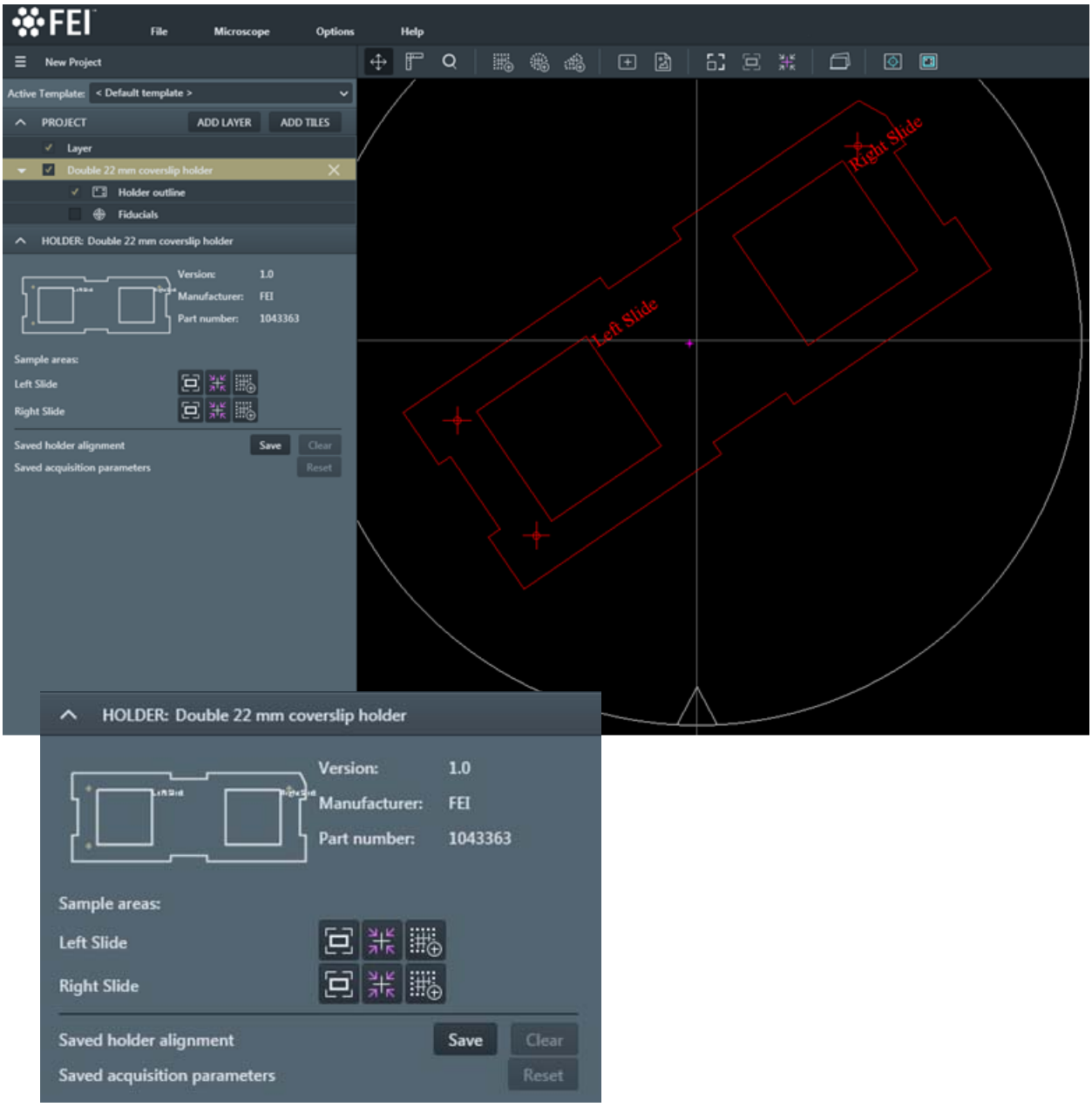
Single 22 mm Coverslip Holder

This is a single 22 mm coverslip holder.



Double 22 mm Coverslip Holder

This carrier holds up to two samples.



Holders Supported for Graphical Overlay

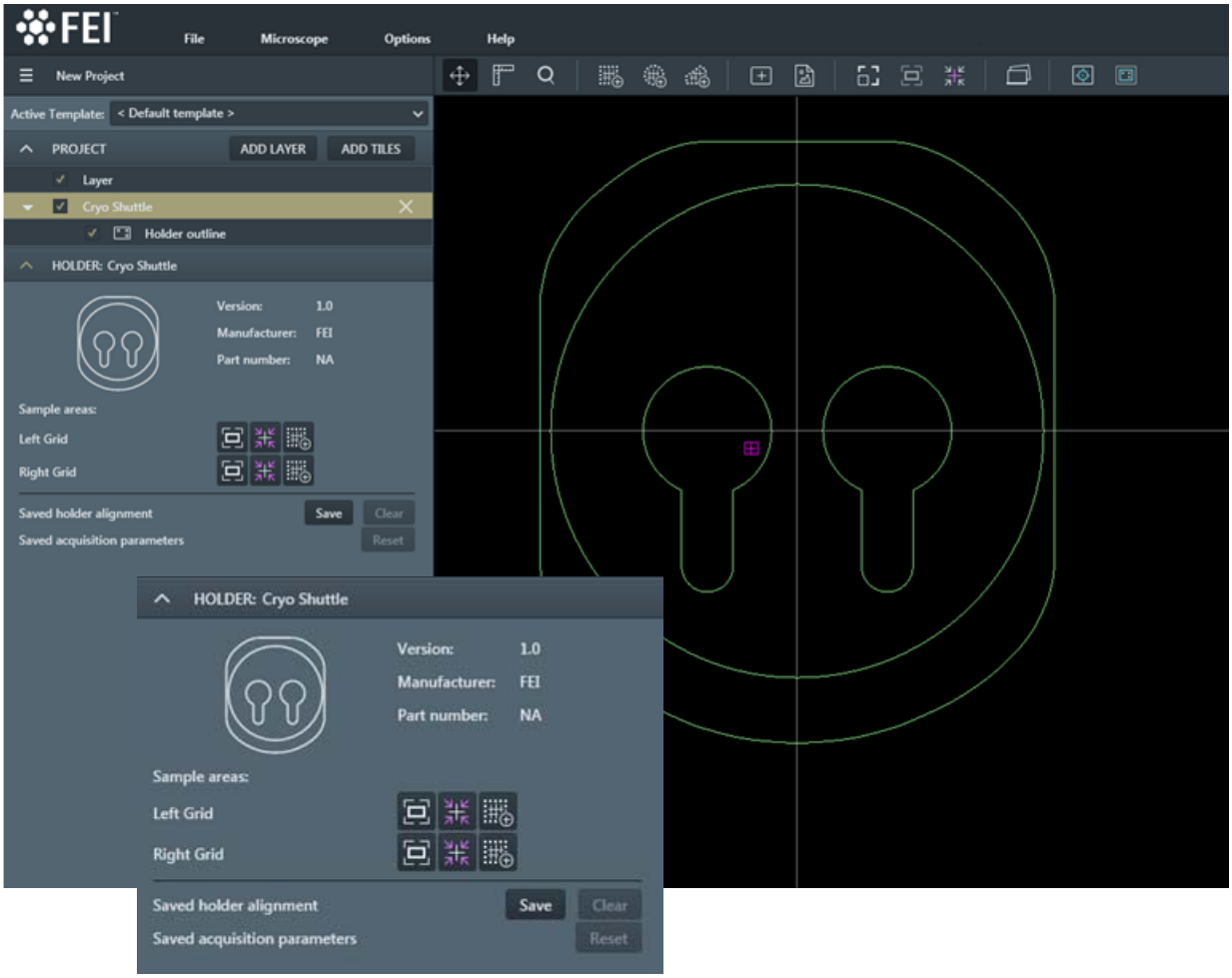
Maps can draw a graphical overlay to assist with navigation. The graphical overlay can be aligned manually to other image layers, but the automated holder alignment is not supported on these layers,

When selected, the holder outline displays in **green** on the viewer, indicating that **automated holder alignment is NOT supported**.

- *"Cryo Shuttle Holder" on page 162*
- *"Cryo SEM Shuttle Holder" on page 163*
- *"Multiwell Slide Holder" on page 164*
- *"Slide 75mm x 25mm Holder" on page 165*
- *"12xthin-section Quanta Mount Holder" on page 166*
- *"14x30mm Round Quanta Mount Holder" on page 167*

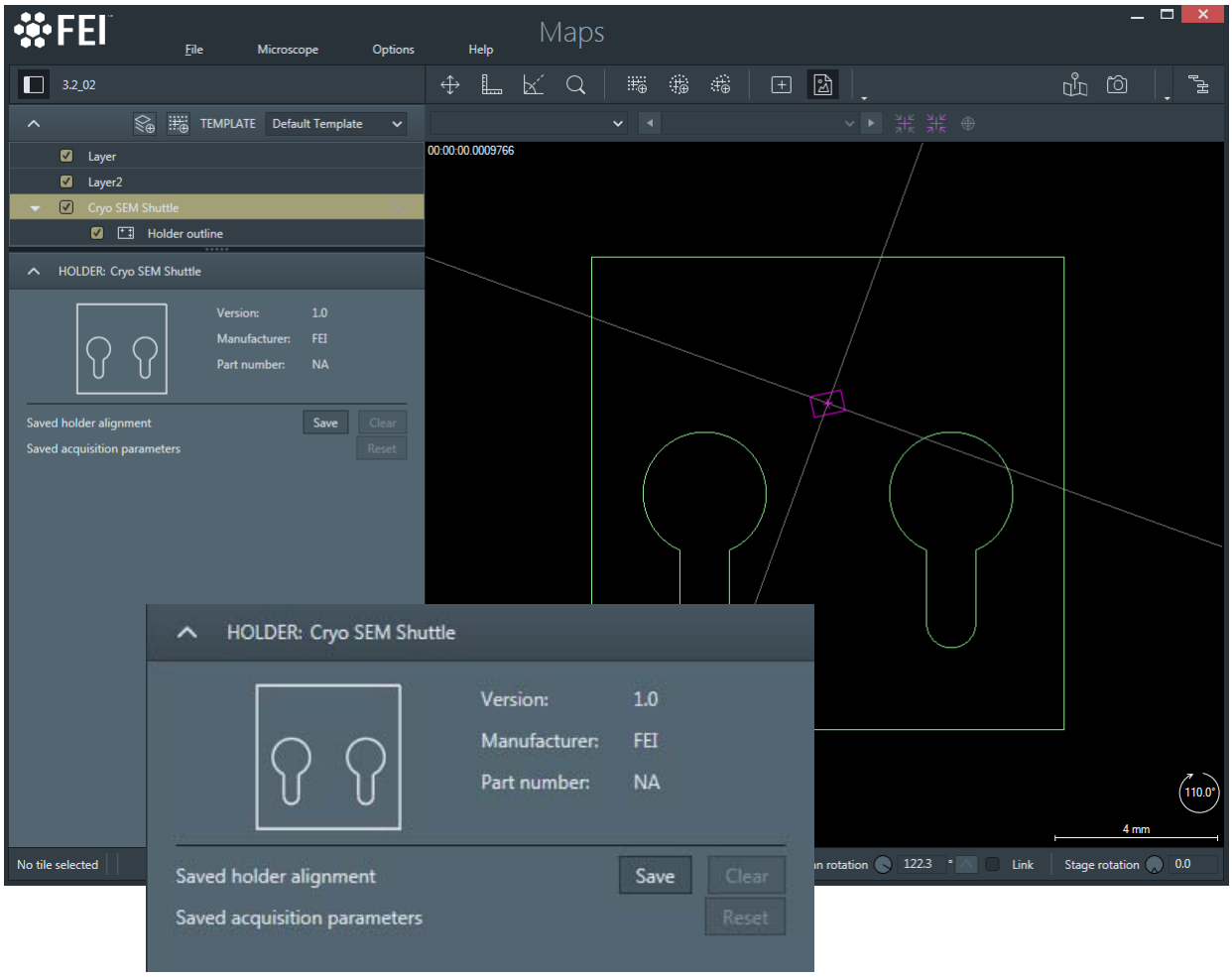
Cryo Shuttle Holder

This holder can be connected to a cooling system for imaging samples at cryogenic temperatures.



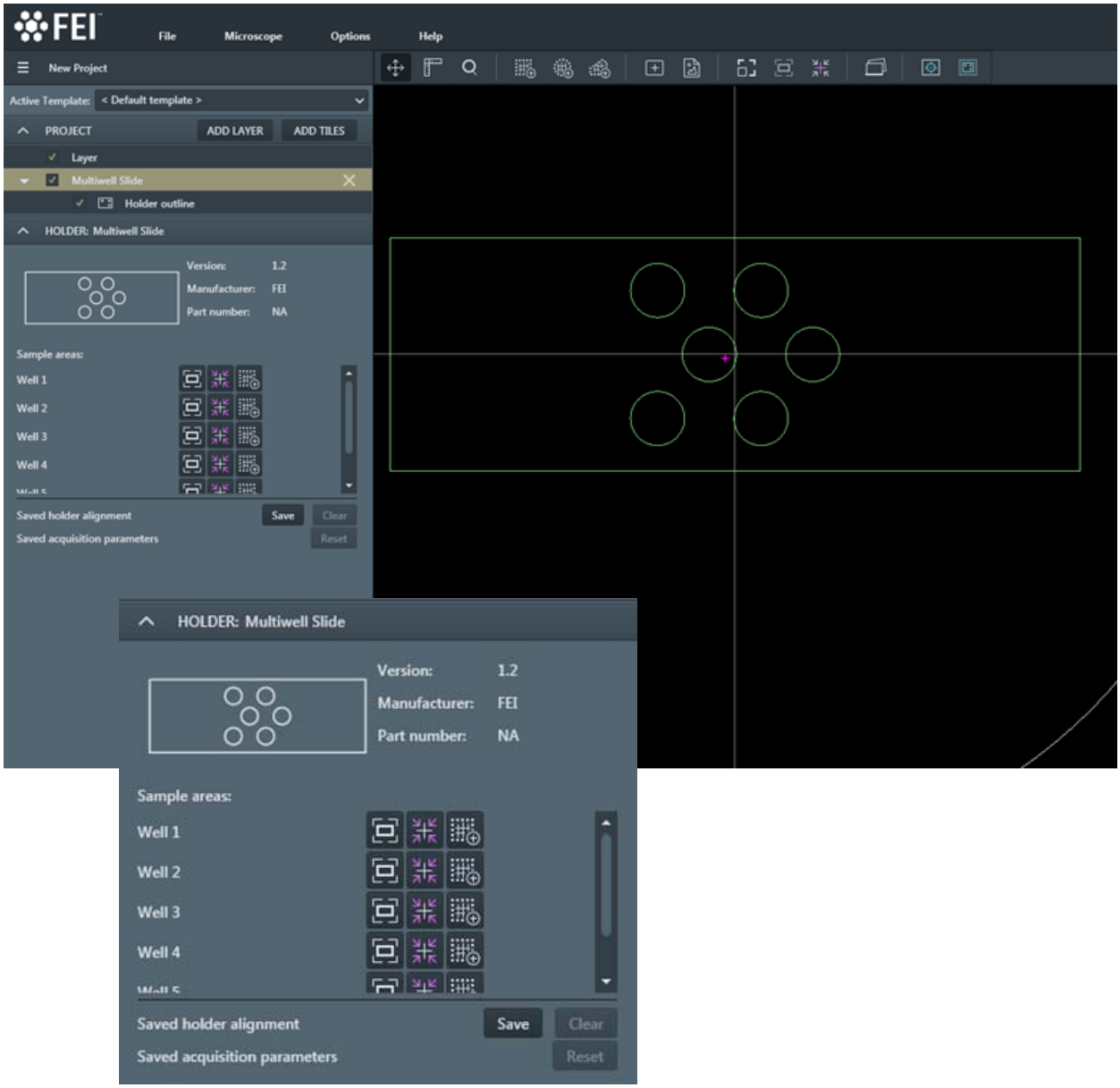
Cryo SEM Shuttle Holder

This holder is used for Cryo applications. It can be connected to a cooling system for imaging and sample preparation at cryogenic temperatures.



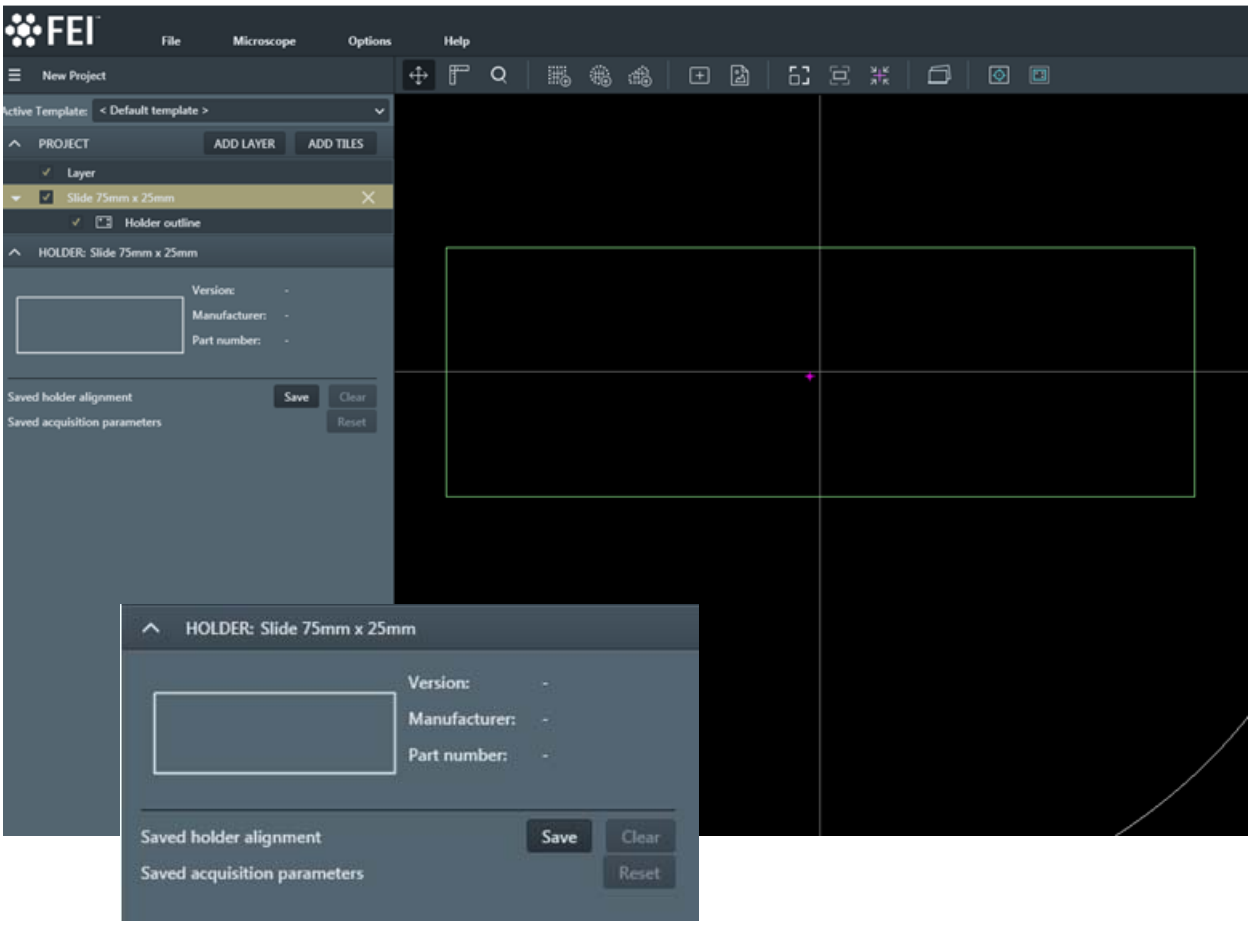
Multiwell Slide Holder

This holder is designed to handle up to six samples and may be used for live cell imaging applications.



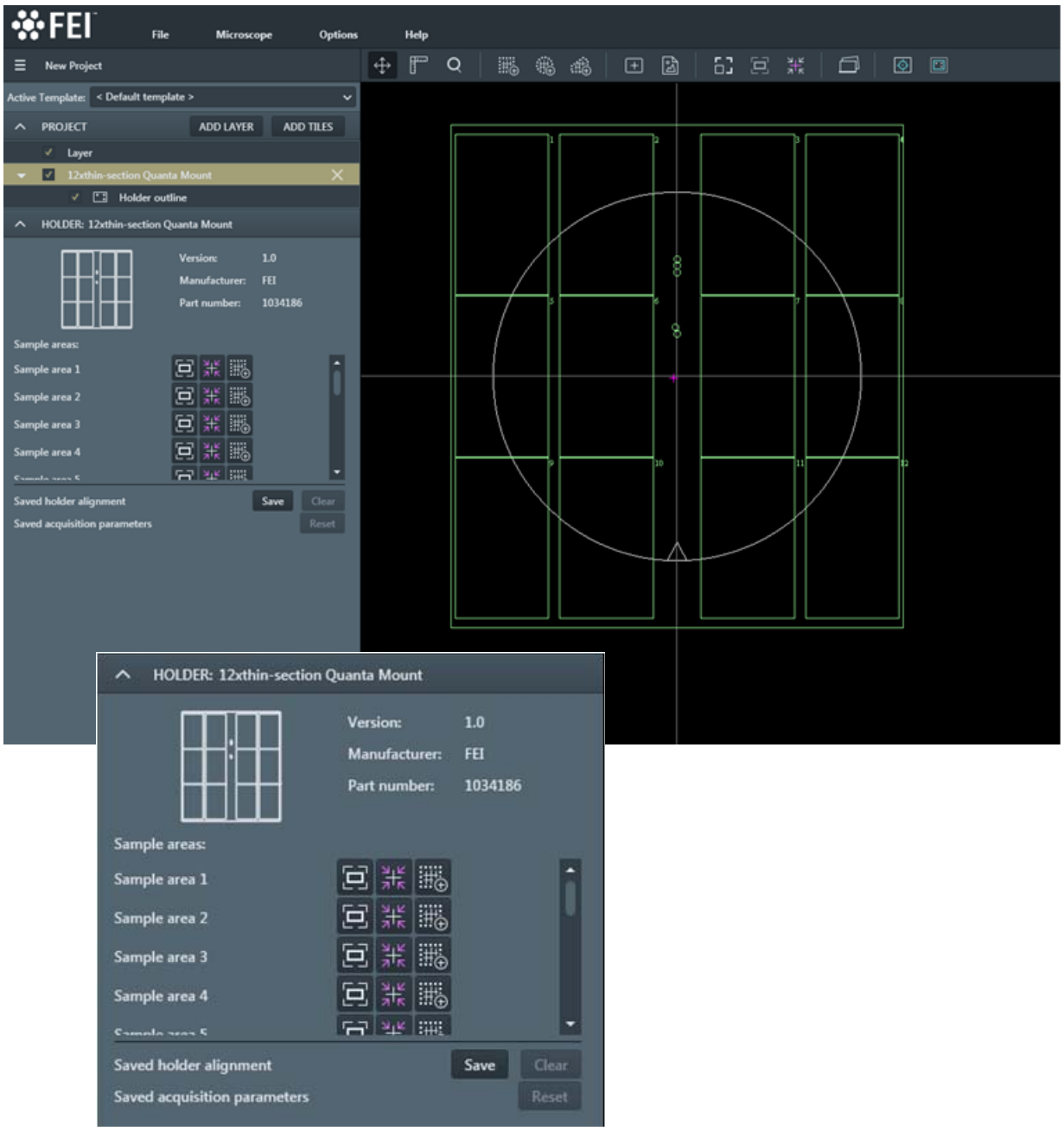
Slide 75mm x 25mm Holder

This holder represents a slide with dimensions 75 mm x 25 mm.



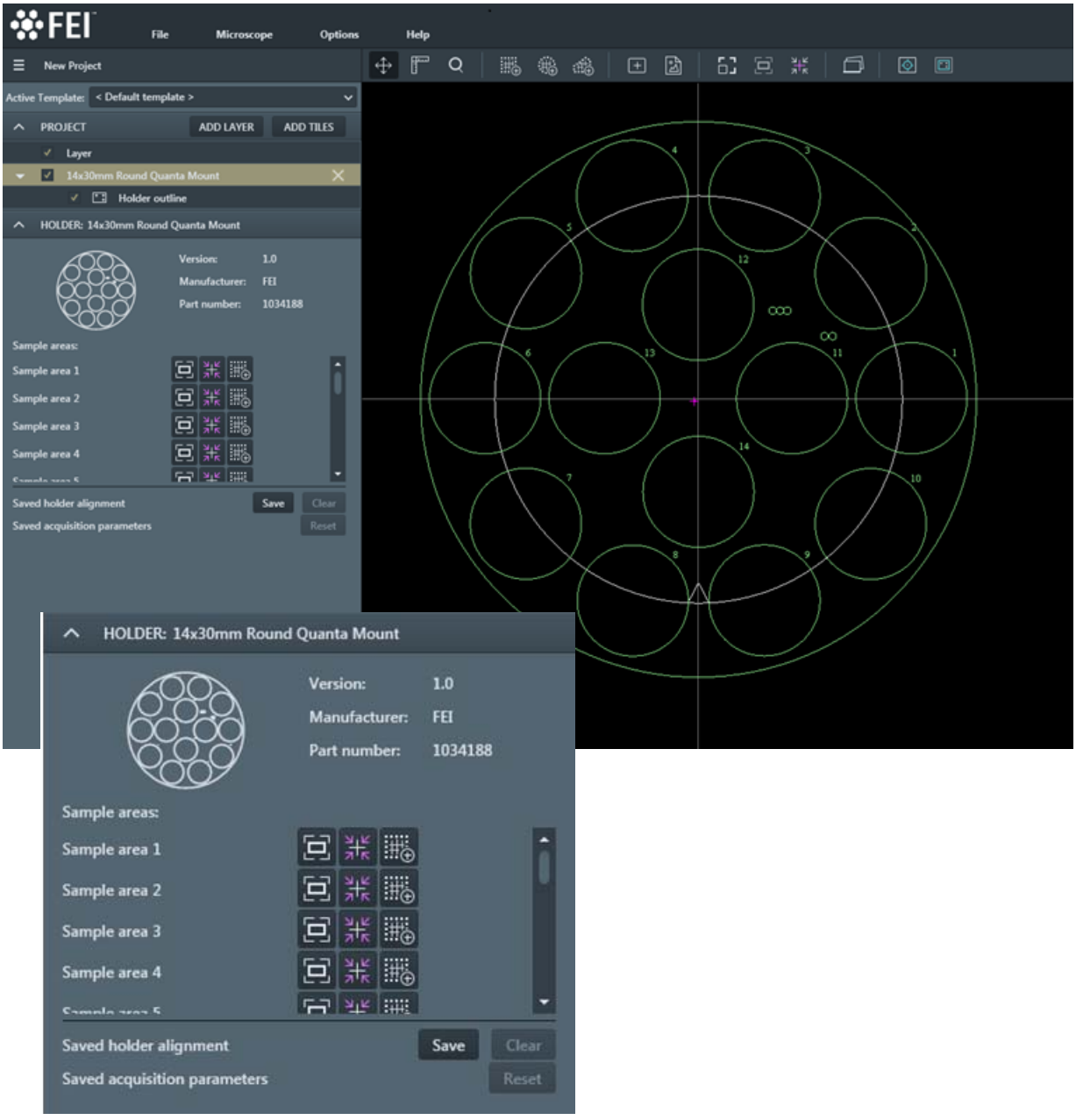
12xthin-section Quanta Mount Holder

This mount holder is designed to handle up to 12 thin mineralogy samples. The mount holder includes a Faraday cup; meets gold, quartz, and copper standards; and has a magnesium calcium position.



14x30mm Round Quanta Mount Holder

This mount holder is designed to handle up to 14 samples of 30-mm resin embedded mineralogy samples. The mount holder includes a Faraday cup; meets gold, quartz, and copper standards; and has a magnesium calcium position.



