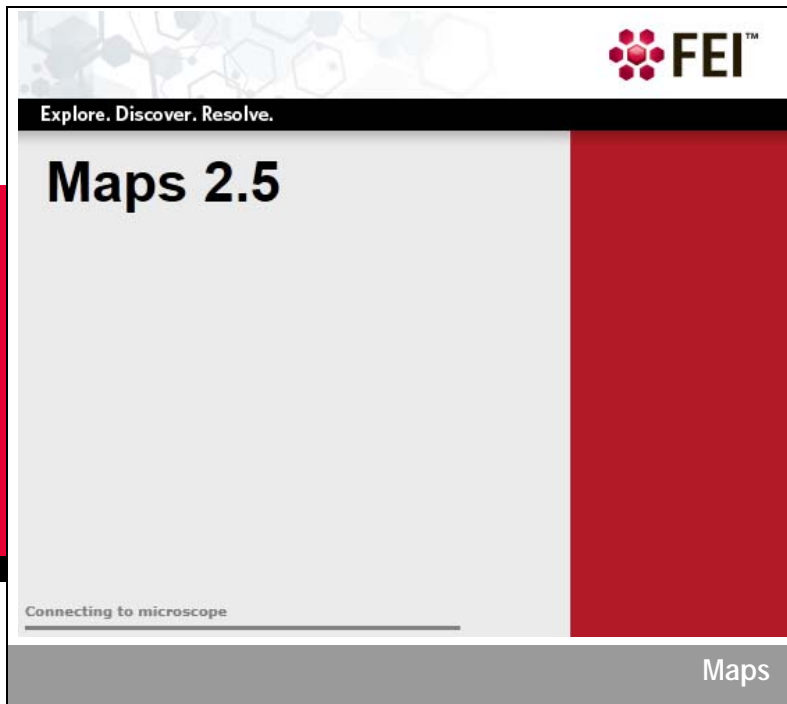




FEI Maps 2.5 SW Application User's Guide

PN 1117107-A



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Document History

Rev A: May 2016

1 Overview

Overview

This document describes the FEI® Maps 2.5 application software.

Topics include:

- *“Introduction” on page 3*
- *“Terminology” on page 4*
- *“System Preconditions” on page 5*
- *“Data Storage Requirements” on page 5*
- *“Project Directory Restructure” on page 6*
- *“Package Options” on page 7*

Introduction

The FEI Maps software application provides users of an FEI system 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.

Maps complements the Correlative Workflow features that enable FEI users 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 in the field of cell biology to use different imaging techniques in order to fully understand the biological question at hand. When images taken