8 Automated Holder Alignment

Overview

This chapter describes the procedure for automated holder alignment (AHA) setup, calibration, and operation for a particular supported holder, on both CorrSight and SEM/SDB systems. It is a guideline for using Maps and the automated holder alignment, and can also be used for CorrSight- or SEM/SDB-only as well as multi-system correlative Maps projects.

NOTE	Automated Holder Alignment is NOT supported for Maps on TEM systems.

Topics include:

- *"Holders Supported for Automated Holder Alignment" on page 168*
- *"Automated Holder Alignment User Interface" on page 169*
- *"CorrSight Holder Calibration" on page 173*
- *"SEM/SDB Holder Calibration" on page 180*
- *"Correlating Projects" on page 193*
- *"Troubleshooting" on page 194*

Holders Supported for Automated Holder Alignment

When selected, the holder outline displays in red on the viewer. They appear in green if automated holder alignment is NOT supported. See *"Sample Holders" on page 157*.

- *"3mm Grid Holder" on page 158*
- *"Multiwell Slide Holder" on page 164*
- *"Double 22 mm Coverslip Holder" on page 160*

All supported holders must be manually calibrated on a given system before Automated Holder Alignment is possible. This is a one-time procedure. After manual calibration is complete, little to no manual action will be required.

Automated Holder Alignment User Interface

Click the **Align to Holder** icon in the tool box and select the appropriate holder from the **Sample Holder** dropdown menu to cause the Automated Holder Alignment user interface to appear below the viewer.

Figure 1 Automated Holder Alignment UI

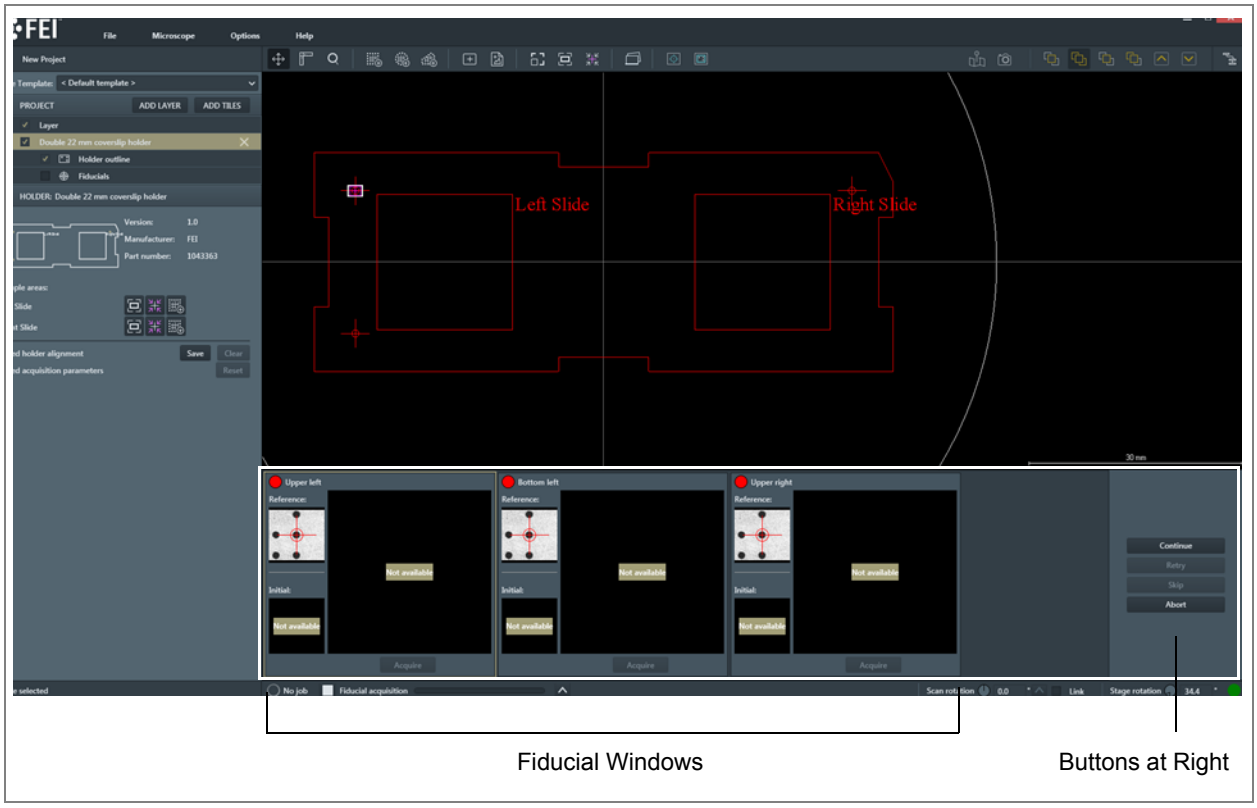


Table 1 Automated Holder Alignment UI Overview (1 of 3)

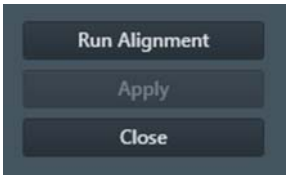
Interface Item	Description
Buttons at right (Before Starting Alignment):	
Run Alignment	Moves the stage to the position where it expects to find the fiducial, auto-focuses, and attempts to match the fiducial.
Apply	Applies the alignment to the project if the alignment is not immediately applied when the automated procedure completes. This can be used if the automated alignment completes but manual adjustment is needed.

Table 1 Automated Holder Alignment UI Overview (2 of 3)

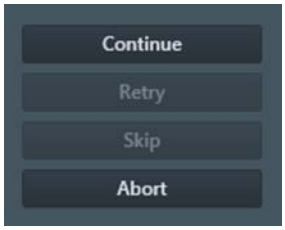
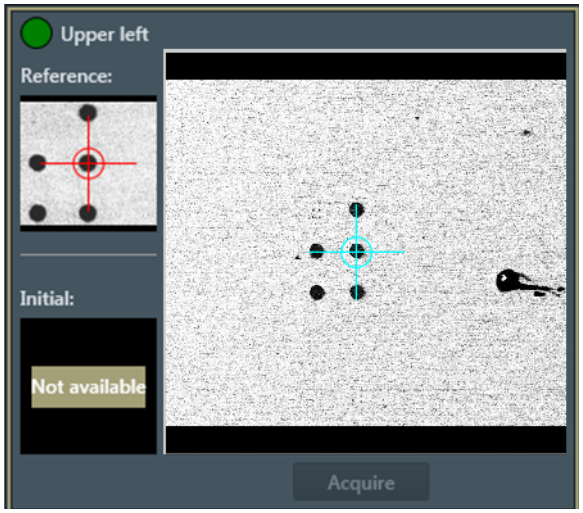
Interface Item	Description
Close	Closes the alignment UI and returns to the main viewer before the automated procedure has begun.
Buttons at Right (During Alignment):	
Continue	On SEM systems, the automated holder alignment requires manual optimization of the image (contrast, brightness, focus etc.). After the stage is automatically moved to the location of the first fiducial and the prompt for image optimization has appeared, use **Continue** to proceed with fiducial matching.
Retry	Autofocuses and attempts to match the fiducial. If the match is successful, the automated alignment procedure will continue to the next fiducial.
Skip	Skips the current fiducial matching procedure and proceeds to the next fiducial. This can be useful if Maps encounters difficulty matching, however, the best practice is to optimize the image for successful automatic matching or to manually select the fiducial position.
Abort	Cancels the alignment procedure currently in progress and returns to the main viewer.
Fiducial Window	
Green/Red Ball	Indicates if the fiducial was found: Green = Found; Red = Not Found.
Fiducial Name	Displays the name of the fiducial. In this example, Upper Left.
Reference	Displays the reference image.

Table 1 Automated Holder Alignment UI Overview (3 of 3)

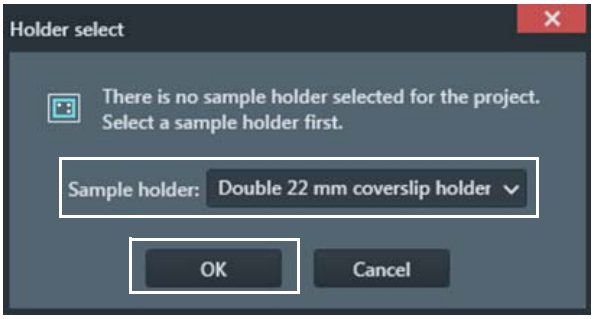
Interface Item	Description
Initial	Displays the result of the previous alignment procedure for a project that has already been automatically aligned.
Current Fiducial Window	Displays the fiducial image acquired during the current automated holder alignment procedure.
Acquire	Acquires an image of the specified fiducial. Maps will move to the expected location for the specified fiducial and attempt to match.
Fiducial Markers for Matching	The fiducial marker is placed on the fiducial image as a point of reference. Maps will align these reference points during alignment. • **Red** markers are used in the reference images to display the reference point that the automated procedure expects to find. • **Blue** markers are placed automatically when a match is successful. • **Light green** markers denote the reference points from the previous alignment procedure and appear in the Initial fiducial images.

Executing the Holder Alignment

When using a sample holder that has already been calibrated on your microscope, the following procedure describes how to execute the alignment sequence.

1. Click **Align to Holder**.

2. Select the same holder that was just calibrated, in this case **Double 22 mm coverslip holder**, and click **OK**.



3. Click **Run Alignment**. The Alignment proceeds automatically.

- If the automated alignment is able to match all fiducials successfully, the holder calibration is complete.
- If the alignment failed to recognize any of the fiducials, then perform the appropriate calibration procedure:
  - CorrSight: see *"CorrSight Holder Calibration" on page 173*.
  - SEM/SDB: see *"SEM/SDB Holder Calibration" on page 180*.

CorrSight Holder Calibration

NOTE	This procedure is applicable to all holders on the CorrSight, only the number of fiducials and fiducial position is different.

CorrSight Preconditions

- Tool running for at least 1 hour
- Transmitted Light channel configured in Maps
- Live imaging functional in Maps
- Camera/Image orientation:

1. Find a distinguishable feature in live imaging in Maps.
2. Click in the live imaging window to center the feature in the FOV.
3. In live imaging, click above and to the right of the feature. Verify that the feature moves down and left.

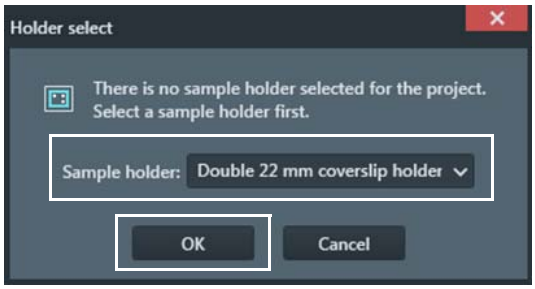
Performing the Calibration

1. Load the appropriate sample holder. In this example, it is **Double 22 mm coverslip holder.**

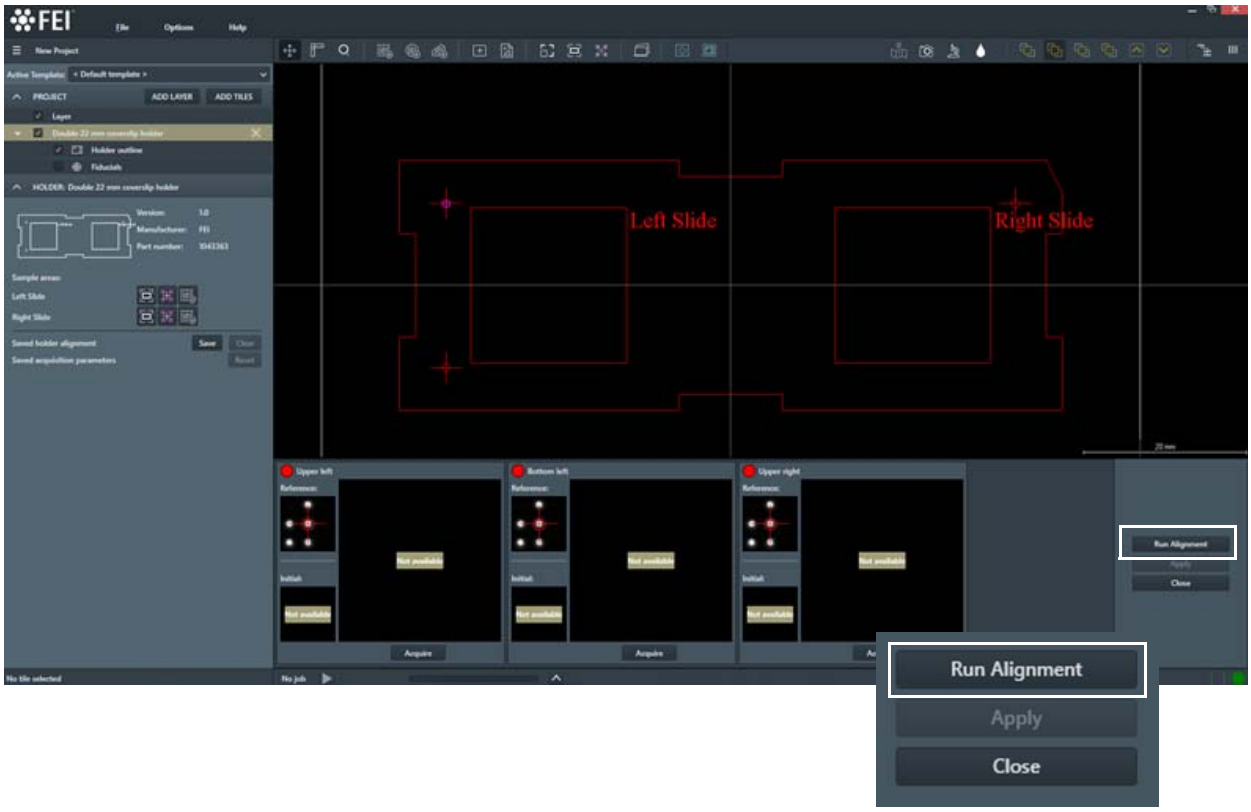
NOTE	Refer to your application specialist for procedural information for each sample holder type. See *"Sample Holders" on page 157* for descriptions of the other types of holders.

2. Create a New Project or open a project that uses the loaded holder type. Create a project by selecting **File Menu** > **New Project…**
3. Click the **Align to Holder** tool box button.

4. Select the appropriate holder from the dropdown, in this example it is **Double 22 mm coverslip holder,** and click **OK**.

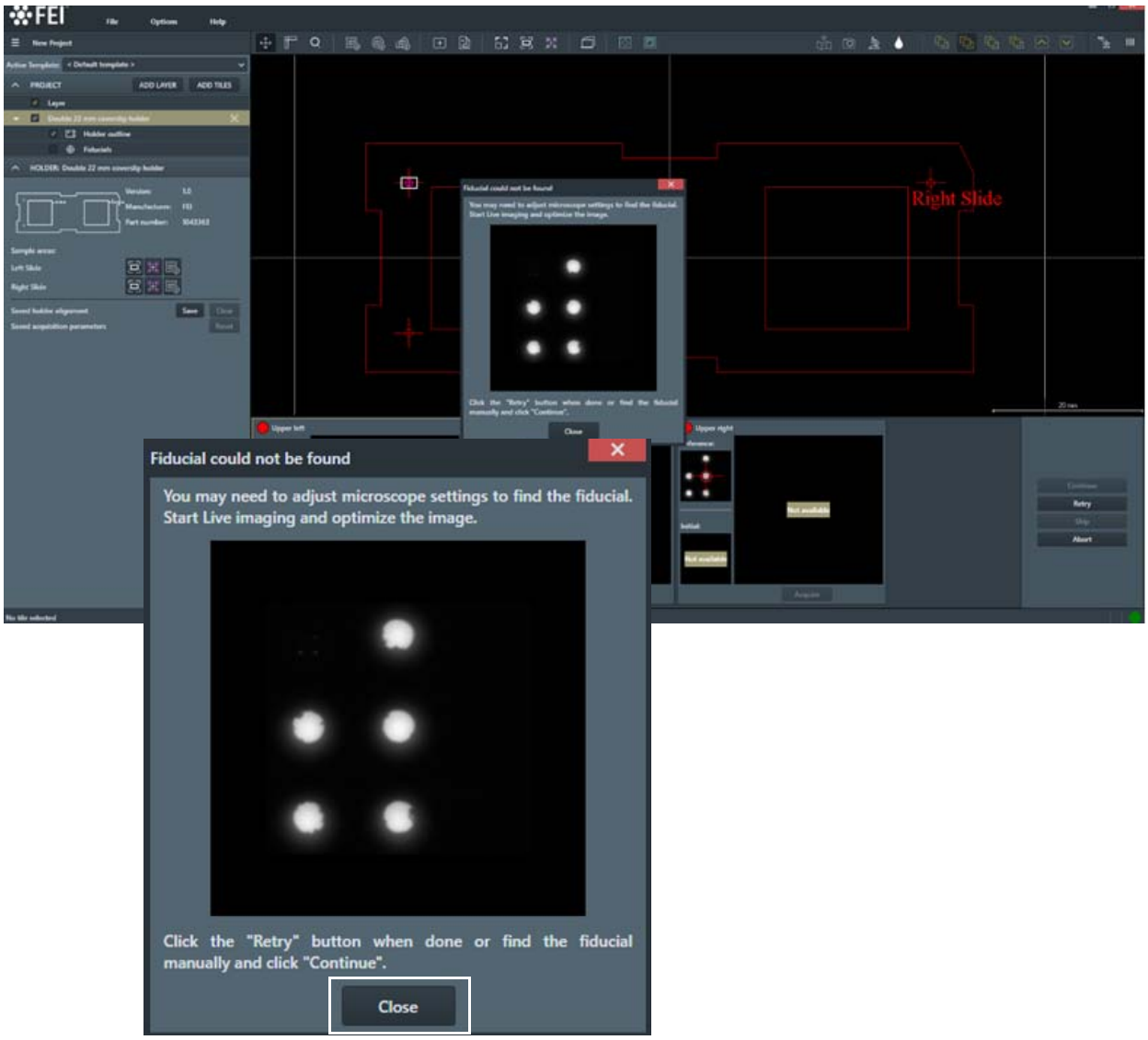


5. In the fiducial alignment controls that appear, click **Run Alignment**.



Maps will move the stage, autofocus, and attempt to match the fiducial. Because the holder has not been calibrated, the match will fail and manual adjustment will be required.

6. Click **Close** in the Microscope Settings dialog that appears.



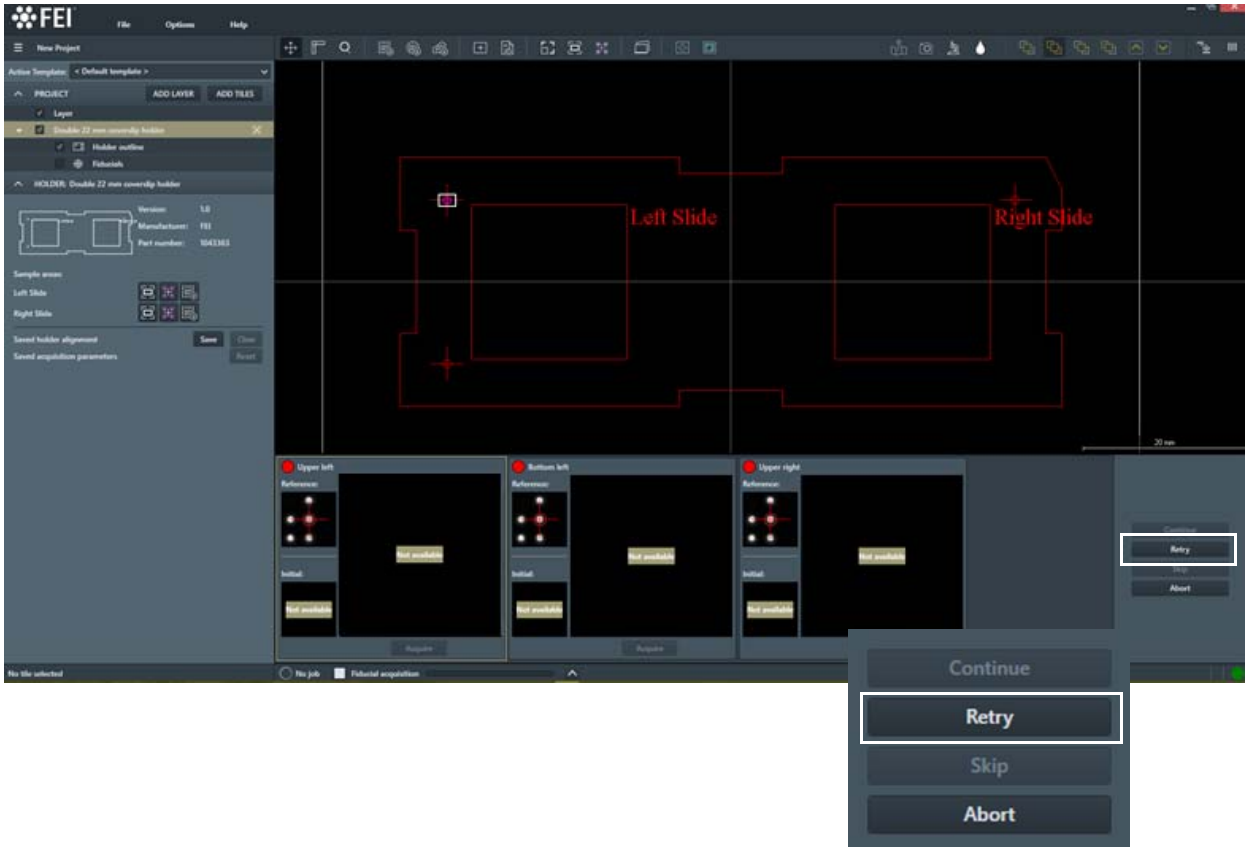
7. Click the **Live Imaging** tool box icon to start Live Imaging in Maps.

8. Locate the correct fiducial by moving the stage. Center the fiducial in the FOV.

NOTE	During calibration, some matches might not select the correct fiducial hole. Retest after calibration and verify that the correct hole is matched automatically.

9. Optimize the image focus in Maps Live Imaging using the mouse scroll wheel. The focus step size is controlled by the **Step Size** parameter in the Microscope controls. See *"Step Size" on page 125*.

10. Click **Retry**.

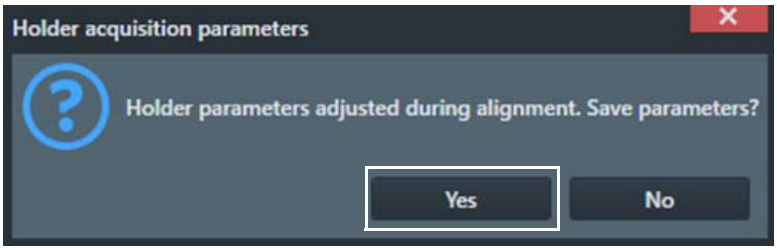


Maps will autofocus and attempt to match the fiducial. If the match is successful, the automated alignment procedure will continue to the next fiducial.

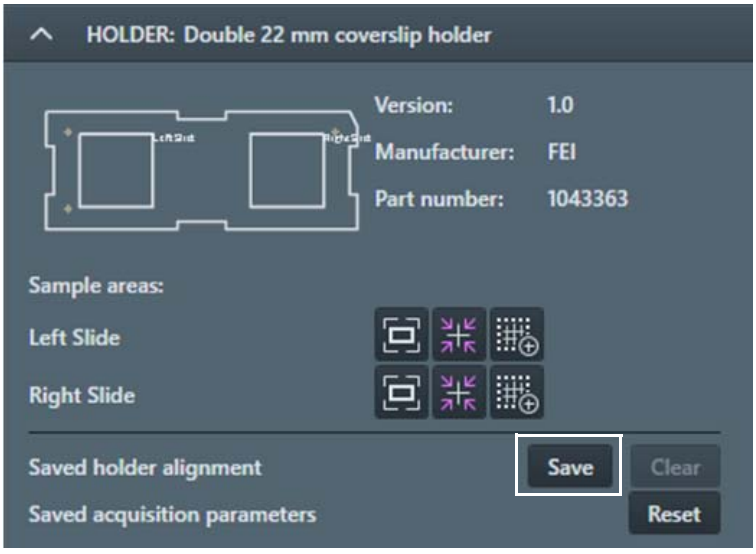
Maps will automatically attempt to match the second fiducial. If the match is not successful, repeat the calibration for the second fiducial (starting from *Step 6*).

Once the second fiducial is recognized, Maps will automatically navigate to the final fiducial (for holders with more than two fiducials). If the match is not successful, repeat the calibration for the third fiducial (starting from *Step 6*).

11. If the holder alignment succeeded and you needed to change the imaging parameters in the course of the alignment, then you will be prompted to save the new parameters. Click **Yes**.



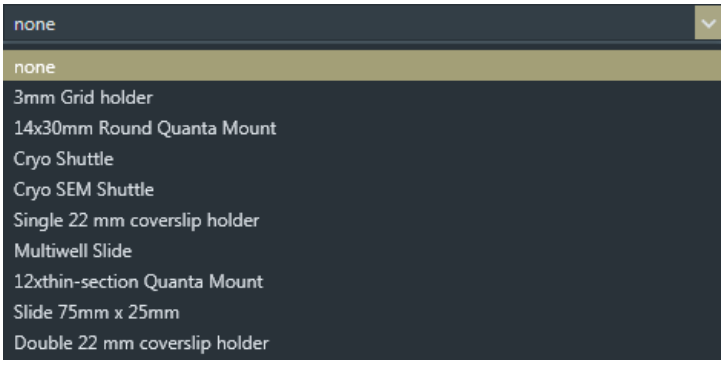
12. After the alignment sequence has completed, click the **Save** button to commit the calibration to the microscope.



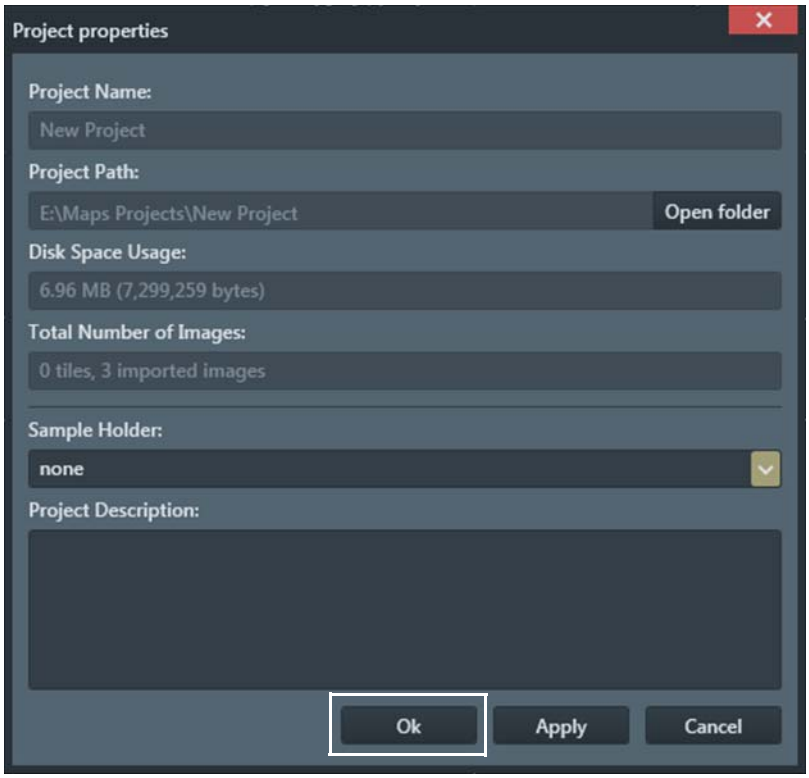
13. Proceed to verify the calibration.

Verifying the Calibration

1. Select **none** from the **File** > **Project Properties** > **Sample holder** dropdown.

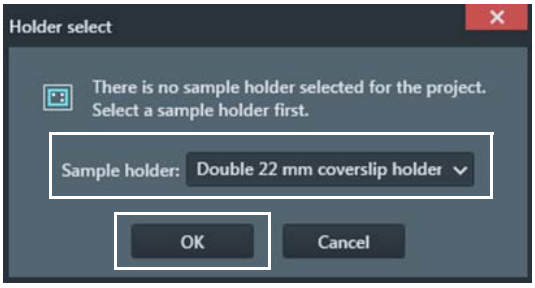


2. Click **OK** to clear the holder from the project. Click **OK** again at the prompt to clear.

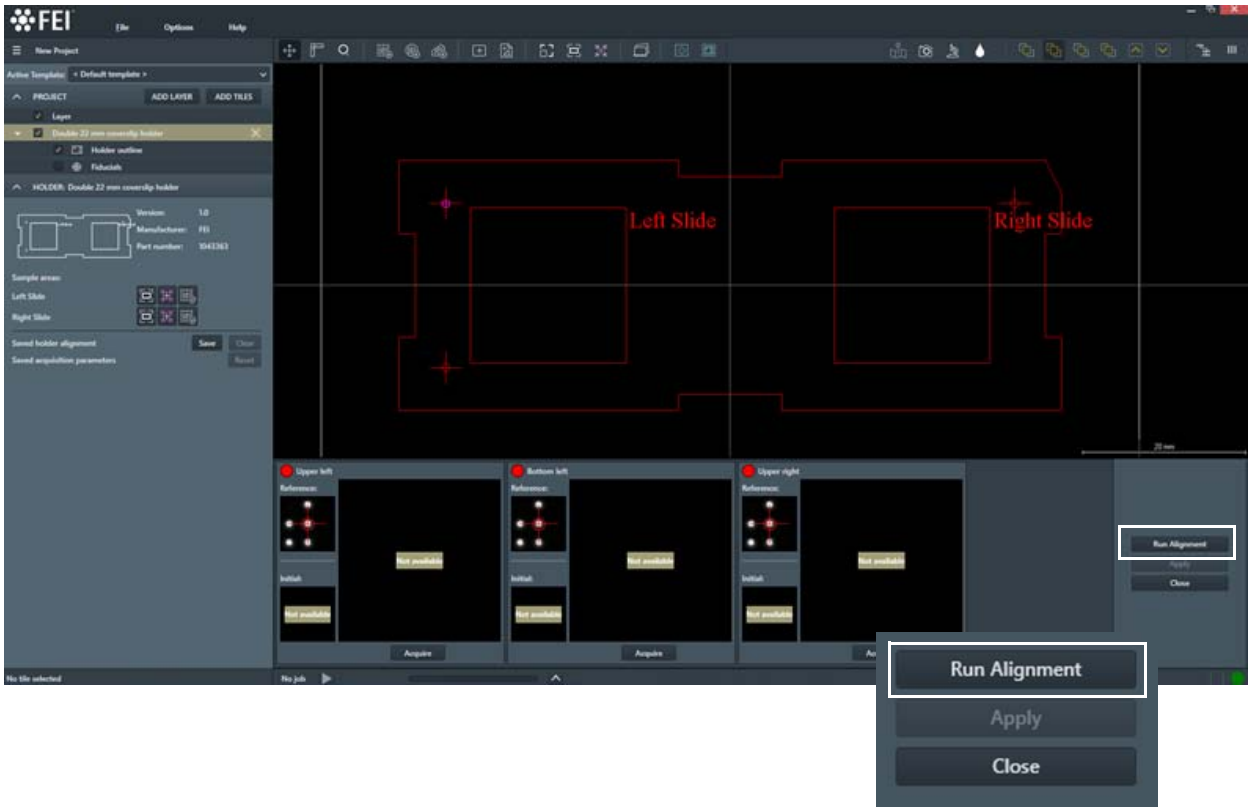


3. Click **Align to Holder**.

4. Select the same holder that was just calibrated, in this case **Double 22 mm coverslip holder,** and click **OK**.



5. Click **Run Alignment**. The Alignment proceeds automatically without manual adjustment.



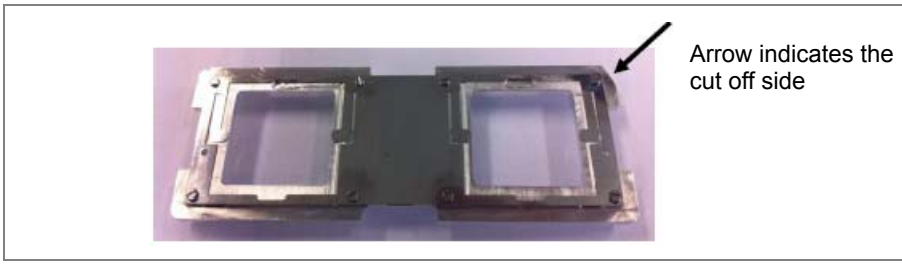
- If the automated alignment is able to match all fiducials successfully, the holder calibration is complete.
- If adjustment is still required, repeat the above procedure.

SEM/SDB Holder Calibration

Mounting the Coverslip Holder and Adaptor to the Stage

Follow this procedure to mount the **Double 22 mm coverslip holder** and adaptor to the stage.

Figure 2 Top View of the Holder



The holder only fits in the adaptor in one position.

1. Withdraw the clamp and place the holder with the cut off side facing the clamp in the holder. The holding device (encircled in red in *Figure 3*) fits the hole (encircled in red in *Figure 4*) in the adaptor.

Figure 3 Bottom View of the Holder

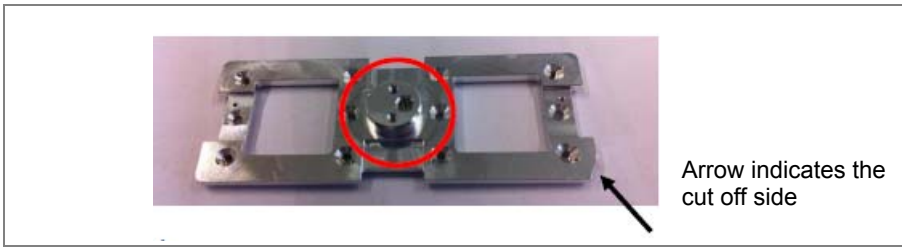
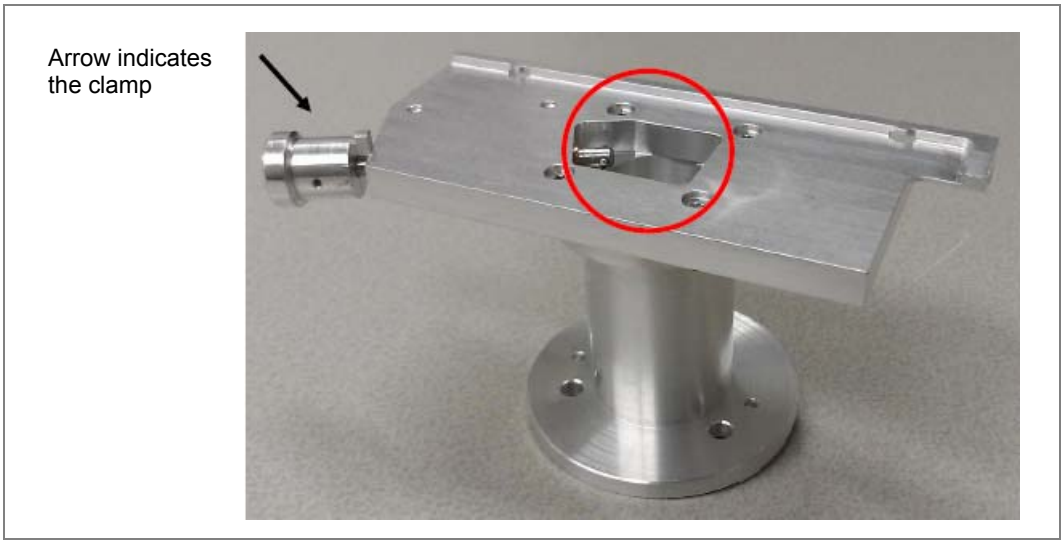
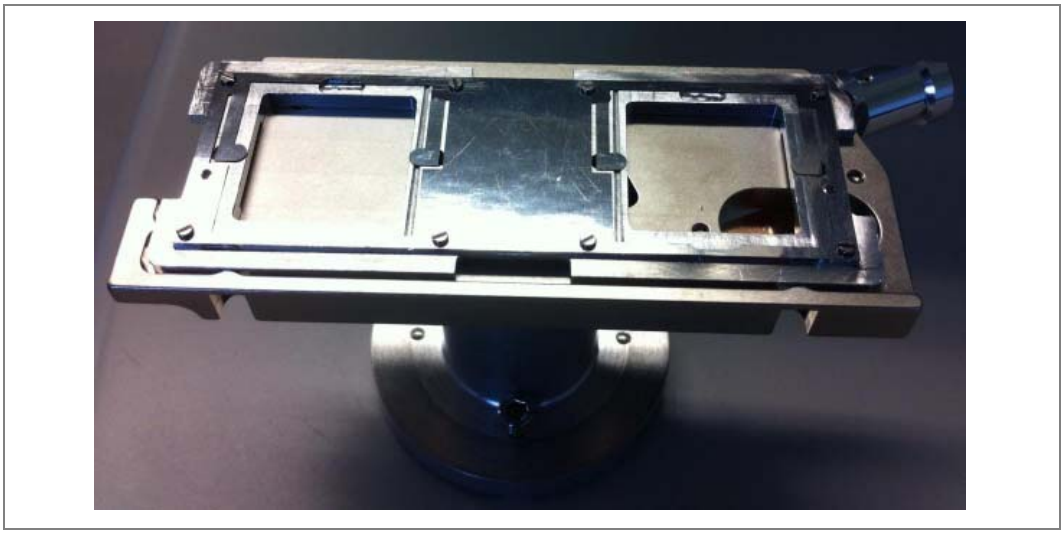


Figure 4 6 inch Stage Adaptor for Double 22 mm Coverslip Holder



2. If the holder is positioned (*Figure 5*), release the clamp.

Figure 5 Holder Positioned on Adaptor



3. Place the holder and adaptor on the stage. This can only be done in one orientation. The adaptor has three screws (encircled in red; *Figure 6*) and two pins (encircled in green) that fit nicely into the stage (*Figure 7*).

Figure 6 Adaptor Base with Screw and Pin Positions

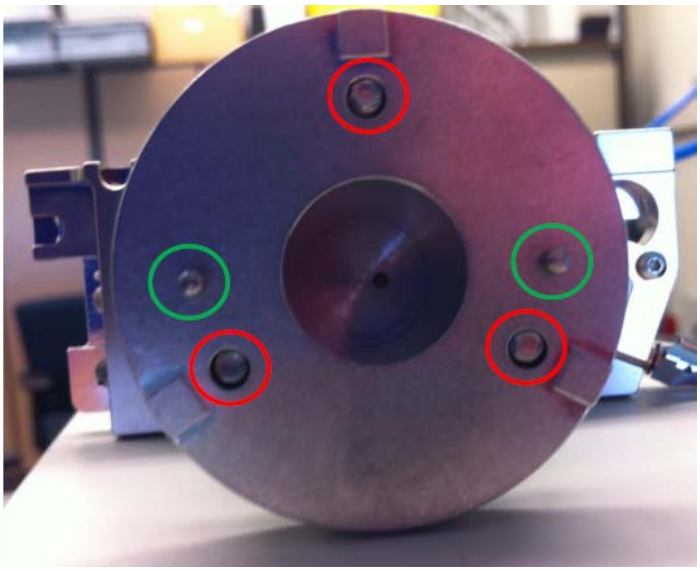
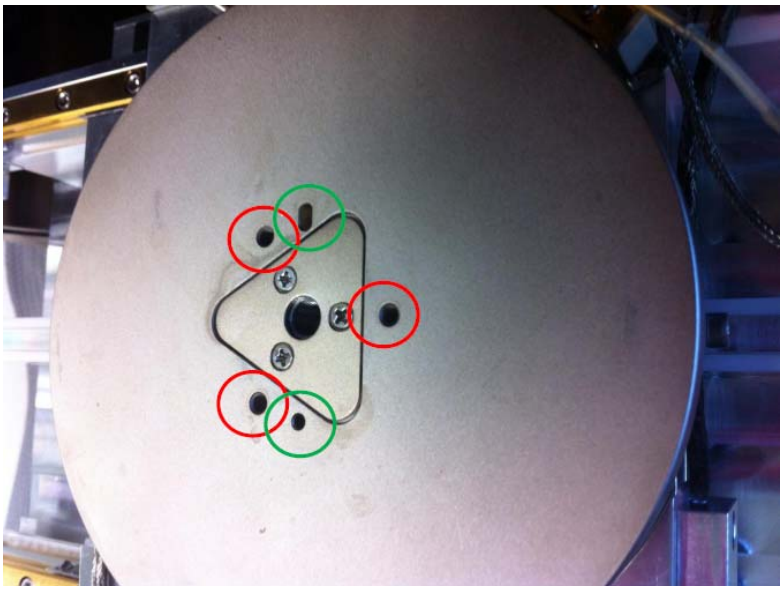


Figure 7 Stage Screw and Pin Placement Indicators



4. Tighten the screws so the stage cannot move.
5. Set the Z coordinate to 0 using the xT UI to make sure the holder will not touch the pole piece when inserting.

6. Close the chamber.
7. Press **Pump.**

NOTE	Refer to your application specialist for procedural information for each sample holder type. See *"Sample Holders" on page 157* for descriptions of the other types of holders.

SEM/SDB Preconditions

Before performing calibration on a Maps supported holder using an SEM/SDB system, verify that the following preconditions are satisfied:

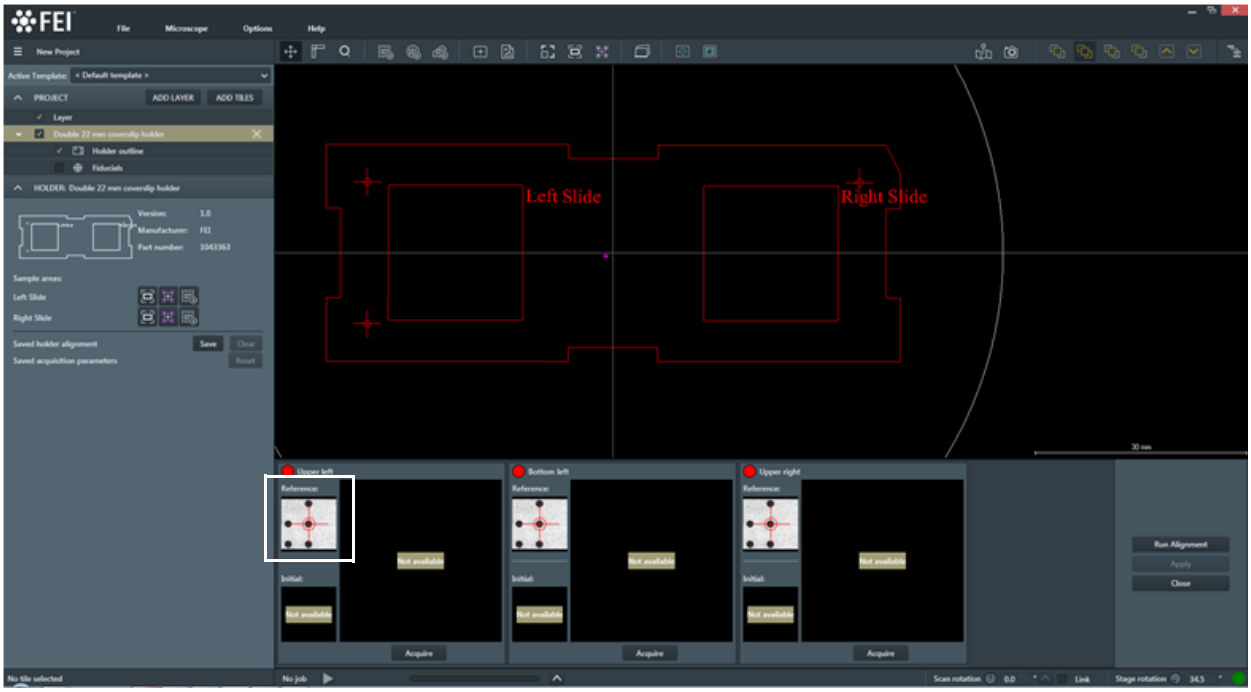
- Home stage
- SEM mode: lowest magnification
- ETD
- BSE
- 10 kV
- 100 pA (Use higher beam current of about 1 nA if you have difficulty matching automatically)
- Eucentric
- Link stage to WD
- Optimize contrast such that the fiducial holes are black and the background is white

Finding the Fiducials and Storing their Coordinates

NOTE	This procedure is only necessary when starting Maps for the first time on a particular SEM/SDB, using a particular holder.

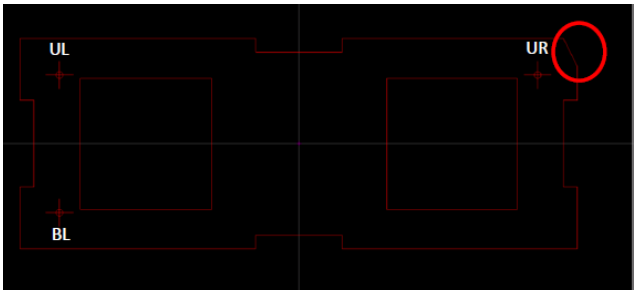
Using the xT UI, find the upper right (UR), upper left (UL), and bottom left (BL) fiducials and store the coordinates on the Stage tab.

NOTE	The fiducial orientation must match the reference image.



The fiducial next to the chamfer/cut off (encircled in red; *Figure 8*) is called upper right.

Figure 8 Upper Right Fiducial



The **upper right** fiducial should be present at coordinates:

- X: 9.5 mm
- Y: –35.0 mm

The **upper left** (UL) fiducial should be present at coordinates:

- X: 9.5 mm
- Y: 35.0 mm

The **bottom left** (BL) fiducial should be present at coordinates:

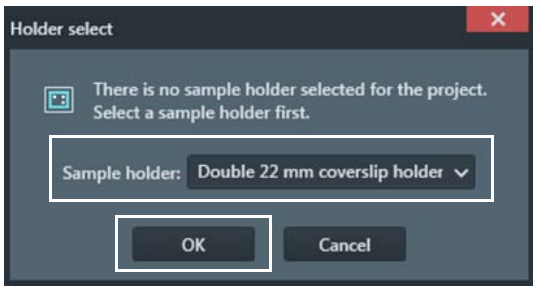
- X: –9.5 mm
- Y: 35.0 mm

NOTE	Depending on the system type, X and Y coordinates might be reversed. ■ If you are entirely unable to find the fiducials on the holder, perform a large overview tile for help in navigation. ■ If the system is equipped with a NavCam, use the NavCam navigation image to assist with finding the fiducials.

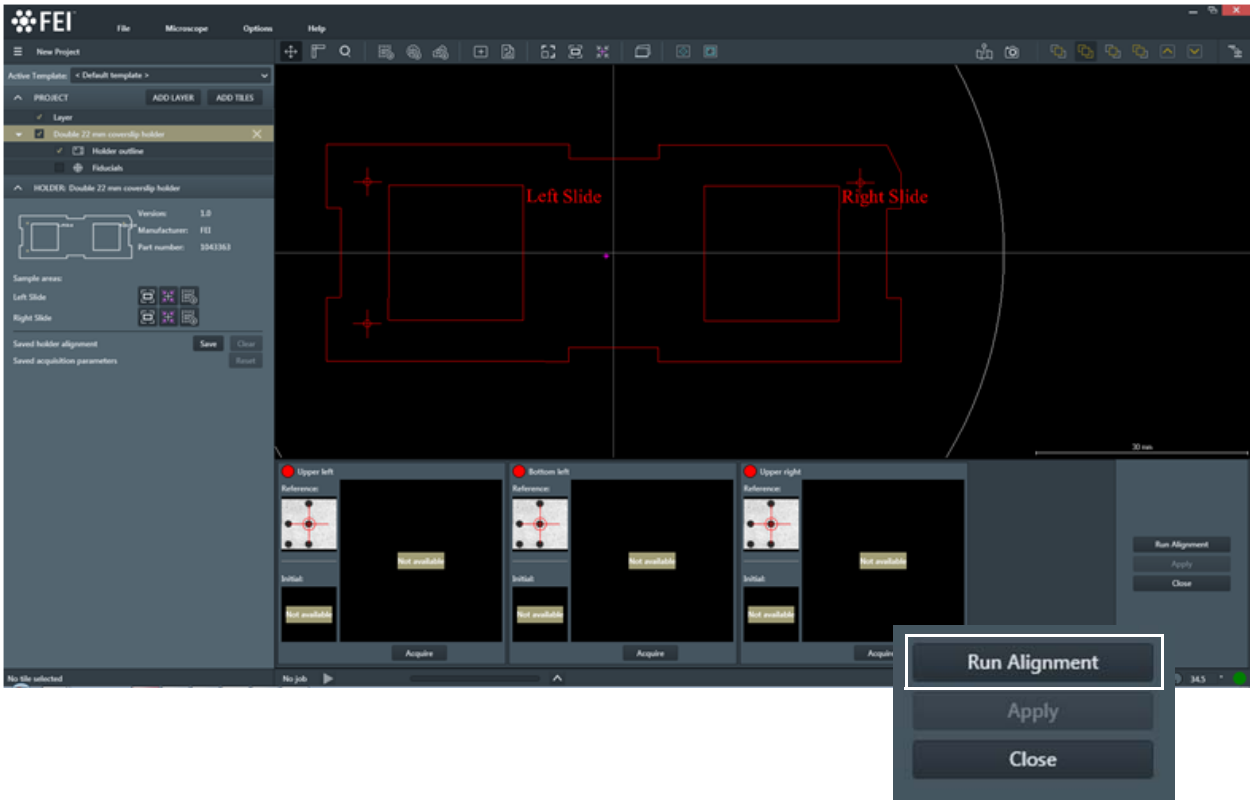
Performing the Calibration

1. Create a new project in Maps.
2. Click the **Align to Holder** icon in the tool box.

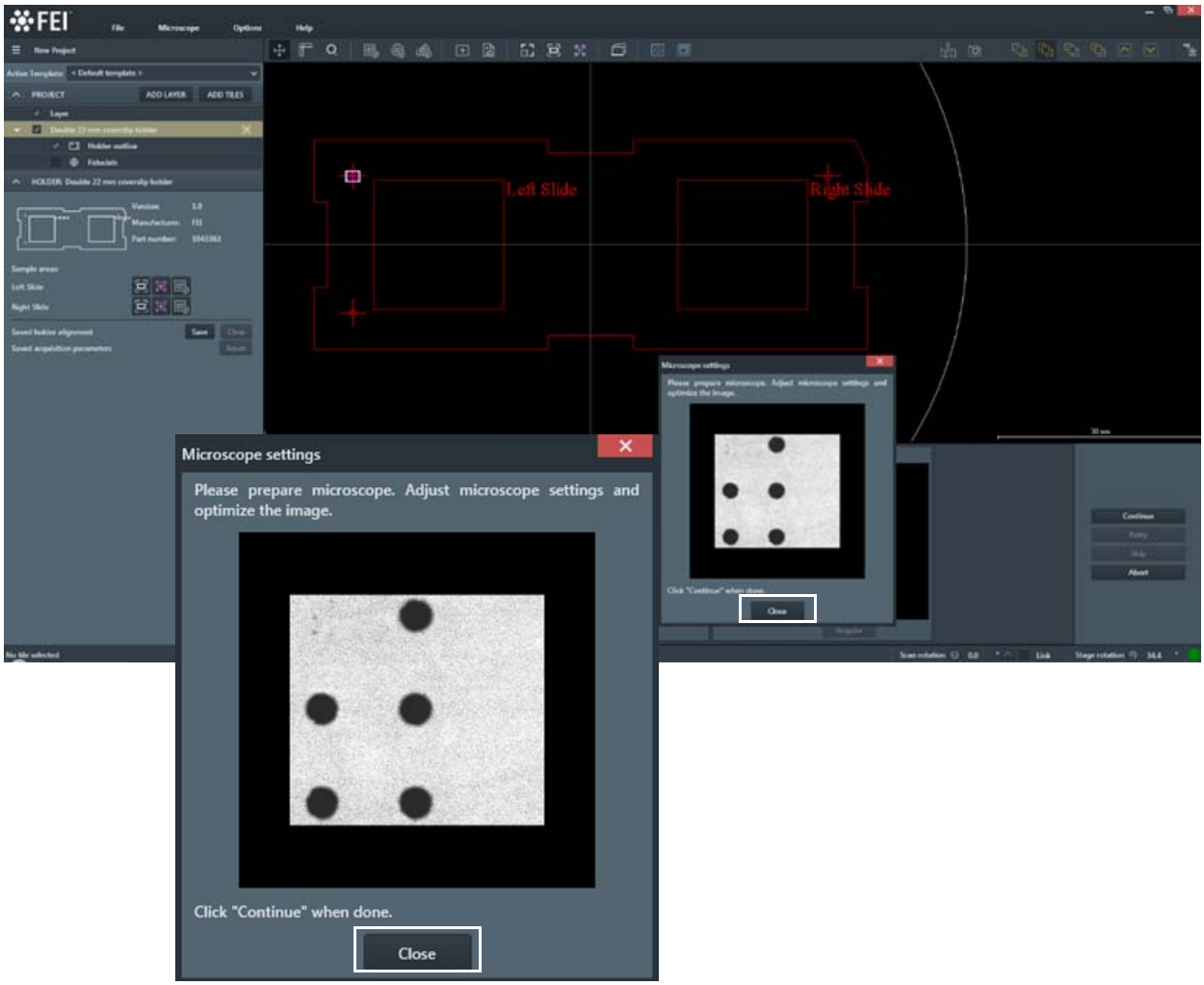
3. Select the appropriate holder from the **Sample Holder** dropdown menu, in this example it is **Double 22 mm coverslip holder,** and click **OK**.



4. In the fiducial alignment controls that appear, click **Run Alignment**.



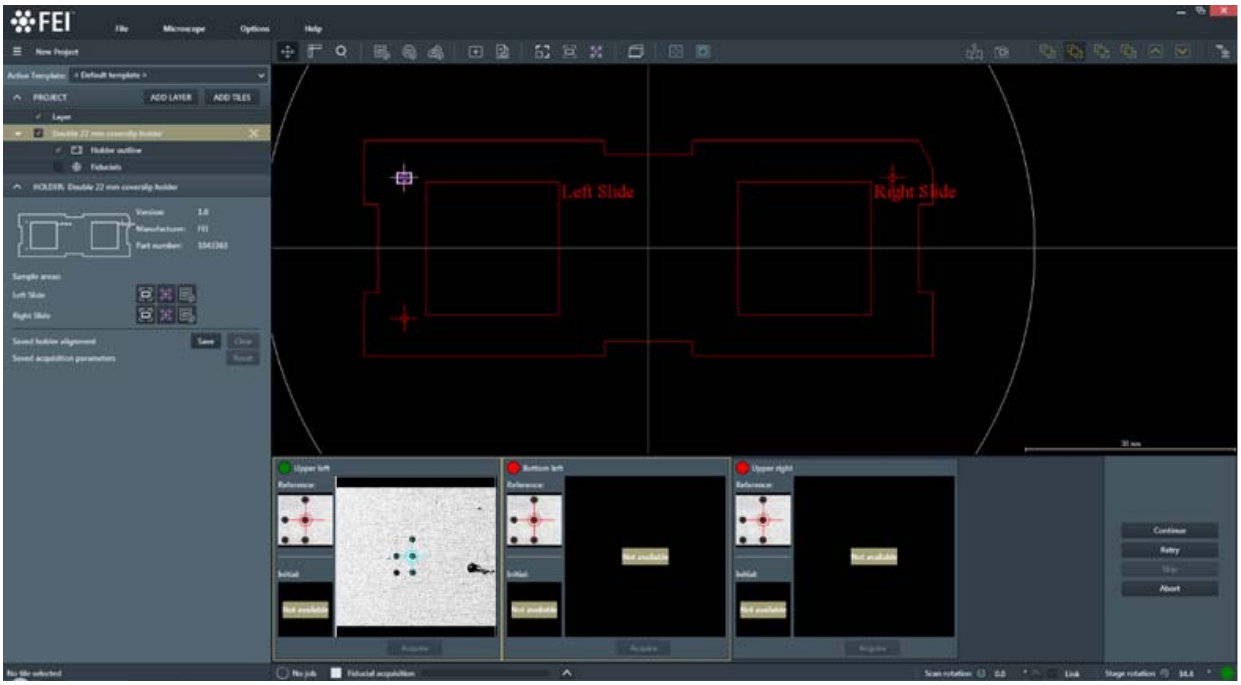
5. The stage drives to the position where it expects to find the upper left fiducial. Maps displays a prompt to adjust microscope settings for optimal fiducial image. Click **Close**.



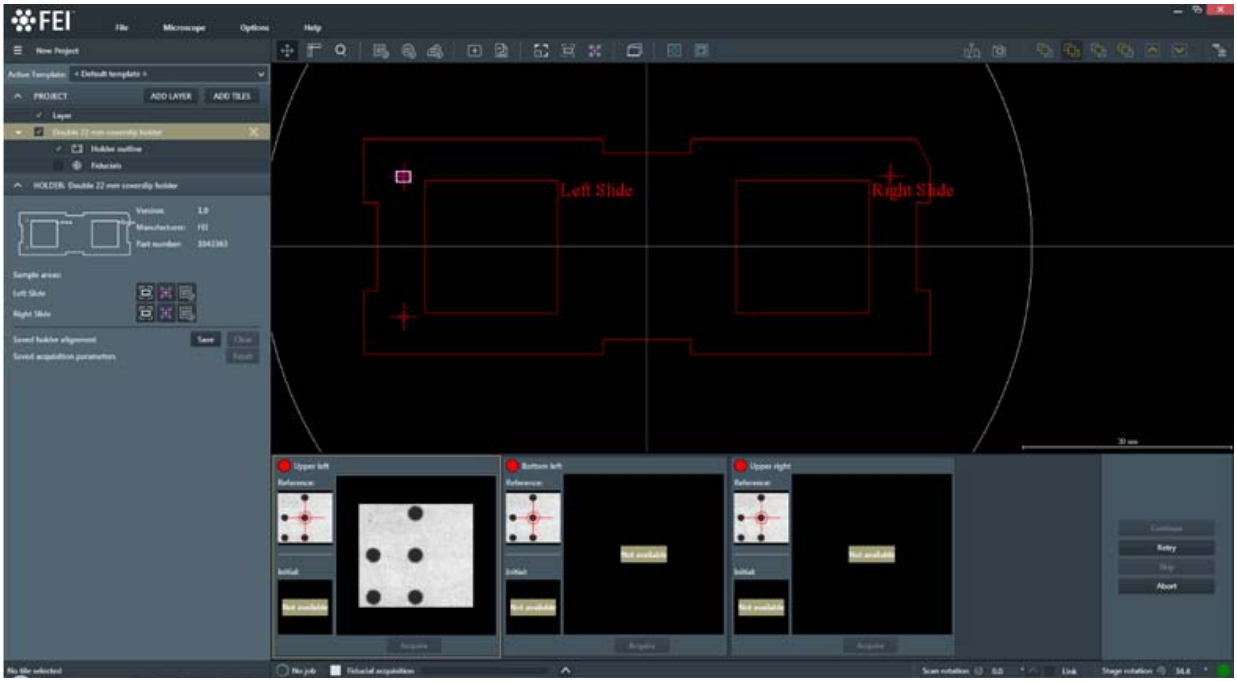
6. Go to the xT UI and find the stored position for upper left fiducial. Select the upper left coordinates and click **GoTo**. The stage navigates to the stored coordinates of the upper left fiducial.

7. Optimize the image (set correct focus and contrast/ brightness) of the upper left fiducial using the xT UI. The optimal image will have completely black fiducial holes against a completely white background. When the image has been optimized, return to the Maps UI and click **Continue**.

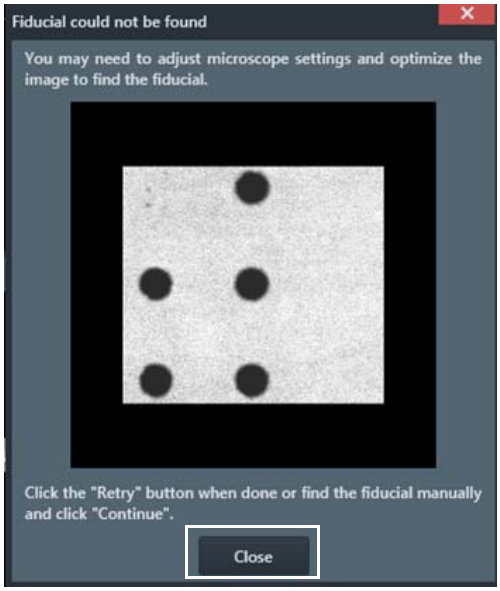
NOTE	If you are having trouble matching, make sure the: ■ Contrast / brightness matches reference ■ Fiducial orientation matches reference ■ HFW is 1.5 mm ■ Sample is clean with clearly distinguishable features and no dust / contamination



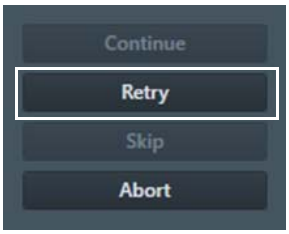
NOTE	During calibration, Maps does not know the orientation of the holder. This can cause false positive matches, as shown in the diagram below. This is normal and these false positives will not occur after the holder has been successfully calibrated.



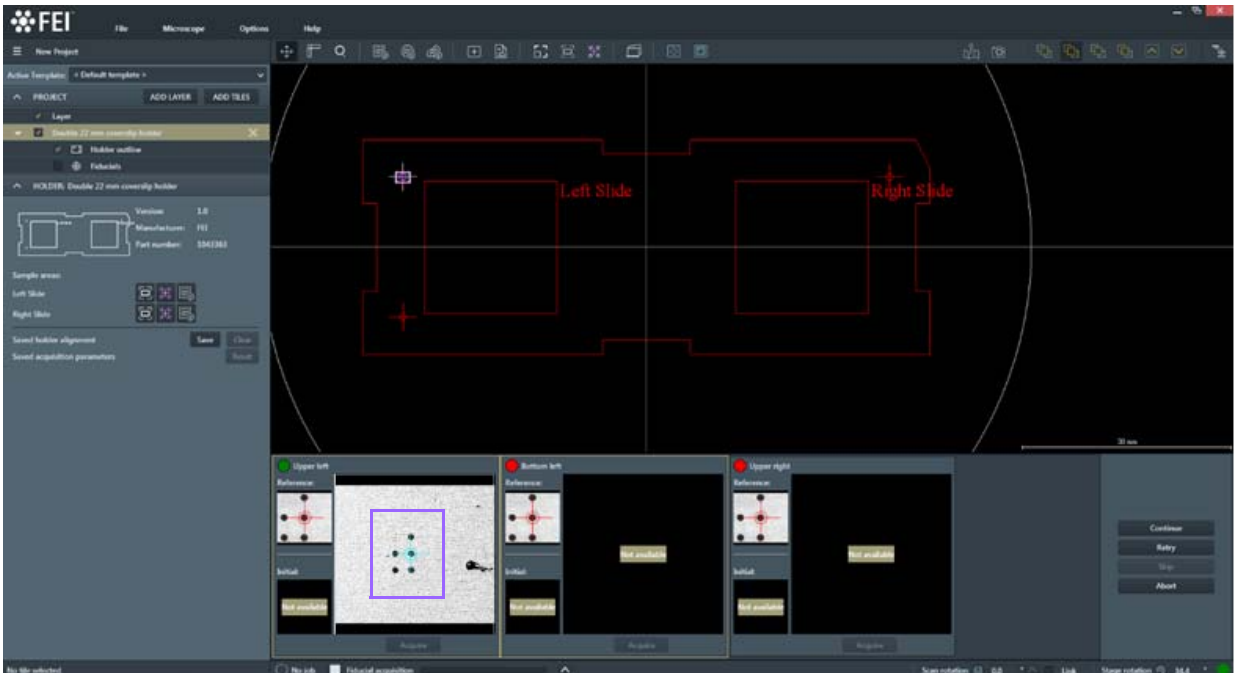
8. The Microscope Settings box displays again if Maps still does not recognize the acquired fiducial. Click **Close**.



9. Optimize the fiducial image again to match the reference image and click **Retry**.



10. If Maps is not able to automatically match the fiducial, double-click to place the blue cross hair on the same fiducial hole that contains the red crosshair in the Reference image.

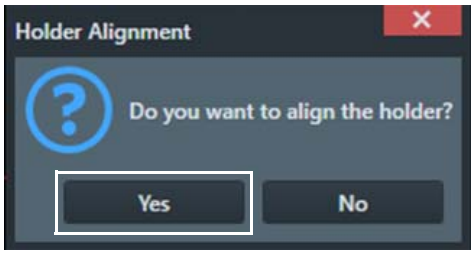


The red button turns green if Maps recognizes the fiducial.

11. If Maps does not automatically navigate to the second fiducial, click **Continue**.

12. Maps will automatically attempt to match the second fiducial. If the match is not successful, repeat the calibration for the second fiducial (starting at *Step 5*). Once the second fiducial is recognized, Maps will automatically navigate to the final fiducial (for holders with more than two fiducials).

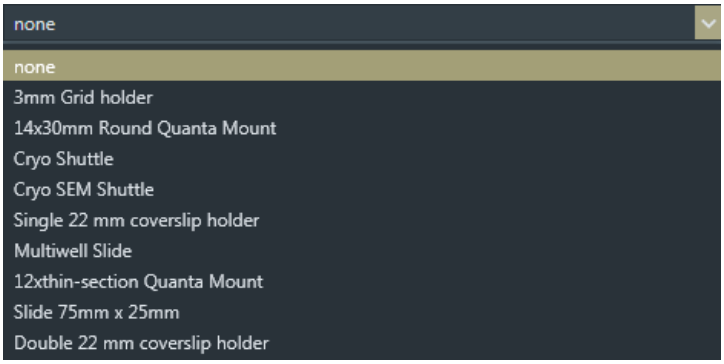
13. Since the automated alignment required manual adjustment, the Calibration prompt appears. Click **Yes** to save the holder calibration.



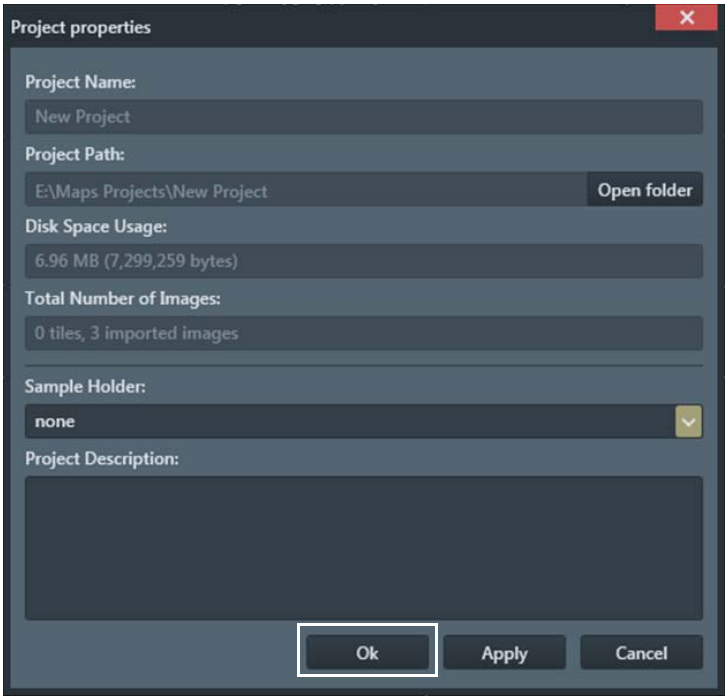
NOTE	If the calibration prompt does not appear, Maps has low confidence in the calibrated fiducial positions and will not allow you to save the potentially bad calibration values. This can occur if there is a large unexpected rotation between fiducials. Perform the calibration a second time, and make sure fiducials are well centered and are oriented properly to match the reference.

Verifying the Alignment

1. Select **none** from the **File** > **Project Properties** > **Sample holder** dropdown.



2. Click **OK** to clear the holder from the project. Click **OK** again at the prompt to clear.



3. Proceed to the procedure, *"Executing the Holder Alignment" on page 172*.

Correlating Projects

Prerequisites for running a multi-tool correlative experiment with Maps using Automated Holder Alignment:

- Sample is mounted on a supported sample holder
- Holder to be used has been calibrated on all systems

NOTE	Manual correlation can be performed on non-supported sample holders using the Maps Global Alignment procedure.

To perform a correlative project:

1. Create a new project in Maps on the first system.
2. Run the AHA (automated holder alignment).
3. Acquire the desired sample data.
4. Close Maps.
5. Make a copy of the entire project directory and move it to the second system's PC.
6. Install the supported holder into the second system.
7. Open Maps on the second system and Import the project.
8. Run the AHA.
9. Acquire the desired sample data.
10. Use alignment to adjust the position of sample data, as desired.
11. Export the results by stitching or saving images to file.

Troubleshooting

Refer to the *Maps Release Notes* for any issues encountered while running Maps.

Problems with Alignment

Misalignment between data in a Maps project can be due to one or more of the following contributing factors:

- Stage positioning error
- Stage drift
- Modified scanning parameters
- Modified column parameters
- Microscope modality change
- Sample moved relative to holder
- Sample changed physically during sample prep
- Misalignment between imaging modes/microscope objectives
- Improper holder calibration or false positives during Automated Holder Alignment

9 Manual Alignment

Overview

This chapter describes manual alignment workflows.

Alignment is used in Maps to adjust project data to the current stage position. For example, this is useful after a sample has been removed and re-installed into a system in a different orientation.

Alignment can be performed on imported images, tile sets, stitched tile set data, layers, and entire projects (Global Alignment). The procedure is identical when aligning each of these different objects.

It is worth noting that objects are never aligned to each other in Maps, but instead are aligned to the current stage position. If two objects do not match up, align the first to the stage, then align the second to the stage. To maintain alignment between objects, place all the aligned objects into a separate layer. Then, you can perform alignment on the Layer and all objects in the layer will be moved in unison.

For Offline alignment where there is no stage present, simply choose one image or tile set as a standard and align all other data to this standard. When the project is reopened on a system, a single Global Alignment will correctly adjust all the project data.

There are four levels of alignment:

- **Align**: Aligns a single tile set or image. Selected from the *"Tile Set Right-Click Menu" on page 46*.
- **Align Layer**: Aligns an entire layer, including all tile sets and images at once. Selected from the *"Layer Right-Click Menu" on page 44*.
- **Global Alignment**: Performs an alignment to align all previously acquired data to the stage. Selected from the *"Tool Bar" on page 30*.
- **Magnification Alignment**: Performs an alignment to align TEM-acquired layers based on magnification. Selected from the *"Layer Right-Click Menu" on page 44*.

Topics in this chapter include:

- *"1-, 2-, or 3-Point (Coarse) Alignment" on page 196*
- *"Fine Alignment" on page 200*
- *"Magnification Alignment" on page 149* (TEM systems only)

1-, 2-, or 3-Point (Coarse) Alignment

Use 1-, 2-, or 3-point alignment to match up a previously acquired image with the current stage position.

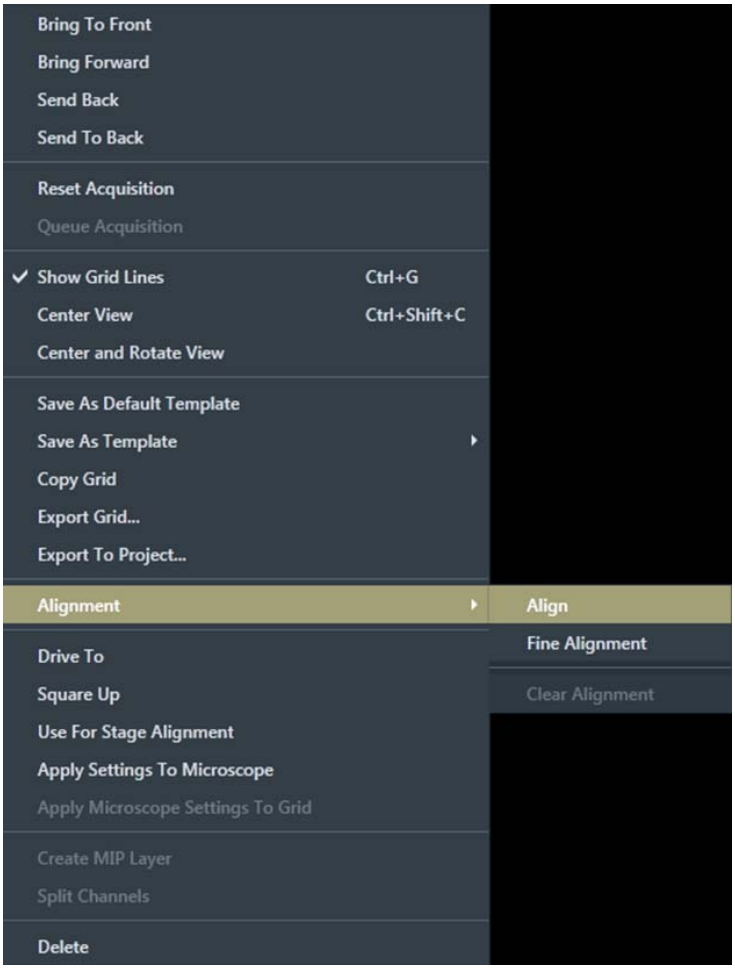
- **1 point:** Adjusts the data using translation only.
- **2 point**: Adjusts the data using translation, rotation, and scale.
- **3 point**: Adjusts the data using translation, rotation, scale, and orthogonality.

Workflow

The workflow described below relates to aligning a single tile set. The same procedure is used for all alignments in Maps.

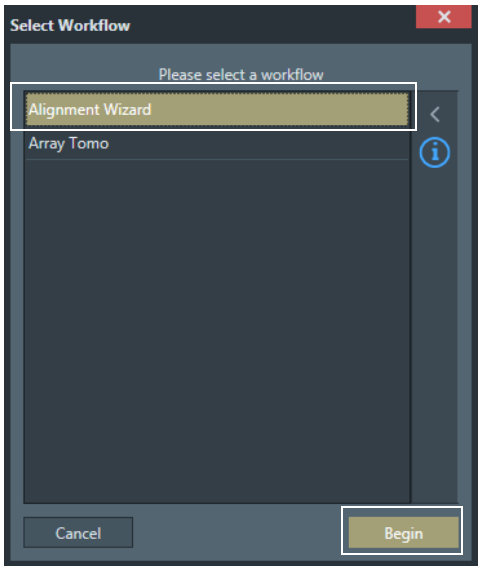
From Tile Set:

1. Acquire a tile set.
2. Right-click on the tile set in the Layer control and select **Alignment > Align**.

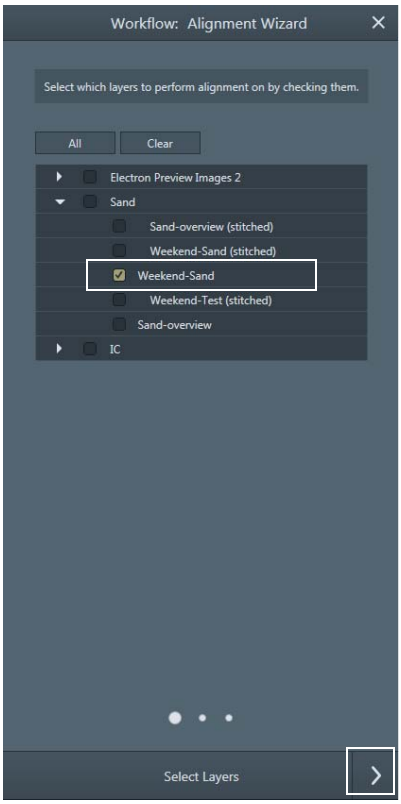


From Workflow:

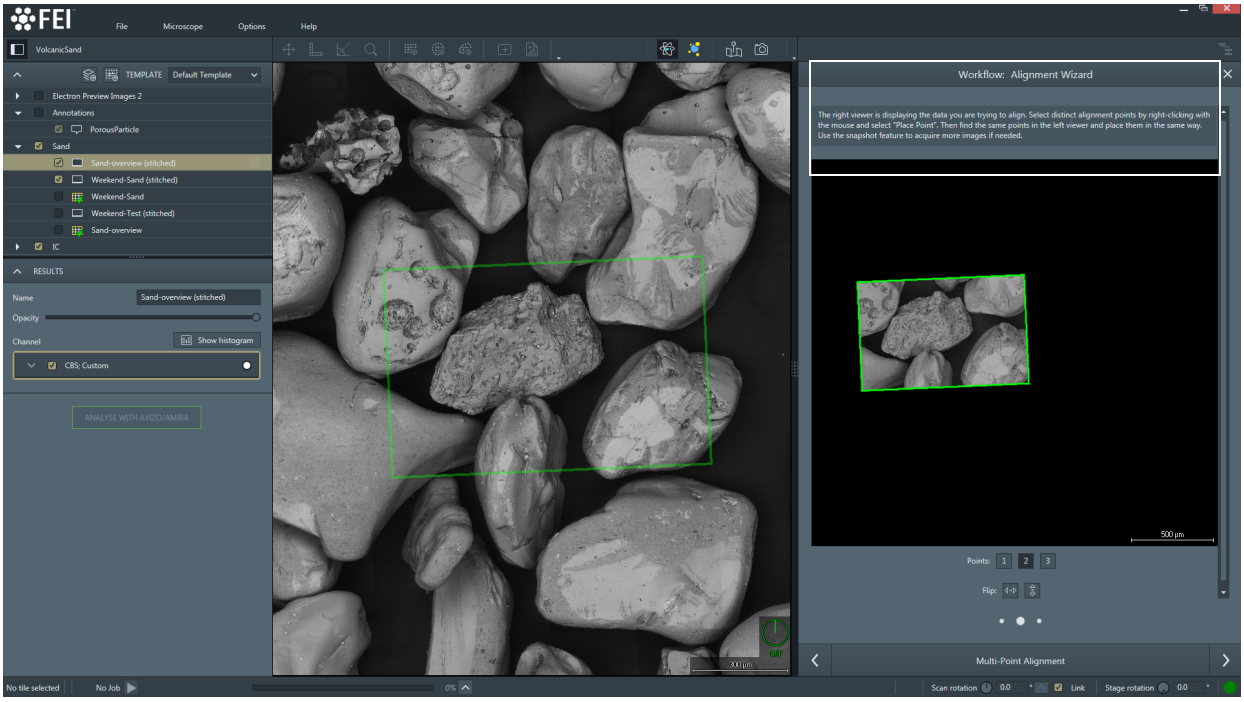
1. Click the workflow button and clickA**lignment Wizard > Begin.** The workflow panel opens and lists layers for alignment.



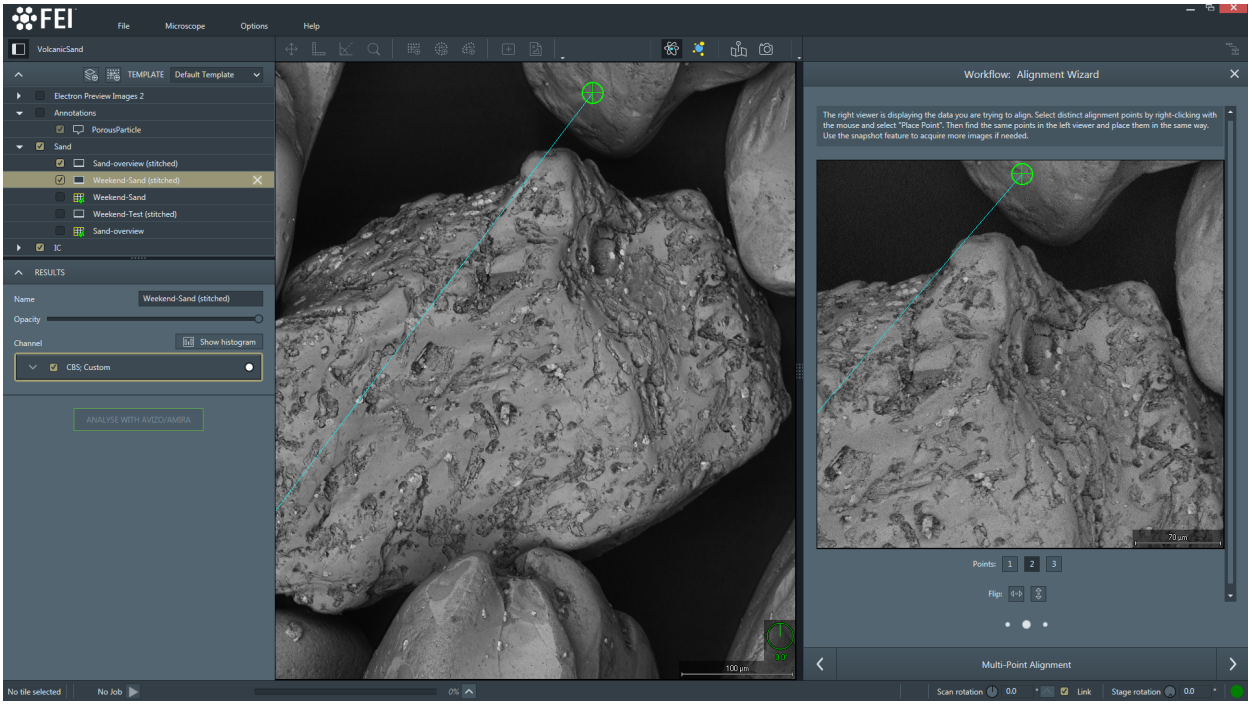
2. Select an acquired tile set to align.
3. Click the right arrow button at the bottom of the workflow panel to go to the next step.



4. Follow the updated instructions in the workflow panel.



5. Follow the guided instructions in the workflow panel to find the same distinct feature in both windows and place Point 1, 2, or 3 in each window. You might have to zoom in close on a feature and grab a new preview image with more resolution to make the best match.



6. Click **Flip** horizontal or vertical to flip the image horizontally or vertically.

7. Select 1, 2, or 3 point alignment.
8. Click the right arrow button at the bottom of the workflow panel to enter Fine Alignment.

Fine Alignment

Use Fine Alignment to fine-tune the alignment of layers and tile sets visually.

The Fine Alignment controls appear when you do the following:

- Click the right arrow button at the bottom of the workflow panel after a 1-, 2-, or 3-point alignment.
- Right-click the Tile Set in the Layer control and then click **Alignment > Fine Alignment**.

Table 1 Fine Alignment Overview

Interface Item	Description
Sensitivity Slider	Sets the sensitivity for all of the fine alignment controls: Left = fine; Right = coarse.
Scale Up/Down Arrows	Scales the image up or down.
Up, Down, Left, Right, Up-Left, Up-Right, Down-Left, Down-Right Arrows	Moves the image in the direction of the arrow.
Rotate Right, Rotate Left Arrows	Rotates the image in the direction of the circular arrows.
Compucentric Check Box	• When selected (the default), performs compucentric rotation. The image is pivoted around its center. • When not selected, the image is pivoted around the center of the stage.
Back Arrow	Returns to the selection of alignment points in the coarse alignment workflow. From there, click **Cancel** if you want to undo the coarse alignment.
Finish Check Mark	Closes the Fine Alignment workflow panel.

10 Correlation

Overview

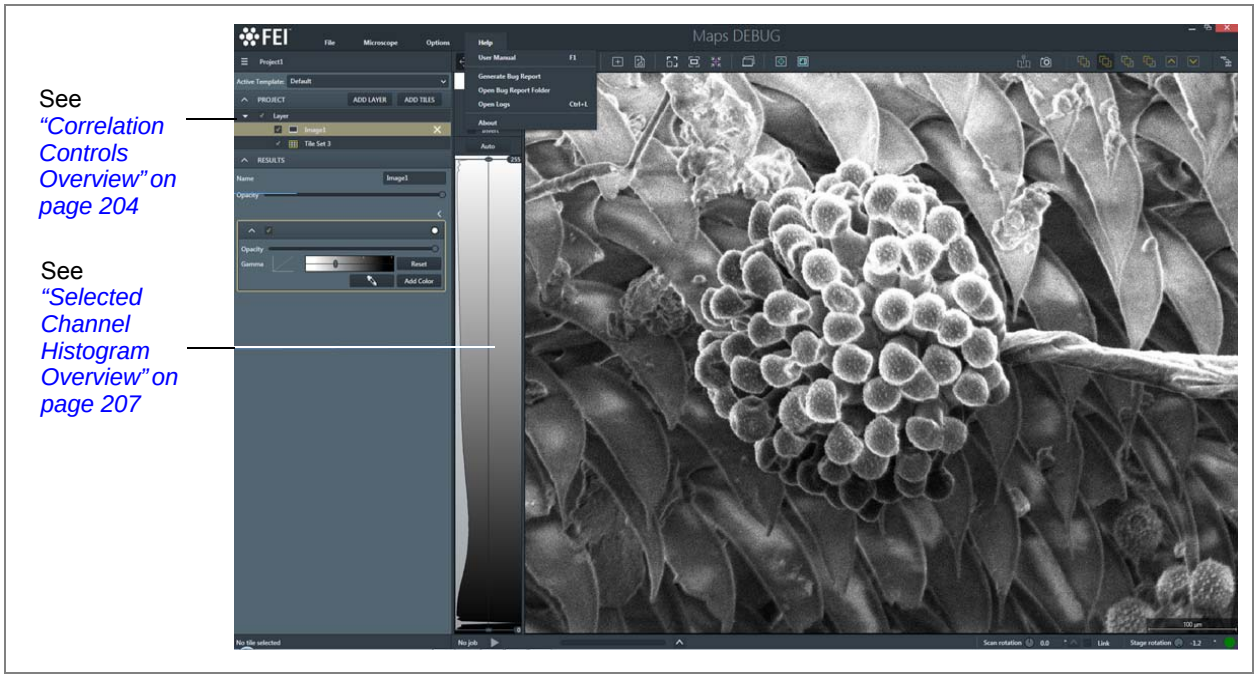
This chapter describes the controls on the Correlation tab to apply simple coloring to the tile set images when they are acquired. This can be used to highlight some features for demonstration or evaluation purposes.

Topics include:

- *"Correlation Controls" on page 202*
- *"Segmentation" on page 208*

Correlation Controls

Figure 1 Correlation Controls



These controls offer post-processing capabilities for multichannel and single channel images.

Figure 2 Correlation Controls

See *"Correlation Controls Overview" on page 204*

See *"Histogram" on page 206*

Table 1 Correlation Controls Overview (1 of 2)

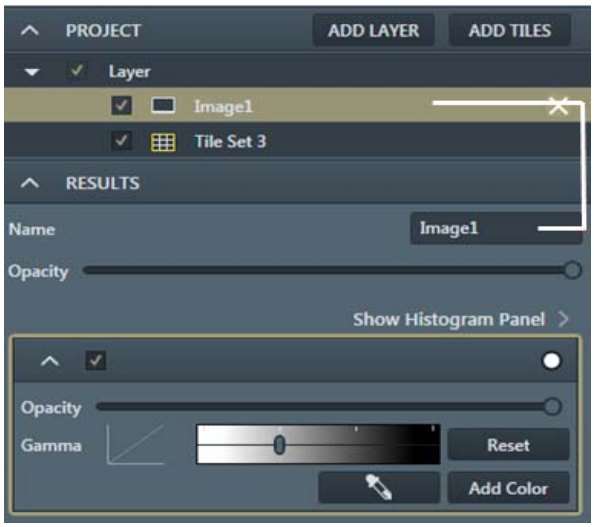
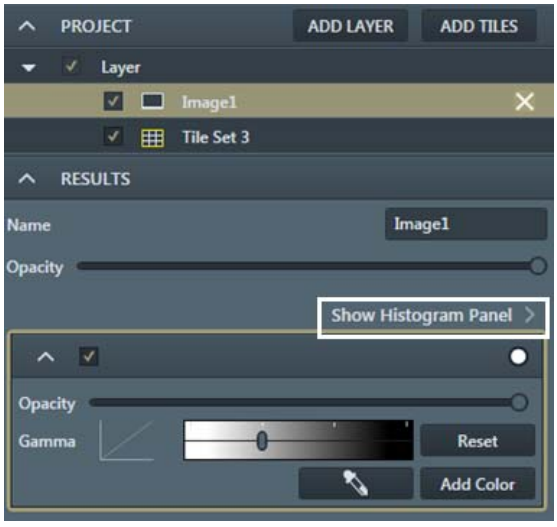
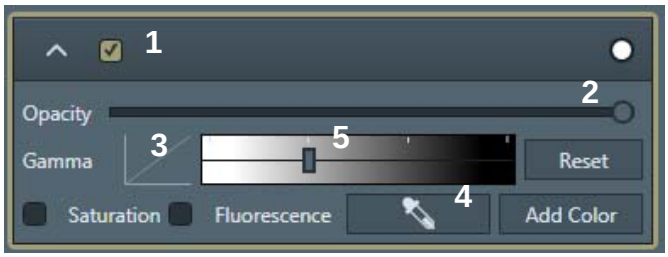
Interface Item	Description
Name	Displays the selected layer item (a tile set or image) from the Layer control. 
Opacity	Sets the measure of opaqueness for the entire image. The less opaque, the more transparent it becomes. Transparent images are useful when overlaying on image over another.
Expand/Collapse Histogram View	Shows/hides the histogram. See *"Histogram" on page 206*. 

Table 1 Correlation Controls Overview (2 of 2)

Interface Item	Description
Channels with Opacity slider and Gamma adjuster	 1. Turns on/off display of that channel 2. Channel opacity slider (adjusts opacity for this channel only) 3. Graphical display of the gamma curve adjustment 4. Channel color selector 5. Gamma slider
Saturation	Select this check box to display saturated pixels in yellow.
Fluorescence	Select this check box to designate this channel as fluorescent. Fluorescent channels are drawn differently; black is considered transparent and colors are added with other channels/images behind it.
Add Color	Launches Segmentation. Displays an additional histogram every time you click the button. See *"Segmentation" on page 208*.
Channel color selector	Select the box to the left of **Add Color** to display the color picker. Choose a unique color for each channel to display in the histogram.

Histogram

Displays a histogram for the selected channel. Click a different channel name to select its histogram and associated color.

Figure 3 Selected Channel Histogram

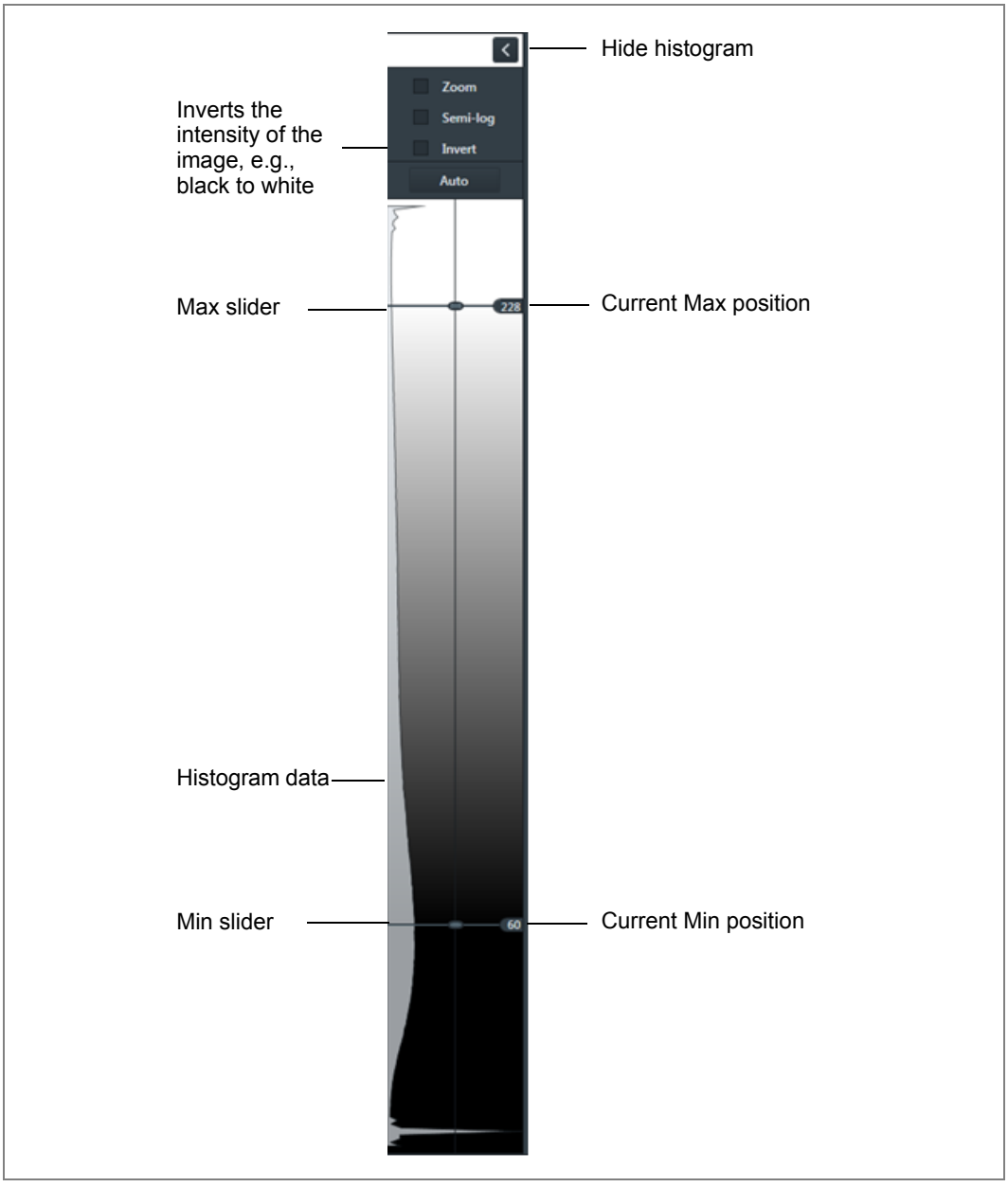
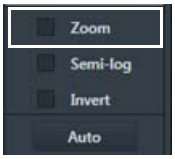
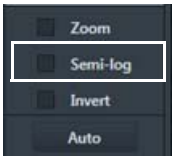
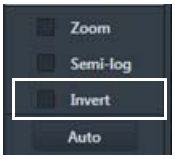
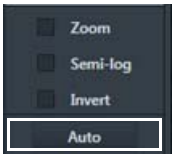


Table 2 Selected Channel Histogram Overview

Interface Item	Description
Selected channel name	Displays the selected layer item (a tile set or image) from the Layer control.
Hide	Hides the histogram. 
Zoom	Expands the histogram area of images with less than 255 colors. 
Semi-log	When selected, changes the histogram intensity display to logarithmic. 
Invert	Inverts the intensity of the image, e.g., black to white. 
Auto	When selected, continuously adjusts the min and max for the histogram display. 
Current max position	Displays the current max slider position.
Max slider	Any data above the max line is white. Gray levels display between the max and min sliders.
Histogram data	Displays the histogram data for the selected channel.
Min slider	Any data below the min line is black. Gray levels display between the max and min sliders.
Current min position	Displays the current min slider position.

Segmentation

Topics include:

- About, below
- Add Color, below
- *"Segmentation Controls Overview" on page 212*

About

Image segmentation is the process of partitioning a digital image into sets of pixels, called segments. The goal of segmentation is to simplify and/or change the representation of an image into something that is more meaningful and easier to analyze.

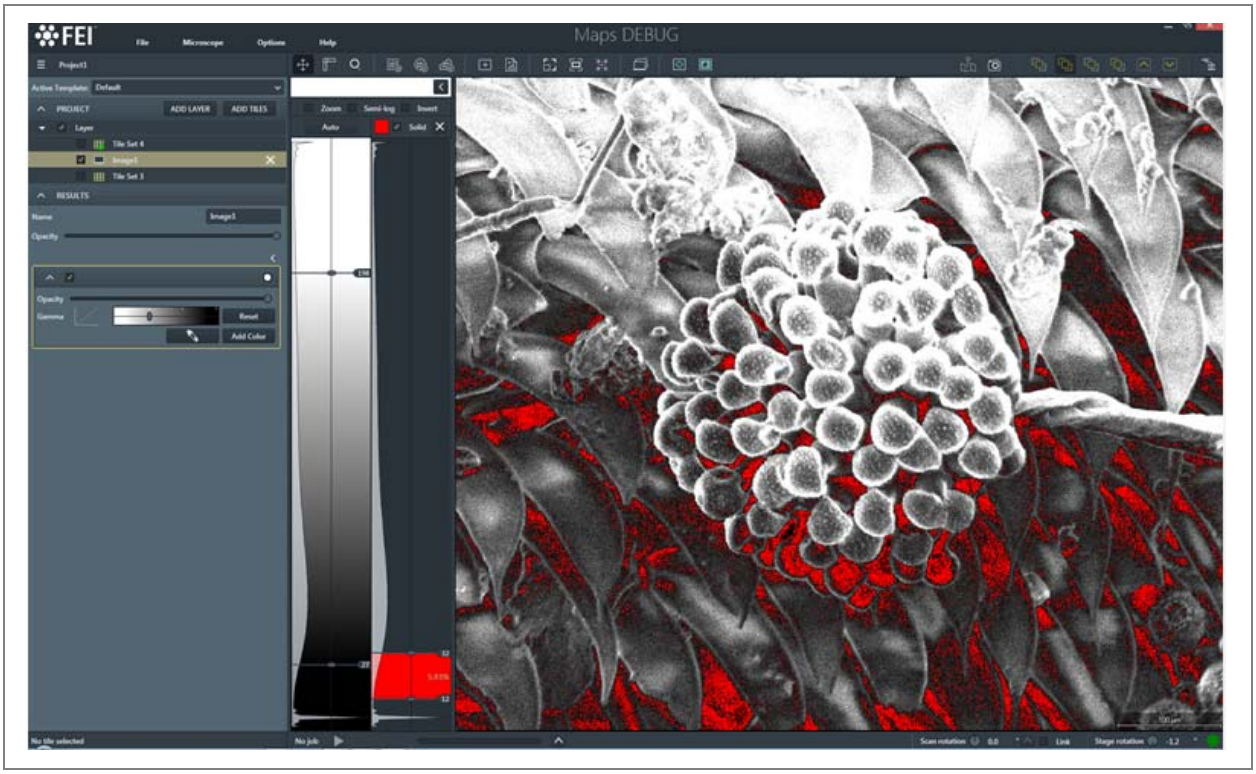
In Maps, images and tile sets can be segmented such that each segment is assigned a color. Segments are defined using the image histogram and a min/max slider that determines the regions of the image to be included in the segment. Additionally, Maps automatically calculates the number of pixels within the segment as a percentage of the total number of pixels and displays the result.

Add Color

1. Click **Add Color** to add an additional image segment.

2. Choose a color from the **Color Selector** and select the **Solid** check box. When not selected, the color will be a gradient. See *"Segmentation, One Color with Gradient" on page 211*.

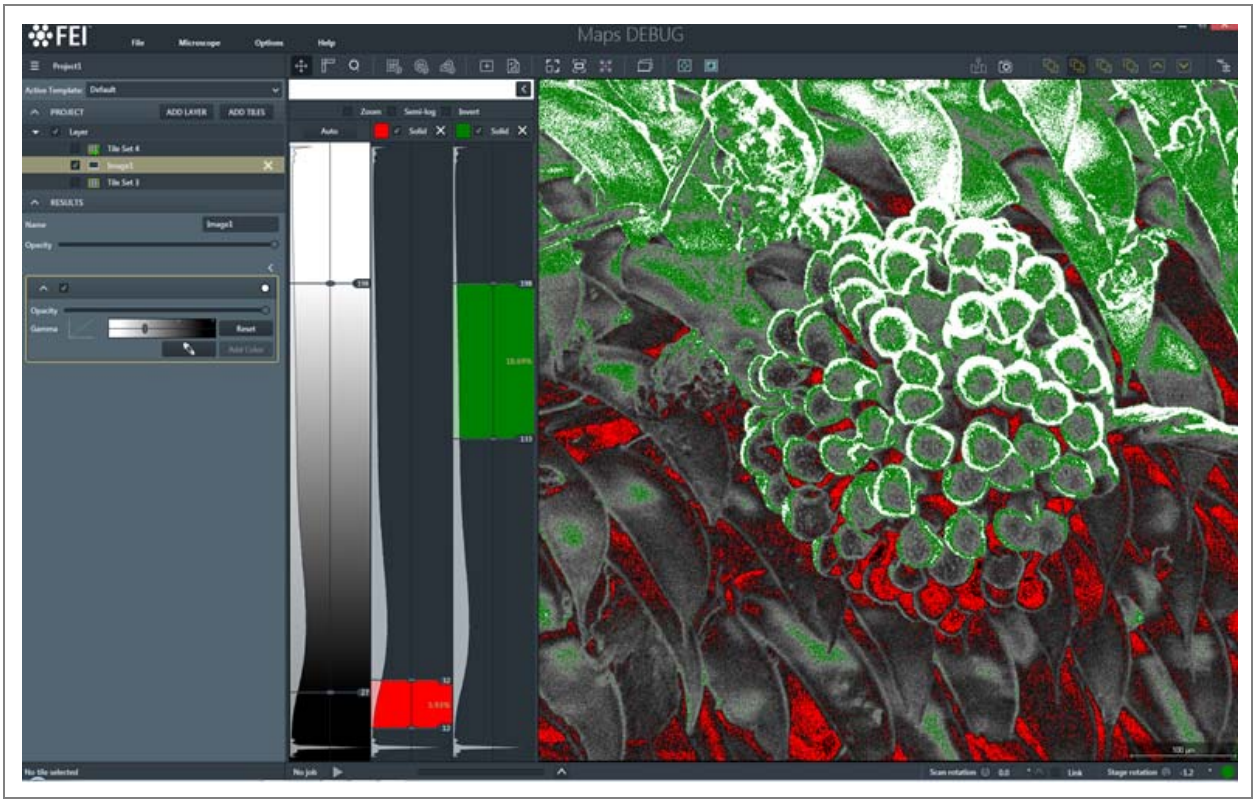
Figure 4 Segmentation, One Color



3. Adjust the **Max** and **Min** sliders to change the range of the histogram data to be assigned to the segment.

4. Click **Add Color** again to add another image segment. In this example, the color green and the **Solid** check box were selected.

Figure 5 Segmentation, Two Colors

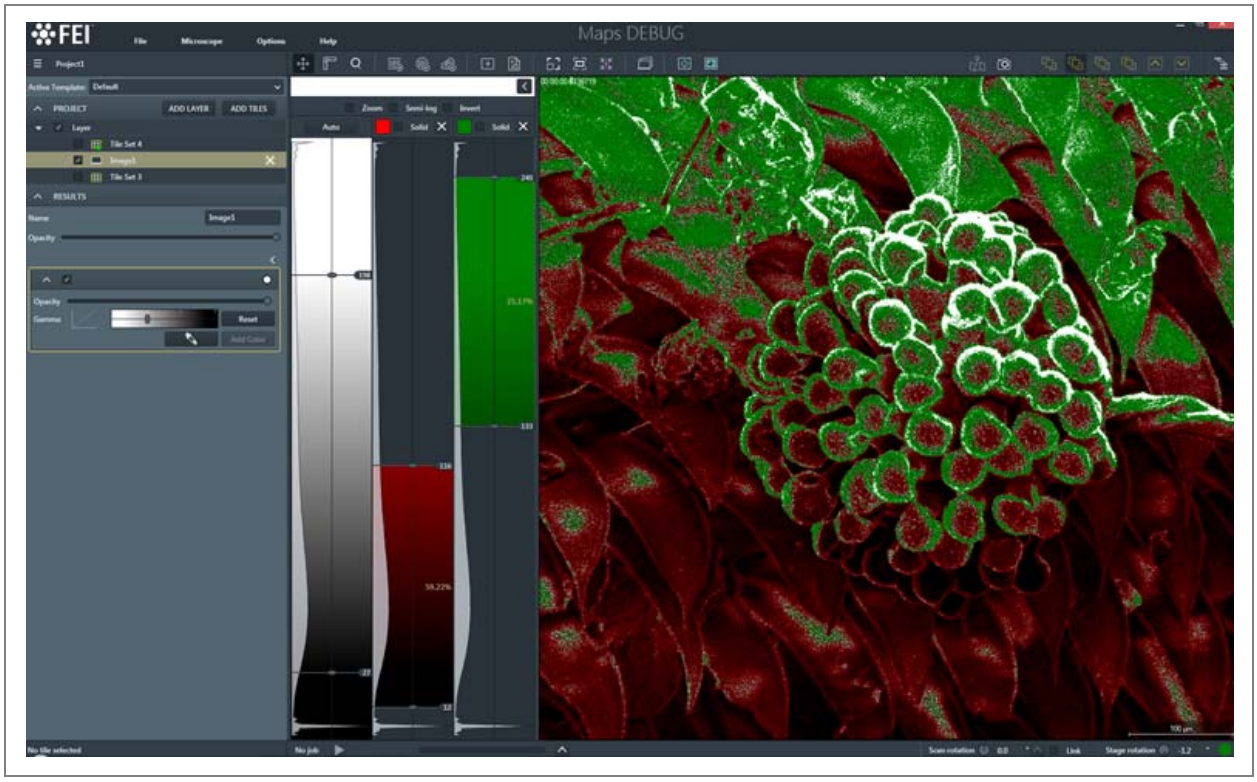


5. Adjust the **Max** and **Min** sliders to select a different range of the histogram data to be assigned to the second image segment.

Segmentation, One Color with Gradient

When the **Solid** check box is **not** selected, the histogram fill color is a gradient from light to dark.

Figure 6 Segmentation, One Color with Gradient



Segmentation Controls Overview

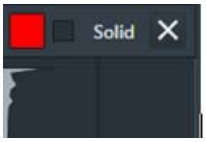
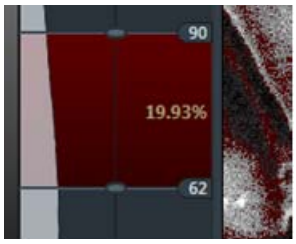


Table 3 Segmentation Controls Overview

Interface Item	Description
Remove	Removes the segment from the image and returns to normal display. 
Color Picker	Displays the color picker for selecting a color, then colors the area between the **Min** and **Max** sliders with the selected color.
Solid	When selected, the data between min and max on the Segmentation histogram will be one color instead of a gradient.
%	Calculates the number of pixels between min and max as a percentage of the entire image. 

11 Maps-Avizo/Amira Connectivity Bridge

Overview

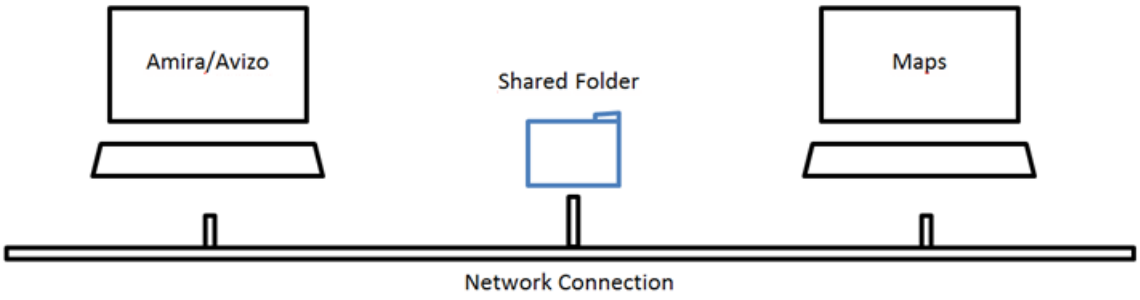
This chapter describes how to set up and use the link between Avizo/Amira and Maps.

Topics include:

- Prerequisite, below
- *"Defining Shared Folder" on page 214*
- *"Avizo/Amira Settings" on page 217*
- *"Connection Messages" on page 218*
- *"Sending an Image to Avizo/Amira" on page 219*
- *"Analyzing with Avizo/Amira" on page 220*
- *"Receiving AOI from Avizo/Amira" on page 221*

Prerequisite

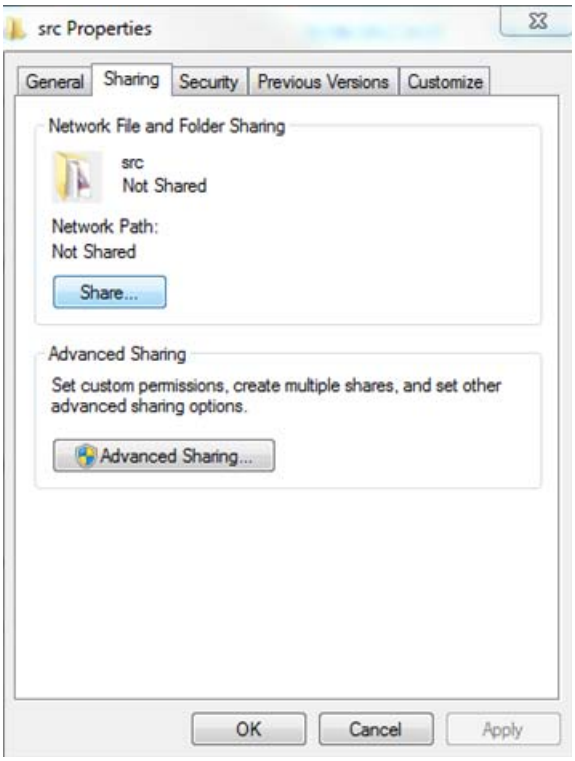
Maps 3.3 and Avizo 9.3 or Amira 6.3 are installed. They must be on the same network.



Defining Shared Folder

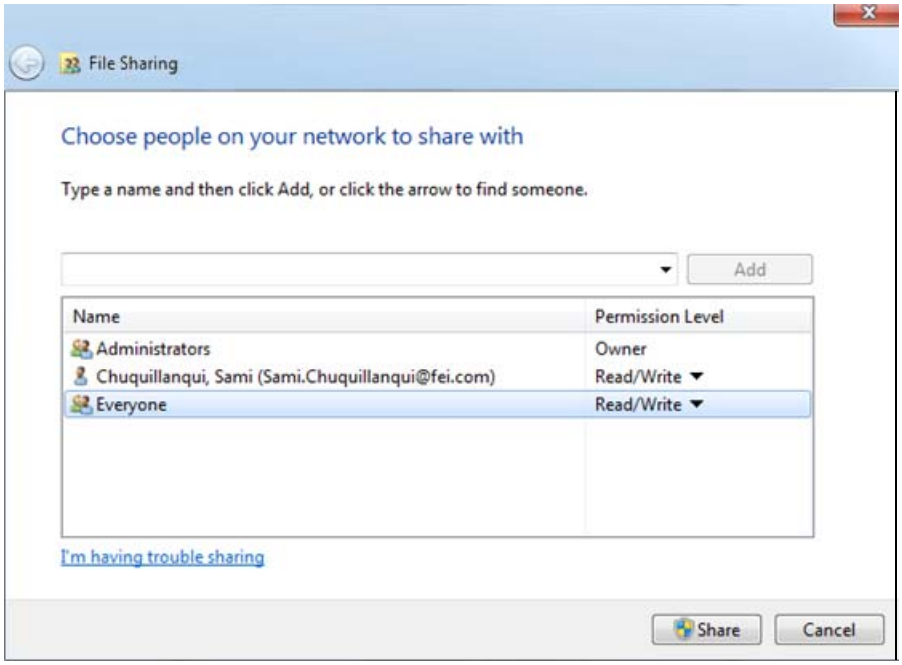
A shared folder is necessary to allow data exchanges. This folder can be located in any place, as long as it is accessible to both software applications. It can be located on one of the two computers.

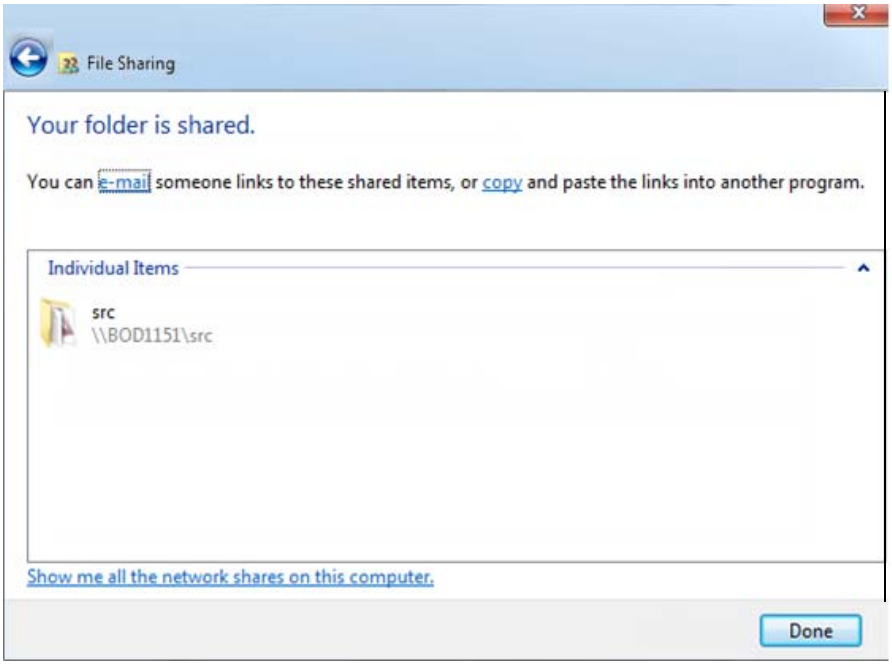
1. To share a folder, determine the folder you want to share.
2. Right-click on the folder and click **Show Properties**.



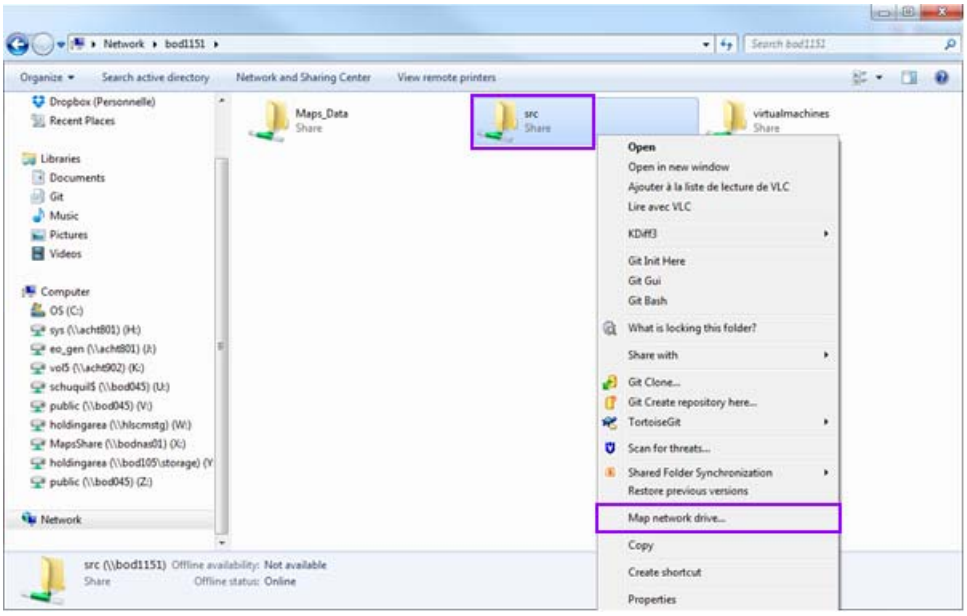
3. Click **Share** in the **Properties** dialog box.

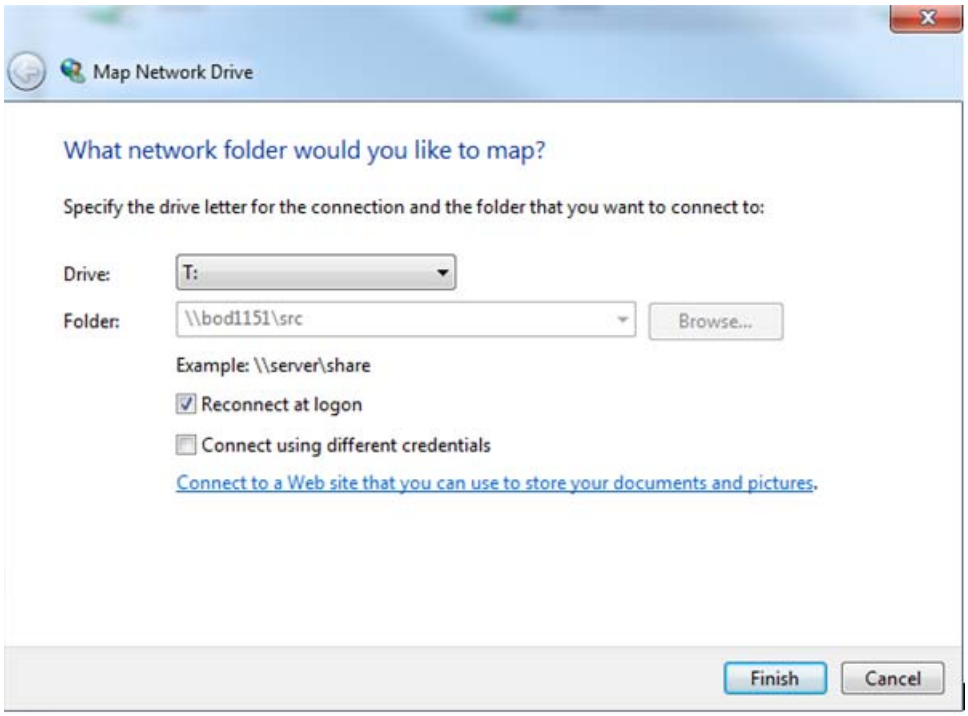
4. Select **Everyone** for Name and **Read/Write** for Permission Level and click **Share**.





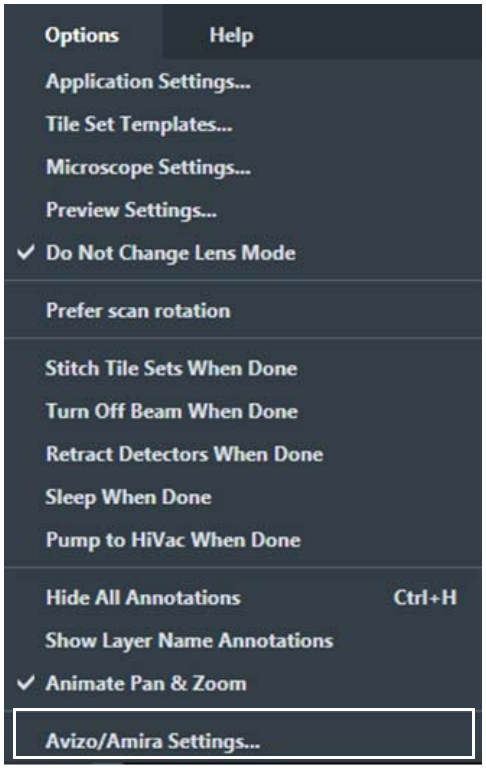
5. Right-click on the shared folder and select **Map Network Drive** to define the shared folder as a network drive to simplify the access to this folder.





Avizo/Amira Settings

Select **Options** > **Avizo/Amira Settings** to display the settings window. Filling those settings is mandatory before using the feature.



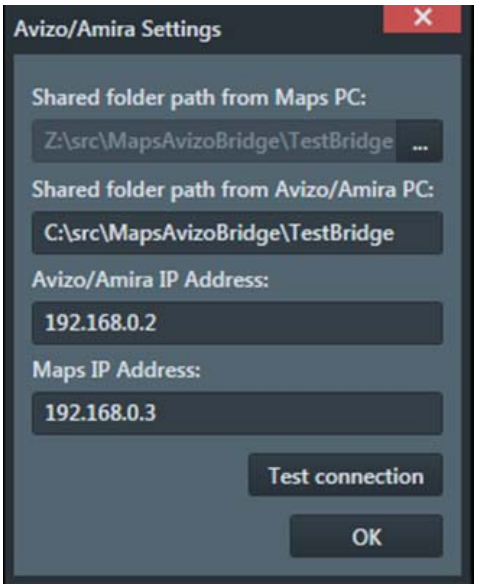


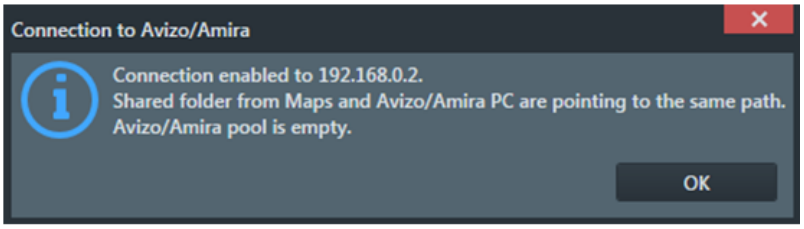
Table 1 Avizo/Amira Settings Overview

Interface Element	Description
Shared folder path from Maps PC	Browse to the shared folder. The selected path must be correct for usage on the Maps PC.
Shared folder path from Avizo/Amira PC	Input the access path to the shared folder. This path must be correct for usage on the Avizo/Amira PC.
Avizo/Amira IP Address	Displays the IP address of Avizo/Amira PC. This input field must be filled-in.
Maps IP Address	Displays the IP address of Maps PC. This input field must be filled-in.
Test connection	Checks the current connection between the Avizo/Amira application and Maps PC.

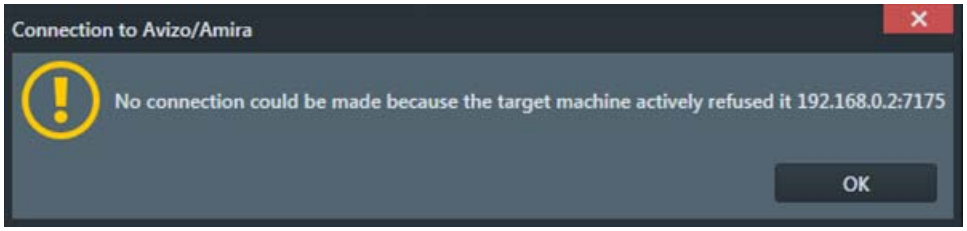
Connection Messages

Connection messages indicate:

- The status of connection with Avizo/Amira
- Maps and Avizo/Amira are using the same Shared folder
- The status of Avizo/Amira pool

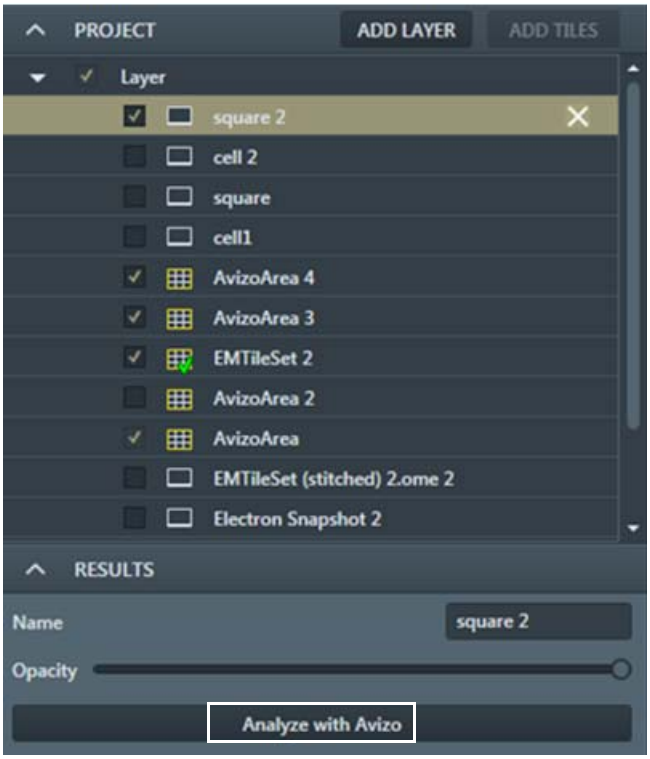


If the Avizo/Amira host IP address is incorrect, or the Avizo/Amira application is not launched, the following message is displayed.

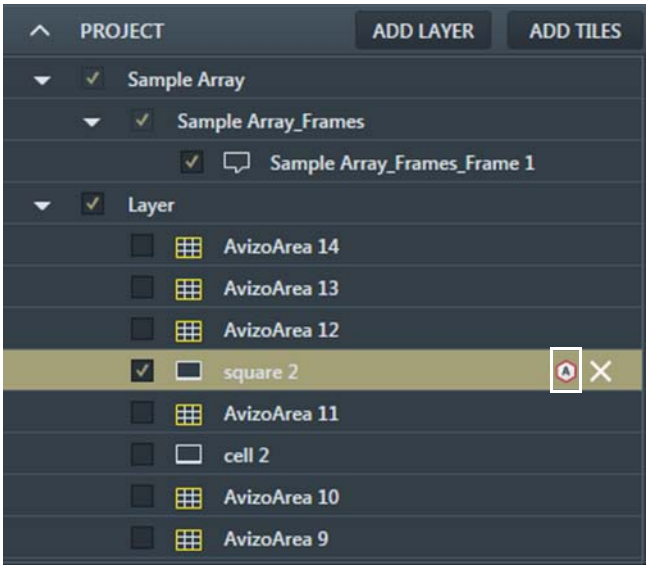


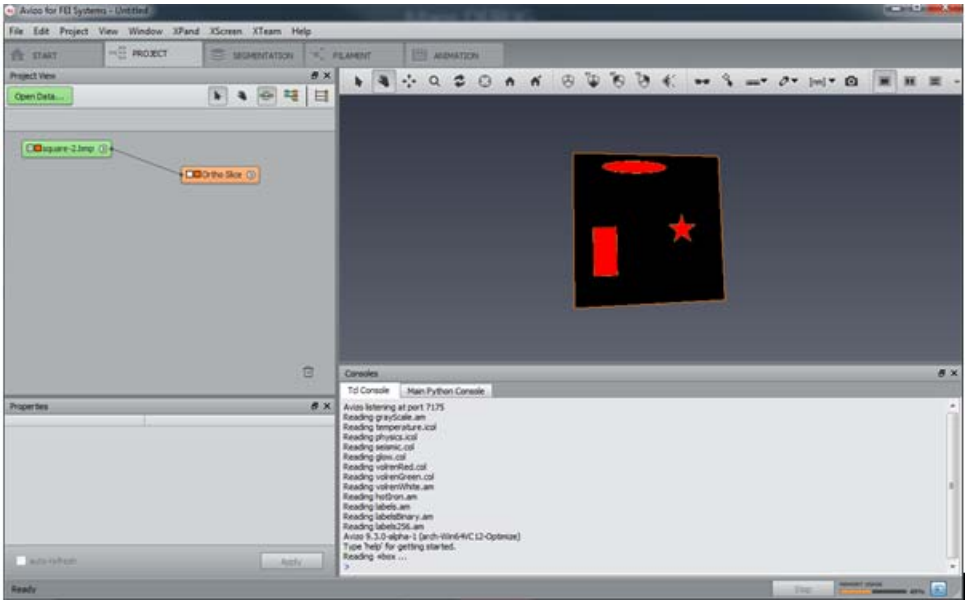
Sending an Image to Avizo/Amira

1. Select an image layer and click **Analyze with Avizo**.



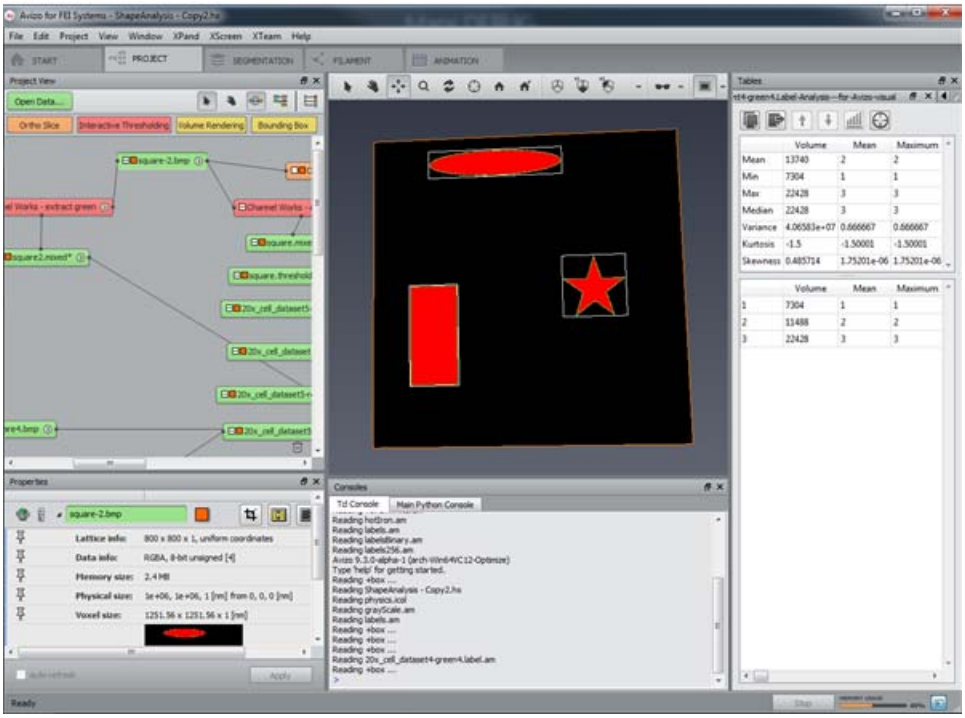
The image layer is displayed into Avizo/Amira. In the Layer control, an icon indicates which image layer is currently analyzed by Avizo/Amira.





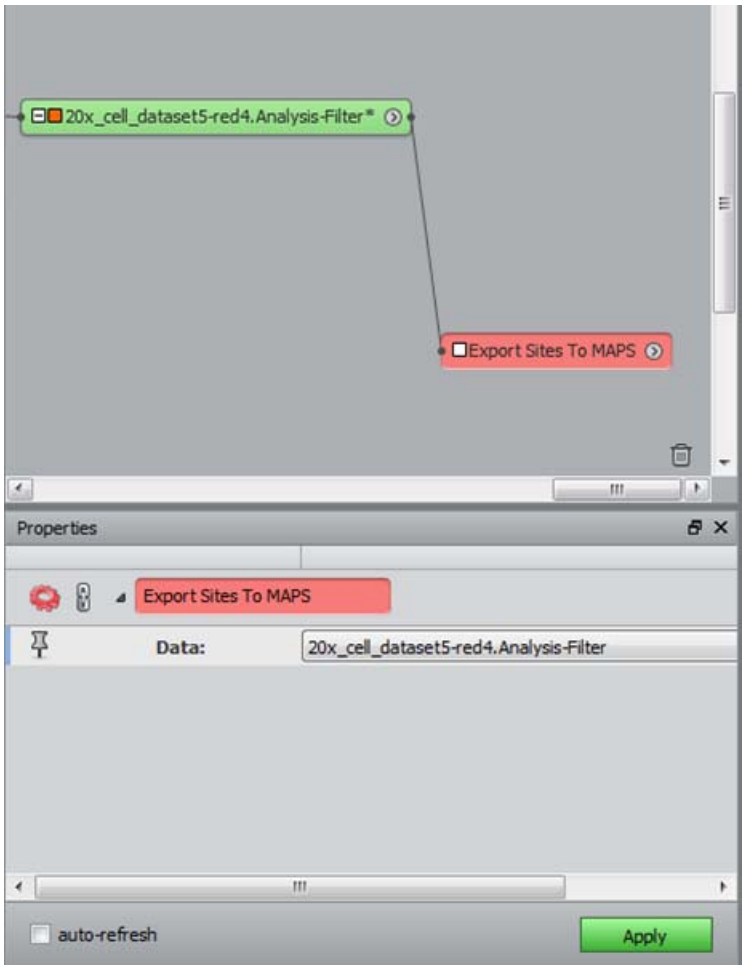
Analyzing with Avizo/Amira

From the displayed layer, you can create an analysis workflow.

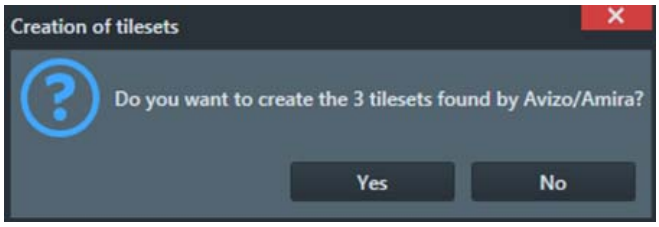


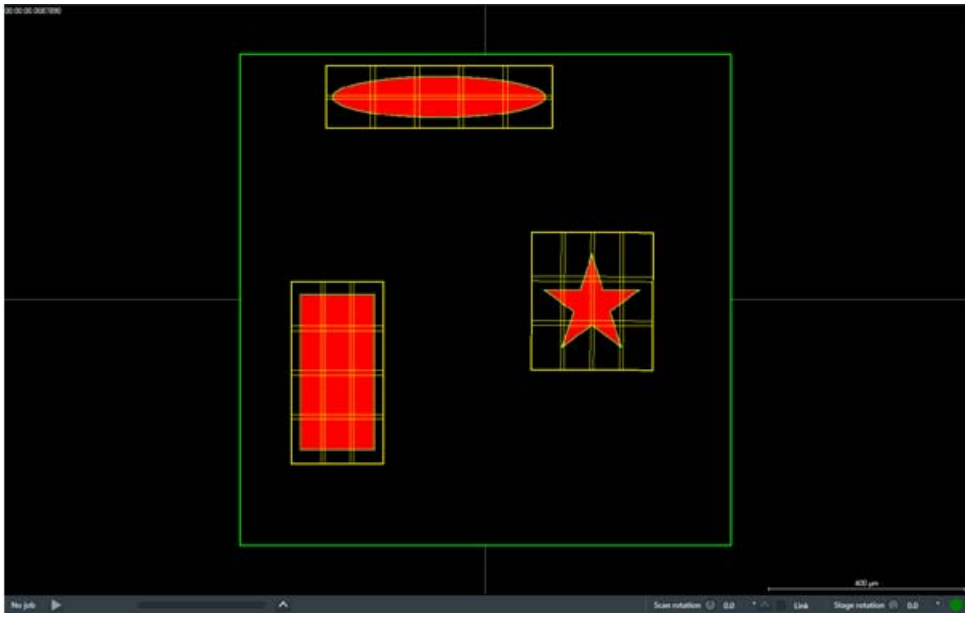
Receiving AOI from Avizo/Amira

In Avizo/Amira, when the specific module **Export Sites to Maps** is executed, the regions found are sent to Maps.

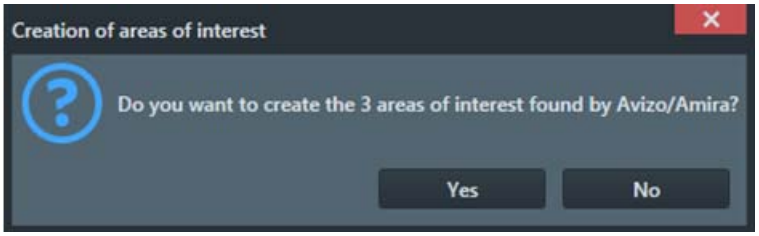


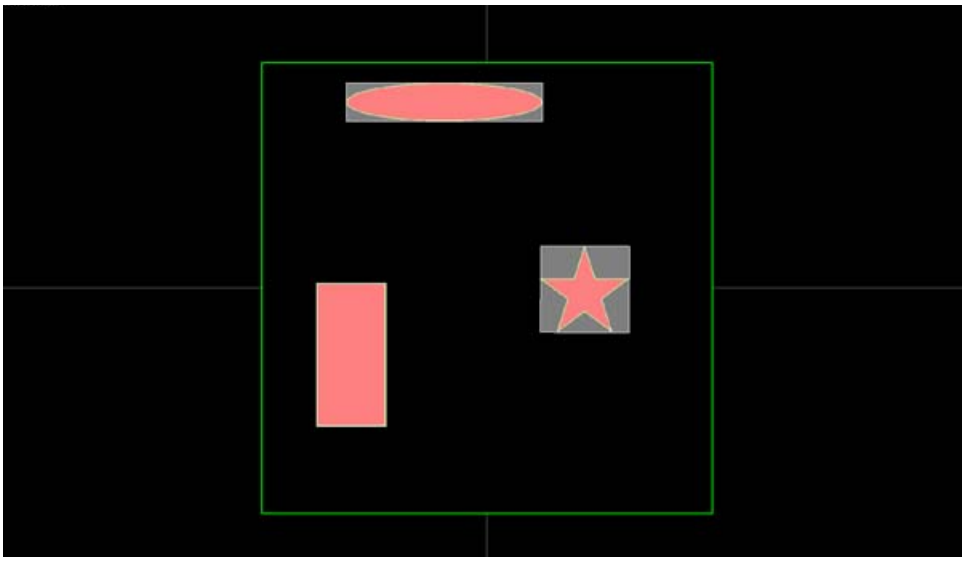
When connected to a microscope, Maps creates tile sets covering the regions sent by Avizo/Amira, using the current Active template.





In offline mode, Maps creates Areas of Interest instead of tile sets.





12 Array Tomography

Overview

This chapter describes how to create an Array Tomography (AT) job using Maps software. Maps AT functionality requires an active AT license.

Topics include:

- *"About Array Tomography" on page 223*
- *"Terminology" on page 224*
- *"Array Tomography Workflow" on page 225*
- *"Extended Controls" on page 242*

About Array Tomography

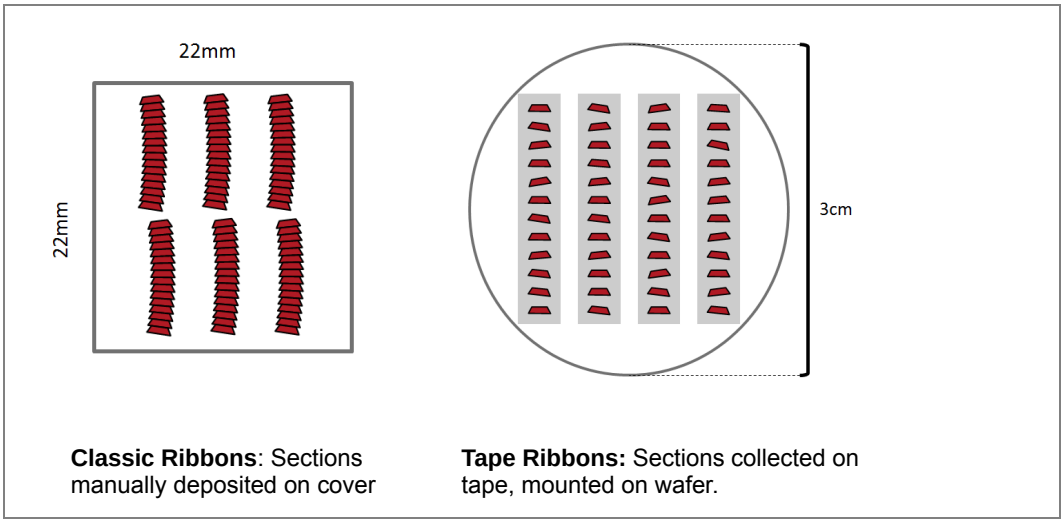
AT is an imaging technique in which 3D image data is reconstructed from serial recordings on serial sections that are spread out on a large sample carrier.

Samples

Samples for AT are produced by serial sectioning of a plastic-embedded sample in a microtome. Sections are deposited on ITO-coated glass substrates (that is, 22 x 22mm cover glasses) or on wafers.

Depositing samples is done manually or by a tape collection device (that is, ATUMtome). The resulting AT samples differ fundamentally:

Figure 1 Classic and Tape Ribbons



Sample Sections and Tile Set Arrays

The main purpose of the AT feature is to easily align high-resolution imaging on areas of interest on all sample sections. The AT feature set consists of a new concept called a **Tile Set Array**. The **Tile Set Array** sets up local coordinate systems around the individual sample sections to create a collection of tile sets across *all* sample sections.

Terminology

- **Classic Ribbons**

  Sample sections are attached to each other and the ribbon is always slightly bent. The angle between each section is constant, for the most part.

- **Serial Section Array**

  The layer group that contains tile set arrays and section outlines. The purpose of the group is to easily contain most of the array tomography data about a sample in one section of the Layer tree.

- **Tape Ribbons**

  Sample sections are regularly spaced and the angle between each slice varies slightly.

- **Tile Set Array**

  A collection of tile sets that all share the same acquisition parameters, but can differ in location and rotation.

- **Workflow**

  A series of steps that are necessary to complete a task.

Workflow Tool Bar

Figure 2 Workflow Tool Bar Buttons

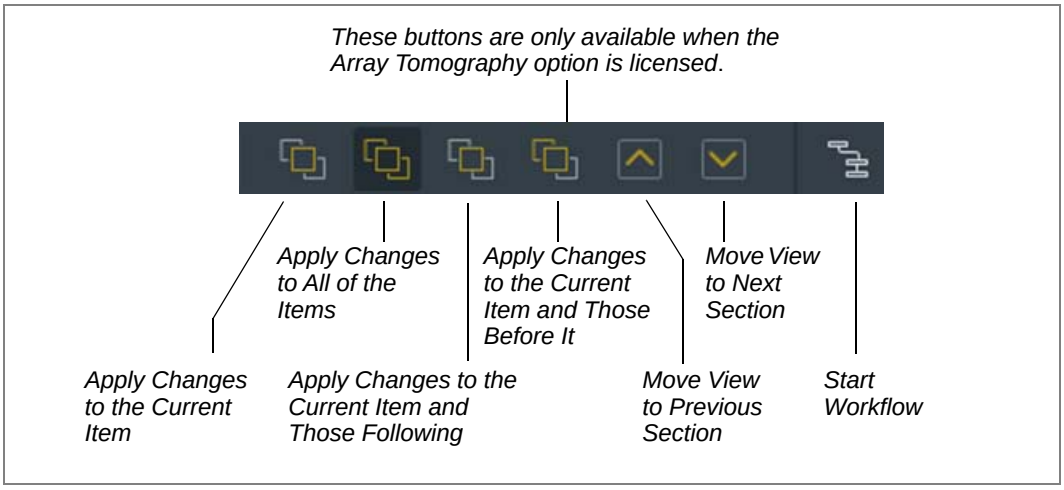


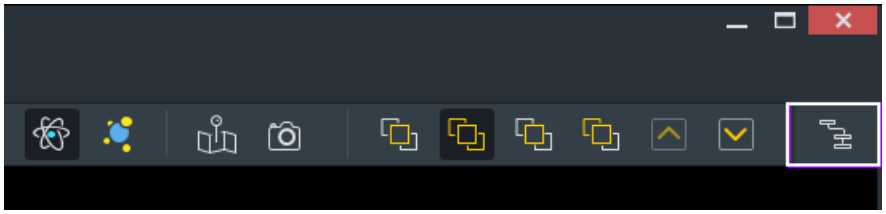
Table 1 Workflow Tool Bar Descriptions

Tool	Description
Apply Changes to the Current Item	Applies changes only to one (selected) tile set.
Apply Changes to All of the Items	Applies changes to all tile sets within the array.
Apply Changes to the Current Item and Those Following	Applies changes to the current tile set and all those following it in the array.
Apply Changes to the Current Item and Those Before It	Applies changes to the current tile set and all those before it in the array.
Move View to Previous Section	*(Enabled when you select a tile set from an array or a section.)* Navigates to the previous section to view the newly acquired data.
Move View to Next Section	*(Enabled when you select a tile set from an array or a section.)* Navigates to the next section to view the newly acquired data.
Start Workflow	Begins the step-by-step workflow sequence.

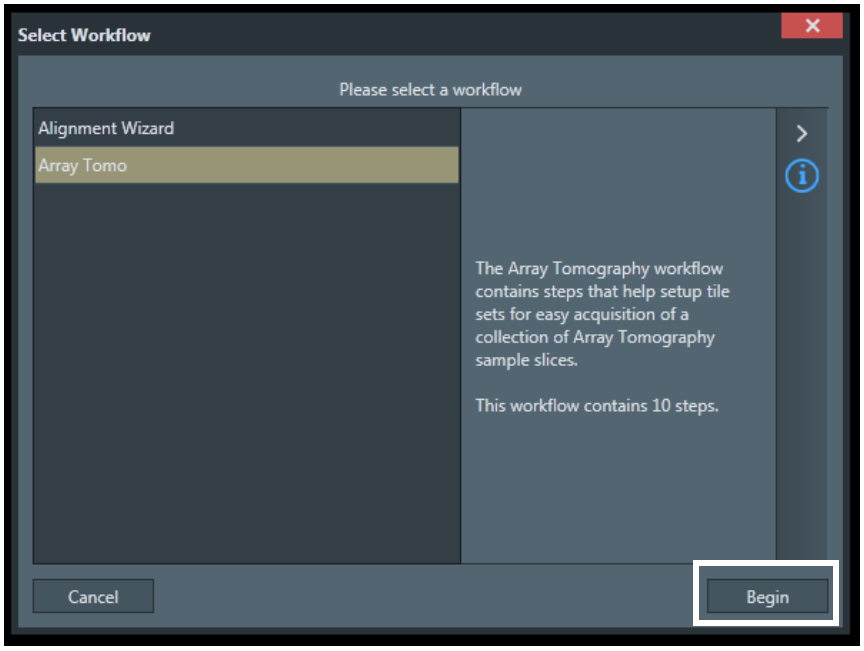
Array Tomography Workflow

An AT job is a new workflow used within the Maps Workflow panel. The workflow panel is a control that contains step by step processes that help make complicated jobs easier. The workflows available depend on your product license.

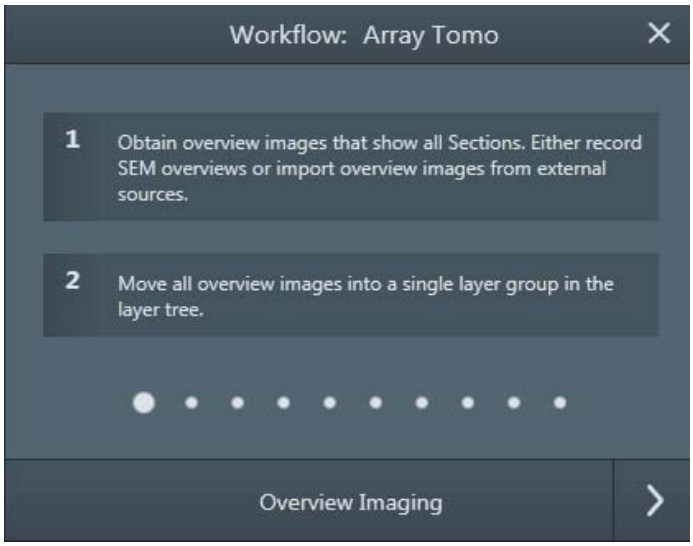
Click the **Start Workflow** button to get started.



In the Select Workflow window that appears, click **Array Tomo** and then click **Begin** to start the workflow.



When a workflow has started, a new interface panel appears on the right side of the main screen.



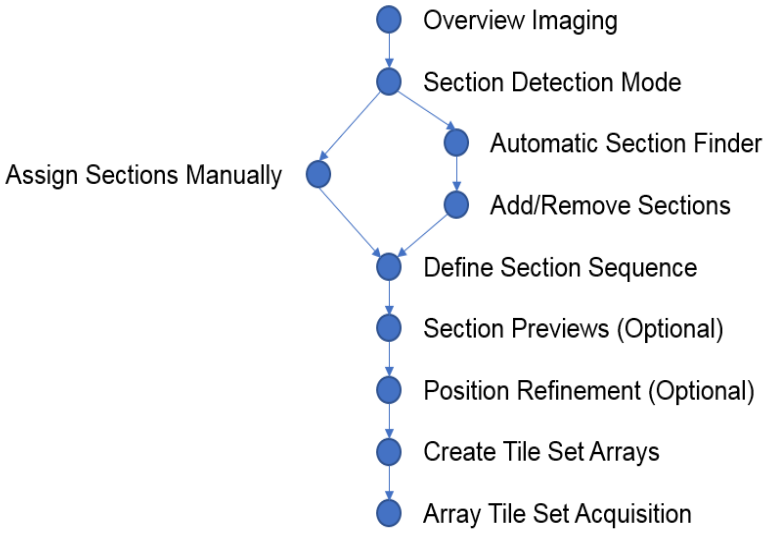
To navigate through the workflow, use the forward and back buttons located at the bottom of the panel. To exit the workflow, click the **X** button at the top right of the panel.

Array Tomography Workflow Steps

The Array Tomography workflow contains two different branches to set up and capture images for an AT reconstruction:

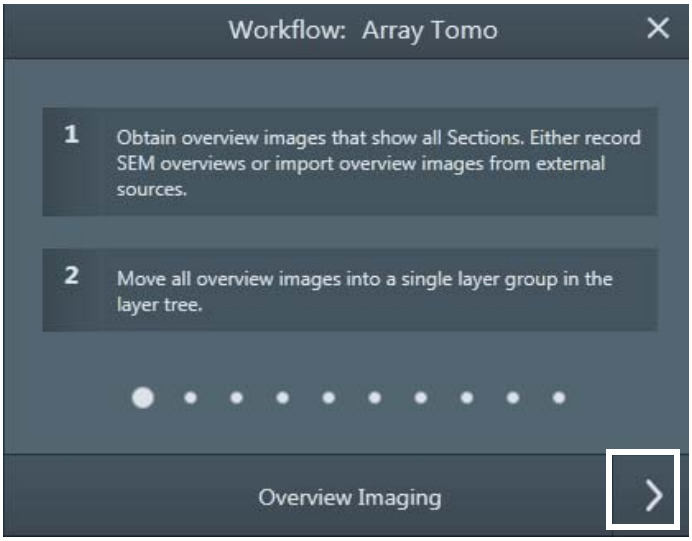
- A branch for mapping out all sections on your sample manually
- A branch for running an algorithm to auto detect the sections.

The following diagram shows the steps for both branches:



- *"Step 1: Overview Imaging" on page 228*
- *"Step 2: Section Detection Mode" on page 228*
- *"Step 3a(1): Automatic Section Finder" on page 230*
- *"Step 3a(2): Add/Remove Sections" on page 232*
- *"Step 3b: Assign Sections Manually" on page 233*
- *"Step 4: Define Section Sequence" on page 234*
- *"Step 5: Section Previews (optional)" on page 235*
- *"Step 6: Position Refinement (optional)" on page 237*
- *"Step 7: Create Tile Set Arrays" on page 241*

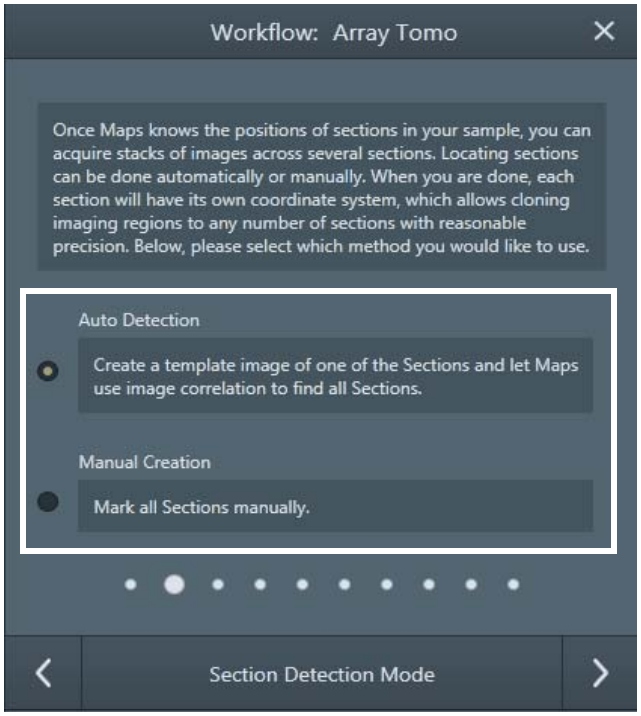
Step 1: Overview Imaging



1. Either take one or several large, low-resolution tile sets, or import images from an external source.
2. Place all overview images into the same layer group in the layer tree. Make sure that the overview images cover all sections on the sample.
3. Click the right arrow button to continue.

Step 2: Section Detection Mode

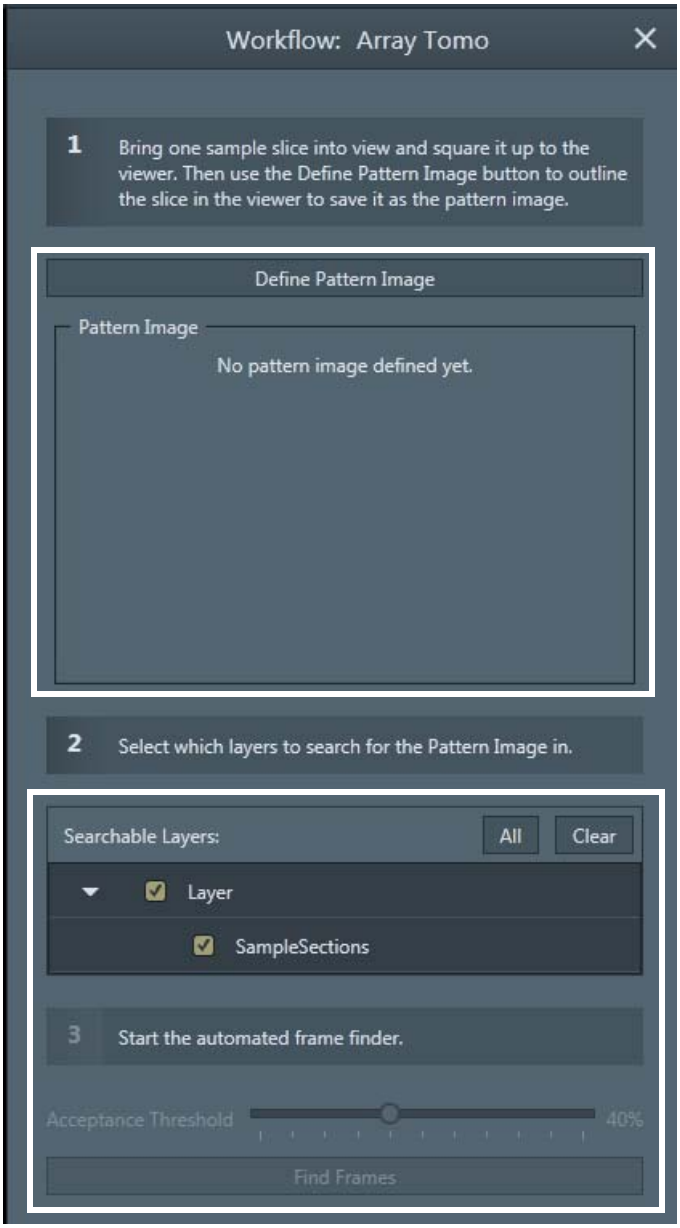
Sections are defined by a rectangular region around them. These regions serve as a local coordinate system for each section and are displayed in the viewer as a brown rectangle. This step determines the mechanism the sections are endowed with, including their coordinate systems. The workflow temporarily branches after this step depending on your selection.



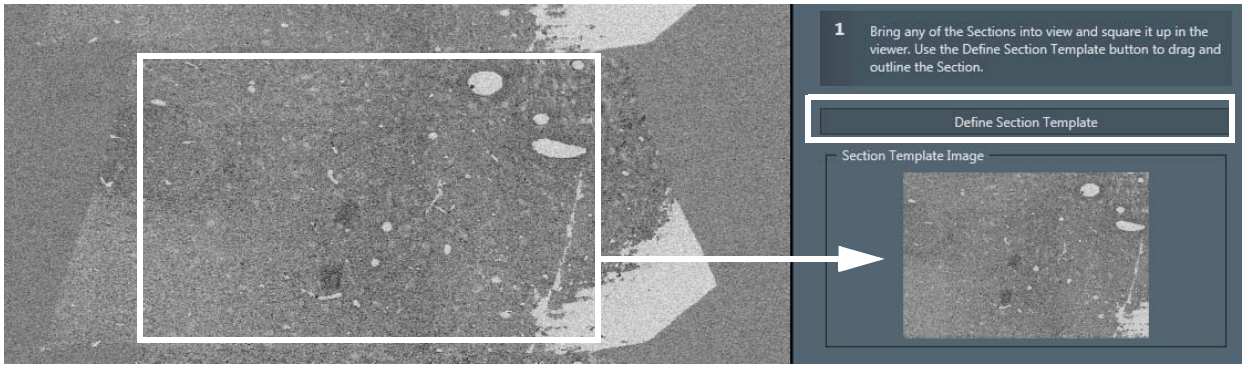
1. Select **Auto Detection** or **Manual Creation**:
   - Select **Auto Detection** to initiate section automation. This workflow branch uses image recognition to search for a user-defined template image of a section within a collection of overview images to create sections.
   - Select **Manual Creation** to manually define each section. This workflow branch requires you to define the size and location of each section of the sample.
2. If you selected **Auto Detection**, then proceed to*"Step 3a(1): Automatic Section Finder" on page 230*. If you selected **Manual Creation**, then proceed to *"Step 5: Section Previews (optional)" on page 235*.

Step 3a(1): Automatic Section Finder

This is the first step in the Auto Detection branch of the workflow.

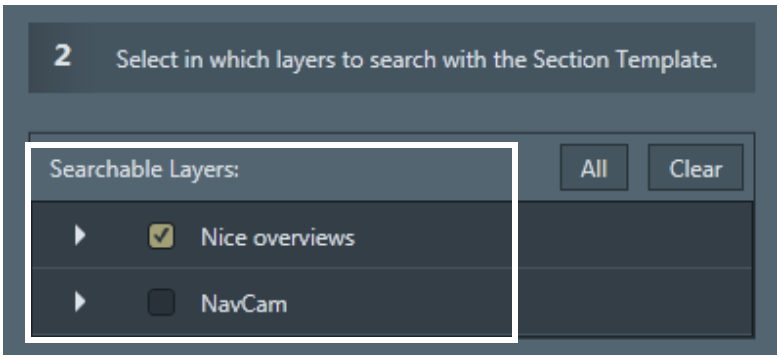


1. Use the mouse scroll wheel to zoom into one section. Then press **Shift + left mouse** to rotate the viewer to make the section *squared up* in the viewer.
2. Click **Define Section Template** and then draw a rectangle in the viewer that encompasses the section. This cuts out an image from the viewer and places it in the Section Template Image box. This is the image that Maps searches for in the overview images. The width/height/rotation of the drawn region is used to define the sample sections.

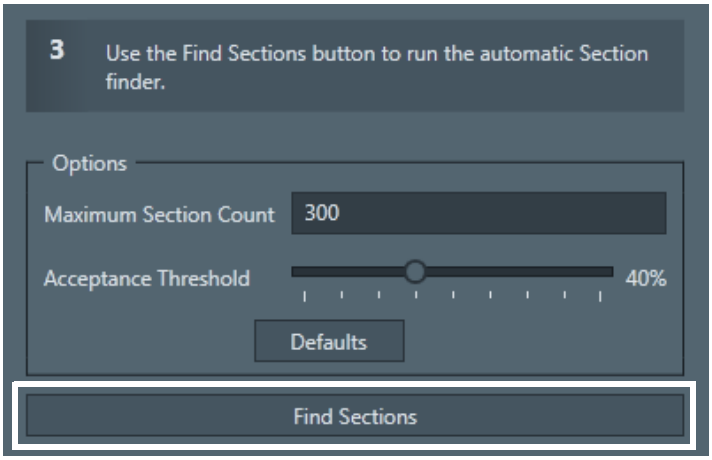


3. Use the **Define Pattern Image** to drag a box around the sample slice in the viewer. This creates a cut-out image of the section that is displayed in the **Pattern Image** box in the workflow panel. This image is used as the search template in the section finder.

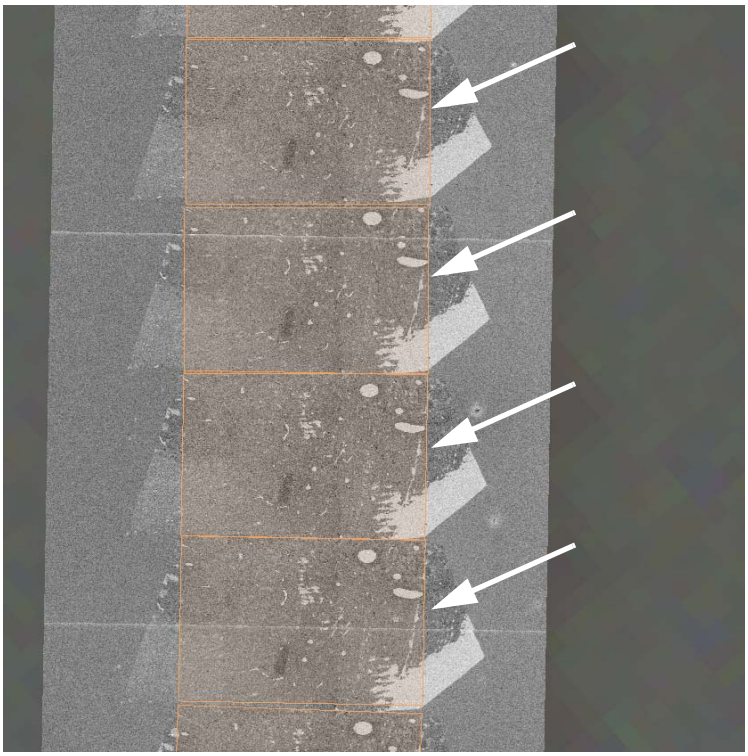
4. Select which overview image layers to search in for the template image.



5. Start the automated section finder by clicking **Find Sections**.



6. Once the automated section finder is finished, the viewer displays the outlined sections.



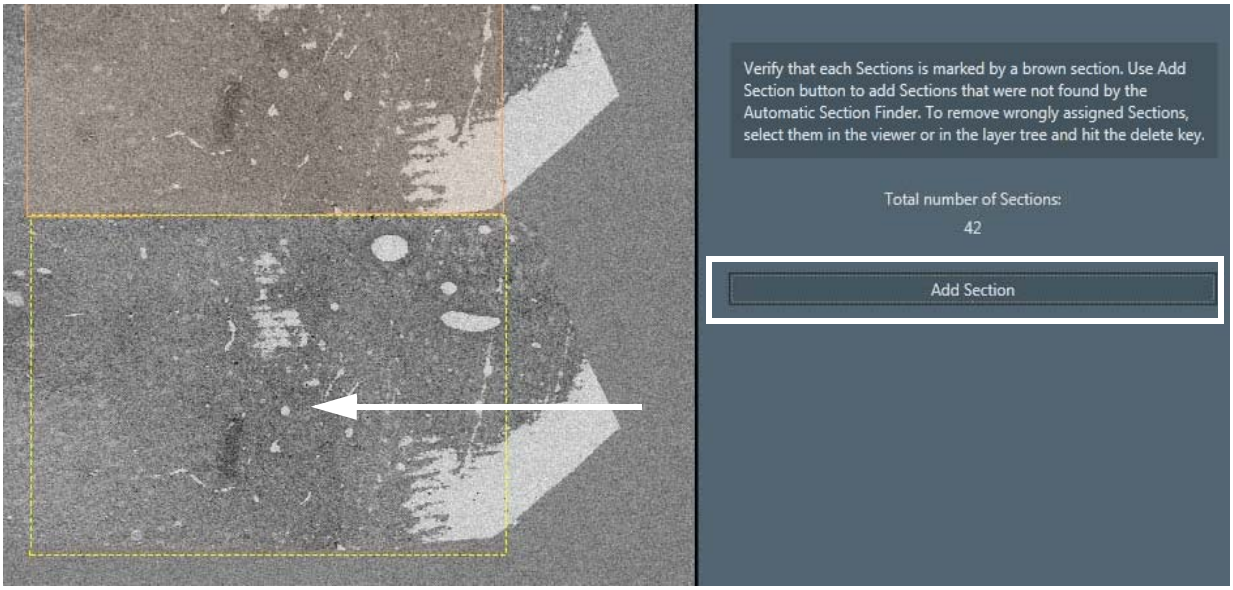
7. Continue to the next workflow step to add/remove sections.

Step 3a(2): Add/Remove Sections

This step removes false positives found in the section finder step and to add section outlines for sections the auto finder may have missed.

- To delete a false positive, select the outline in the viewer and then press Delete.
- To add a missing section, click **Add Section** to generate a preview outline of the missing section and then mouse-click the viewer to place the outline.

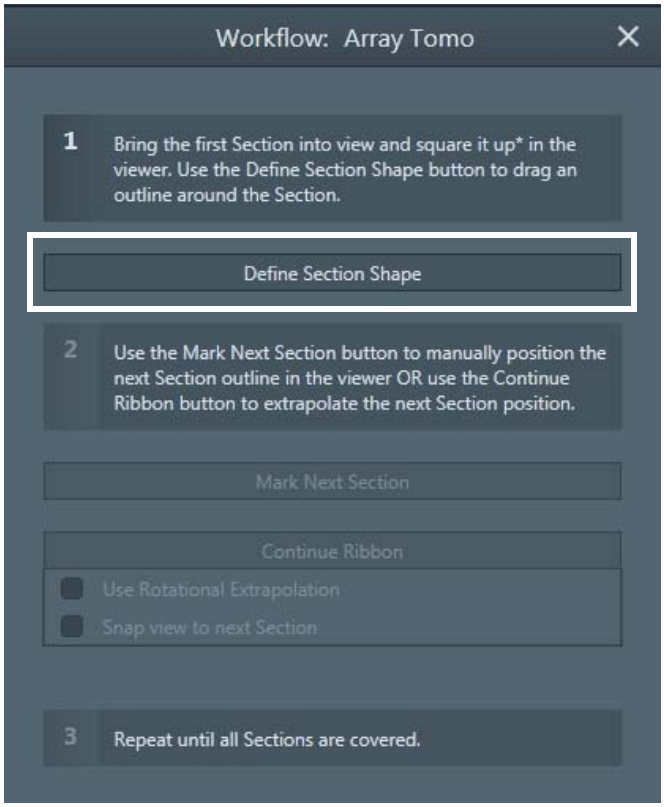
TIP	Since the tool always places the section squared up, square up the section needing an outline in the viewer.



8. Outline all missing sections, and then continue to the next workflow step.

Step 3b: Assign Sections Manually

Manually create a section over each of the sections.



1. Bring the first section into view and square it up in the viewer.
2. Click **Define Section Region** to enable a section definition tool.
3. In the viewer, drag a rectangle around the first section. This creates the first section and defines the section size for all following sections.
4. To create sections for the rest of the sample, click **Place Section** or **Add Section**.
   - **Place Section**: This allows you to click in the viewer to place a section. There will be an outline of the section surrounding the mouse to help position the section precisely over the section.

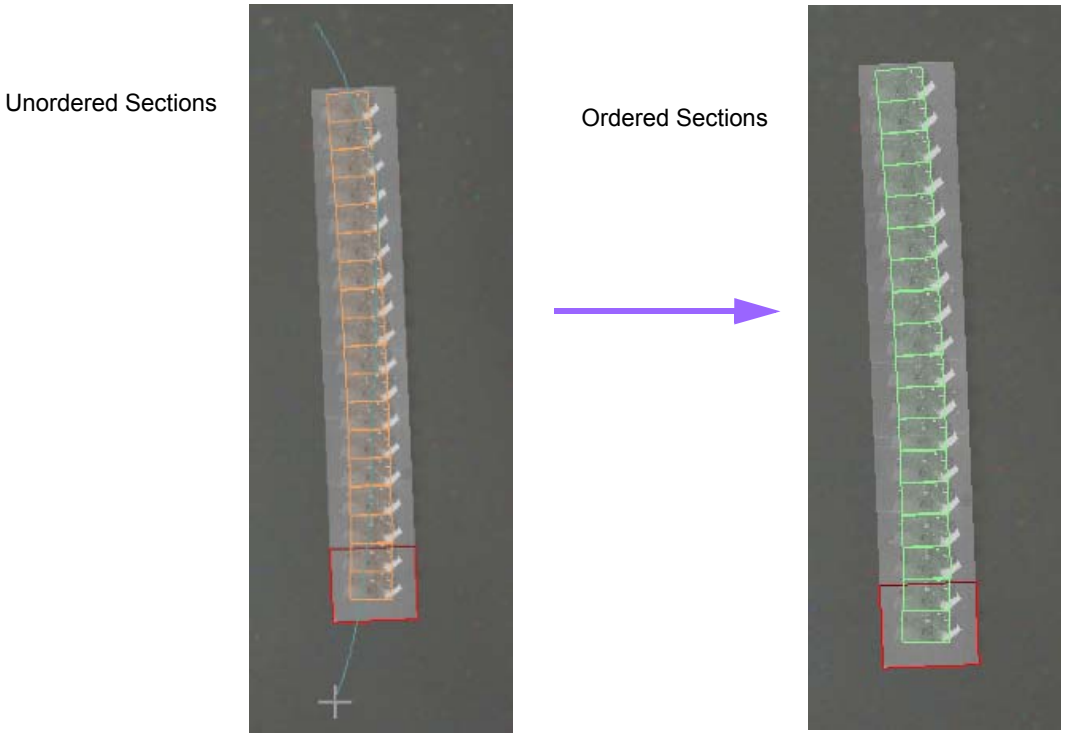
     To adjust the rotation of the section to the sections, use the lollipop handle at the right side of the section. You can also rotate the image in the viewer so that the section borders are parallel with the monitor edges before placing the section. This method is recommended for **Sections on Tape**.
   - **Add Section**: This places a new section down in the viewer. Its position is extrapolated from the positions of sections that were already added. The first two sections are placed near the center of the viewer.

     Adjust the position and rotation of these by clicking **left-mouse + drag** in the viewer and by using the lollipop tool for rotation. Section number three and subsequent sections will use the offsets of the two previous sections for placing them in an approximately correct position.
     - If you select the check box for **Use Rotational Extrapolation**, then the new section also takes into account the rotation offset between the previous sections.
     - If you select the check box for **Snap View To New Section**, then the viewer will align to the newly-created section when it is created to help verify placement. Use **Add Section** when working with a **Classic Ribbon** sample.
5. Fine adjust the position and rotation of all sections to save time later in the workflow.
6. Repeat this sequence of creating sections until all desired sample slices have been covered.

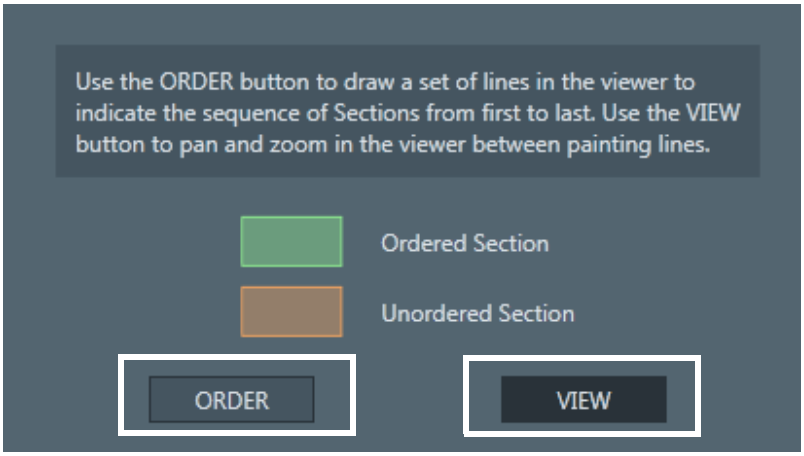
Step 4: Define Section Sequence

In this step, you will set up the order of the sections. Sections that have not been ordered will appear in the Unordered Sections list and those ordered will show up in the Ordered Sections list. The ordered sections list will also display the previous index to show what is changing.

The ordering tool is a viewer tool that allows you to draw a line through a collection of sections within the viewer to determine the order. Those sections closest to the start of the line are first and those sections near the end of the line are at the end of the sequence. See images below.



1. To use the ordering tool, click **ORDER** and then start drawing the sequence lines in the viewer.

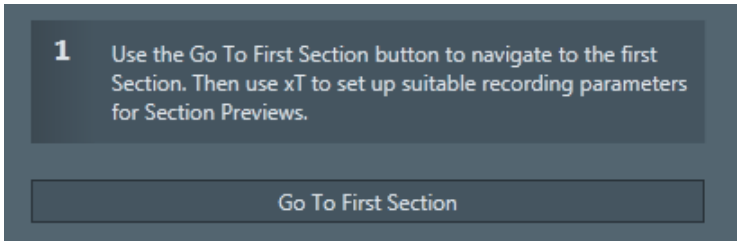


2. To change your current view, click **VIEW** to enable panning around again.
3. After you order all sections, continue to the next workflow step.

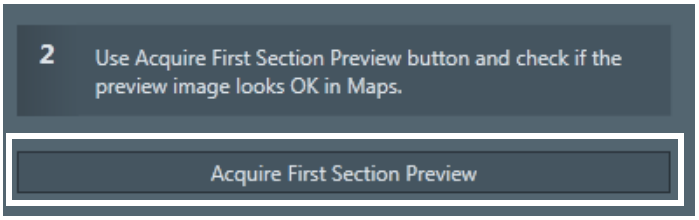
Step 5: Section Previews (optional)

While this step is highly recommended, it is optional. If you run the *"Step 6: Position Refinement (optional)" on page 237*, this step is required.

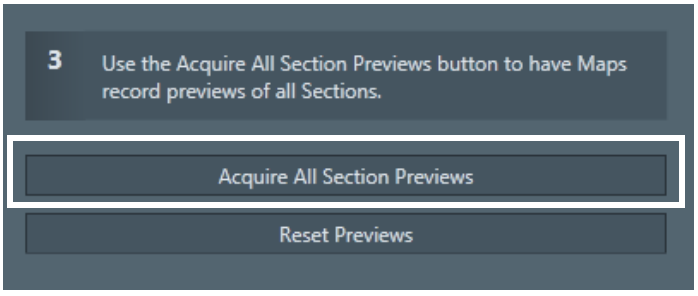
1. Click **Go To First Section** to bring the view and drive the stage to the first section.



2. Use xT to set up satisfactory imaging settings to take a higher-resolution image of the section compared to the overview images.
   - The imaging area for previews is always the size of the section outline +10% margin. It is recommended to choose the Horizontal Field Width (HFW), so that the preview can be recorded as a single image. However, you can select a smaller HFW (Maps will then use tiling to cover the area of the section).
   - The recommended imaging parameters include: 500 nm pixel size and 3µs dwell time.
   - Set the working distance, imaging mode, high tension, and beam current to the same values that will be used for high-resolution imaging.
3. Click **Acquire First Section Preview** to have Maps create and acquire a tile set at the first section.



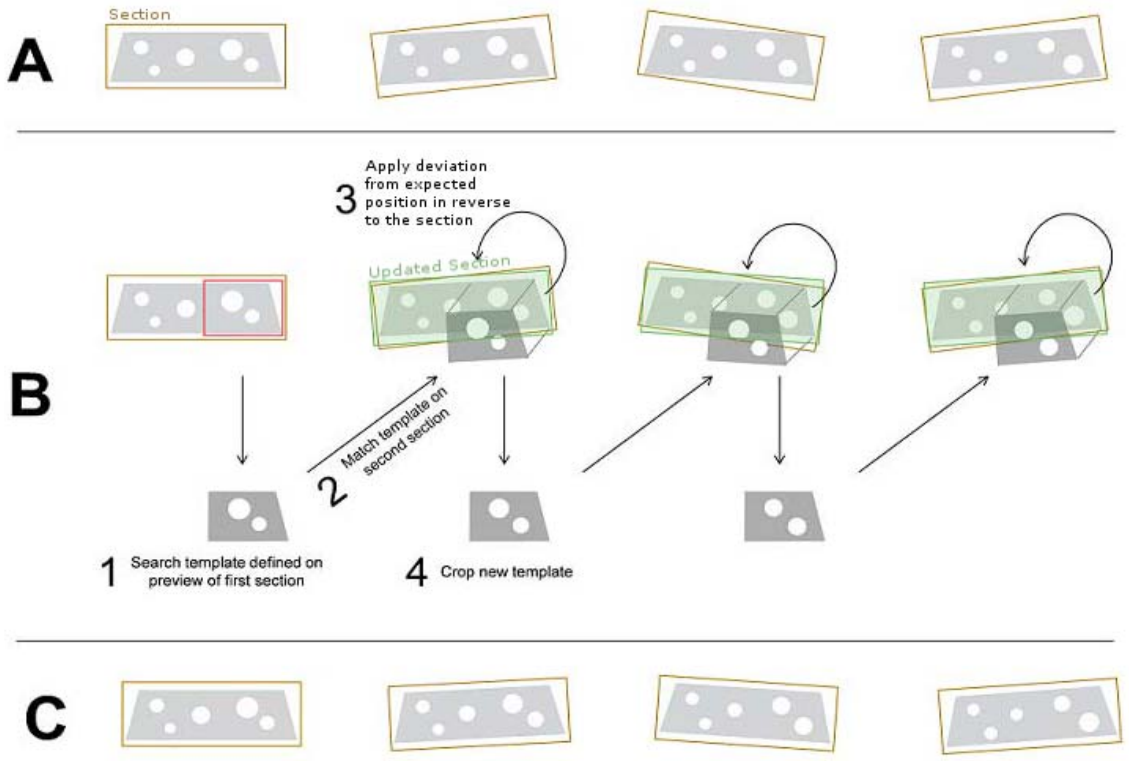
4. Ensure that the tile set acquires the images as desired. If everything looks good, then click the **Acquire All Section Previews** to have maps automatically create and acquire matching tile sets across the remaining sections.



5. You can always stop acquisition at any time and click **Reset Previews** to restart preview acquisitions from scratch. When resetting, you have the option to save the acquired data to a new layer group, in case you do not want to delete it.
6. Once you have acquired all section previews, continue to the next workflow step.

Step 6: Position Refinement (optional)

If you acquired the section previews in *"Step 5: Section Previews (optional)" on page 235*, then this step is an option. This step is highly recommended to increase the accuracy of the placement of tile sets later.



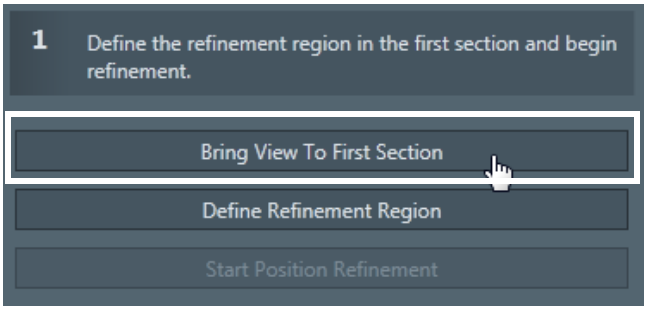
The above schematic shows how the refinement process works.

- Row A: This shows section outlines created on the basis of overview images, which do not match perfectly.
- Row B: You select a sub-region on the first section and use it as a search template on the second section. The offset position of the sub-region relative to the section outline is stored. Template matching in the second section begins in that stored offset relative to the second section outline.

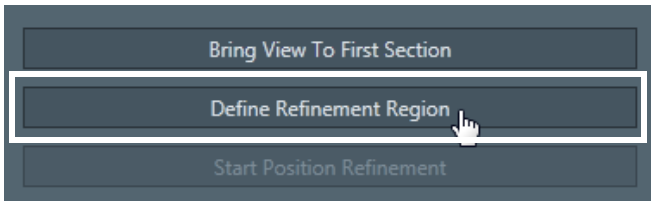
  If section outlines were matching perfectly, then the search template would match exactly at this step. If a location with a better match can be found within the search image, then the position of the second outline is adjusted accordingly.

  A new template is automatically cropped from the second section based on the matching results. This process is repeated from section-to-section until the entire set of sections has been refined.
- Row C: After refinement, section outlines match much more precisely.

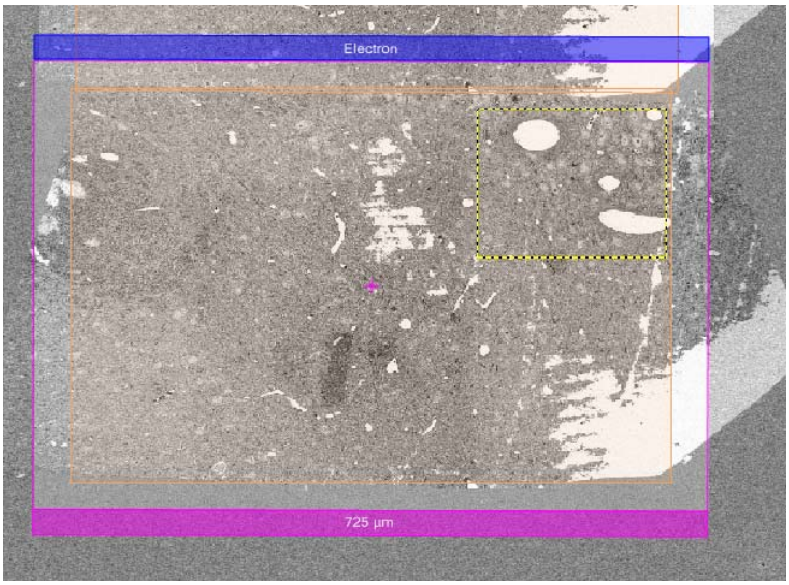
1. Bring the view to the first section by clicking **Bring View To First Section**.



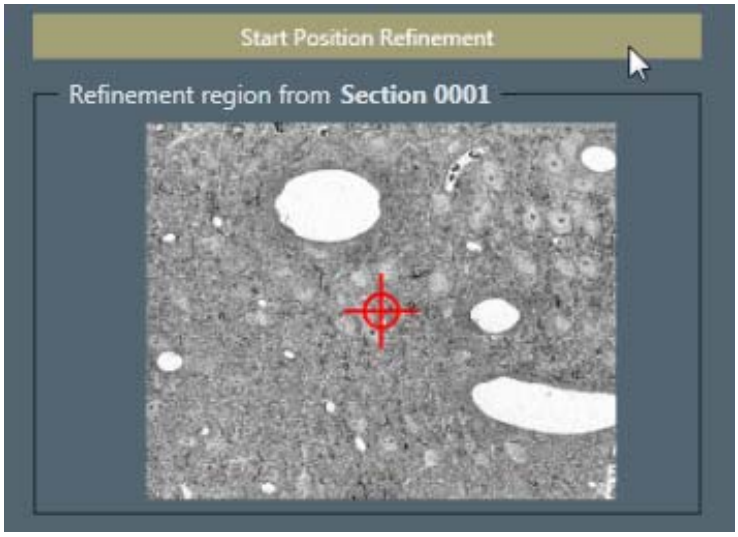
2. Click **Define Refinement Region** to define a refinement region in the viewer that will be used to begin the position refinement.



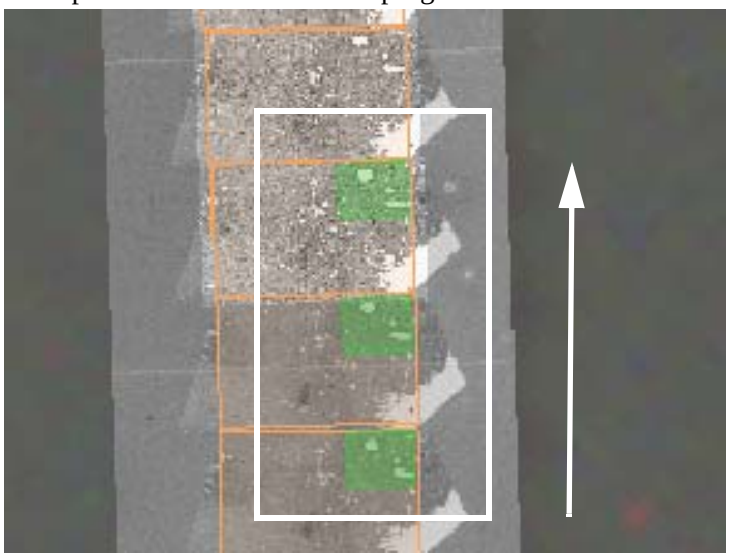
3. Drag the yellow/black border box to define the refinement region. The refinement region image cutout then displays in the box labeled **Refinement region from {section}**.



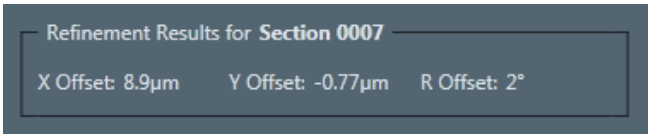
4. Start the refinement process by clicking the **Start Position Refinement**.



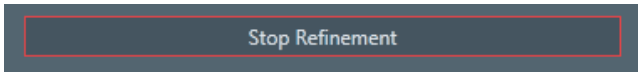
While refinement is running, green rectangles are placed in the viewer where the refinement process found its match. The green rectangles appear from the bottom to the top of the screen to indicate progress.



- The refinement results (correction offset) are displayed in the **Refinement Results** box.



- To stop a refinement run, click **Stop Refinement**, which is highlighted in red.

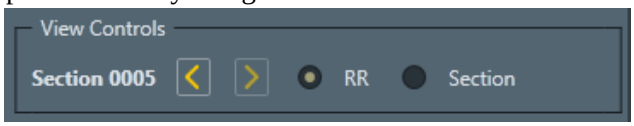


5. If the refinement fails to find a match for a given template, then the viewer will be brought to the section that currently failed to find a match. It will also prompt you to manually place the cutout.

   The goal here is to move/rotate the yellow cutout in the viewer to the correct location. The correct location should be in the current section and match the image currently shown in the **Refinement region from {section}** image box in the workflow panel.

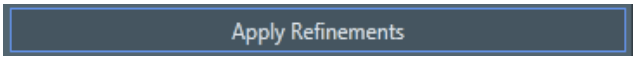
   To help target a center spot, a green crosshair displays within the box in the viewer and a red crosshair displays over the image in the panel. Once the rectangle in the viewer is placed in the best spot, click **Continue Position Refinement** to continue the refinement run.

6. After you have refined all sections, you can easily check how well the refinement placed itself by using the **View Controls**.



   - Click the two arrow buttons to view the next or previous **RR (refinement region)**/**Section** available. You can use this function to check the accuracy of the refinement region placement.
   - If you find a refinement region was found in the wrong location, then click **Change Refined Placement**. This allows you to change the position/rotation of the refinement region in the selected section (indicated in **View Controls**). The refinement results override the selected section.

7. After you have checked the refined sections, click **Apply Refinements** to apply the refinement offsets to the section outline placement. Now, tile sets in and around the position refinement region will have much better section-to-section position stability.

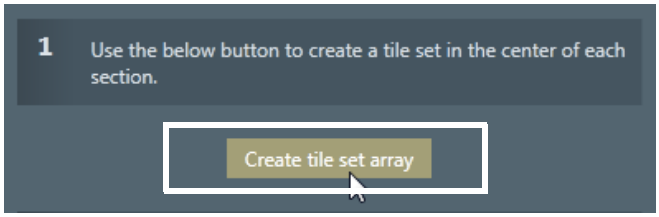


NOTE	Applying the refinement results is a one-time action and is not reversible.

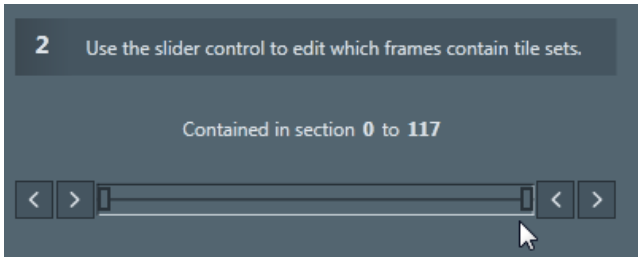
8. When satisfied with the section outlines placement, continue to the next workflow step.

Step 7: Create Tile Set Arrays

1. Click **Create tile set array** to create a tile set array across all sections with a single click.



2. All the tile sets created within this array are linked. If the position, rotation, or imaging parameters of one tile set changes, then the rest of the tile sets will be updated. To adjust how many sections the tile set array spans, click the slider and drag to adjust the control bar.

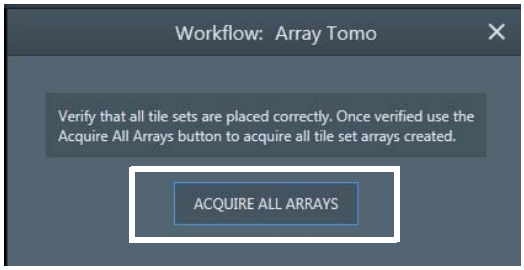


NOTE	There is an exception to the tile set linking functionality in the array for one of the imaging parameters. The **Fixed** parameter in the auto-focus strategy list on the Auto-Functions tab is a per-tile-set setting. This allows you to manually focus and store the found focus position for each tile set. This allows you to work with samples on which auto-focus does not work.

3. Repeat the tile set creation until all Regions of Interest (ROI) are covered. If several tile set arrays are defined, each array can have different parameters.
4. Continue to the next step to begin acquisition of your newly-created tile sets.

Step 8: Array Tile Set Acquisition

1. Double check that all tile sets have been created.
2. Click **ACQUIRE ALL ARRAYS** to start acquisition of all the tile sets across the sample.

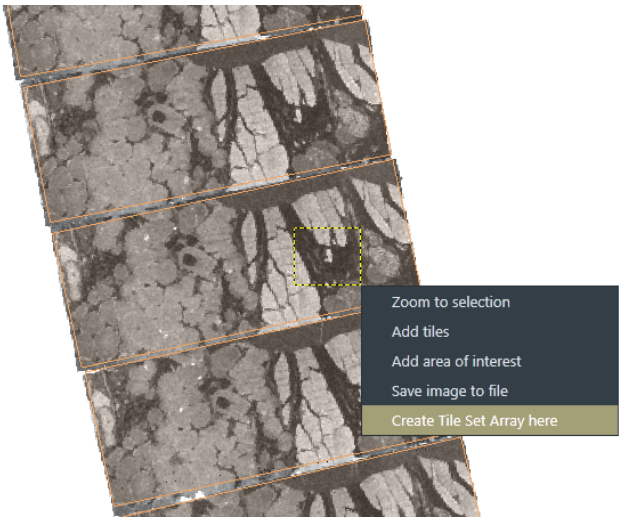


Extended Controls

Viewer Controls

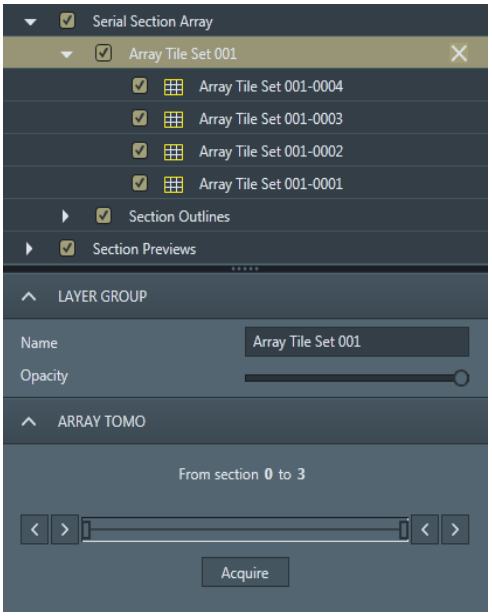
You can create, modify, and acquire a tile set array outside of the workflow as long as there is a collection of mapped out sections.

1. To do so, perform an area selection (**Alt + left-click drag**) within a section and select **Create Tile Set Array Here** in the menu that appears.



This creates a new layer group for the tile set array under the **Serial Section Array** layer group and populates it with one tile set per section.

2. To modify which sections contain a tile set from this array, select the tile set array Layer Group (Tile Set).

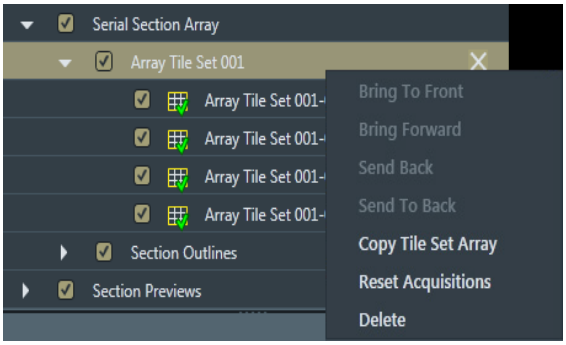


3. Click the slider and drag to adjust the slider control bar in the Array Tomo pane to add or remove tile sets from sections. Alternatively, you can use the single incrementing buttons on either side of the slider for more precise adding and removing of tile sets.

   When you add new tile set, it is added in the same relative location in its containing sections as the other tile sets in the array. This will ideally keep the tile set in the same location on the sample, thus making it easier for you to inspect the changes through the slices.

4. Click the **Acquire** button to start the acquisition of the whole tile set array.

Tile Set Array Layer Group Context Menu

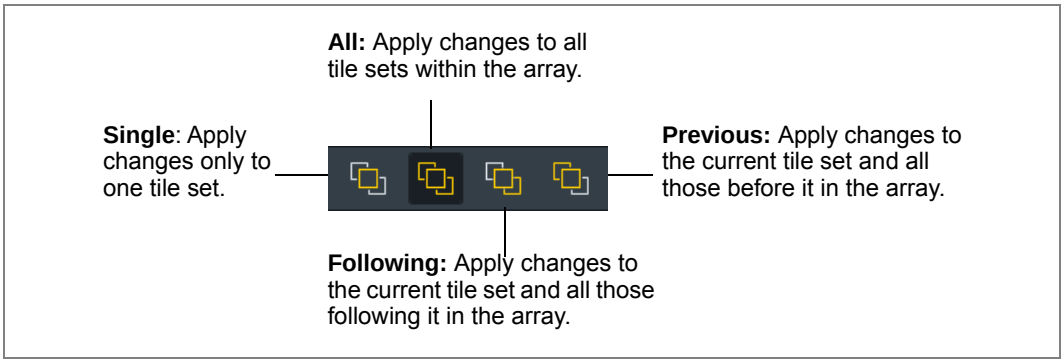


- **Copy Tile Set Array**: Creates a copy of the existing tile set array group. Places it within the serial section array group with a name chosen by you.
- **Reset Acquisitions**: Resets all tile sets acquisition within the tile set array group.

Editing the Tile Set

All tile sets within one array will share acquisition parameters automatically, but location and rotation changes can differ between each tile set. To better handle these changes, use the Edit Mode buttons in the tool bar.

Figure 3 Tile Set Edit Mode Buttons



Viewing the Data

Once all tile sets have been acquired, you can navigate through the sections to view the newly acquired data.

Select a tile set from an array or a section to enable the **Toggle View** buttons in the tool bar.

Click the **Toggle View** buttons to move the view between sections.

The viewer rotation and offset from the selected section are maintained when moved to the next or previous section. This allows you to quickly view a collection of tile sets or sections at a custom zoom and rotation.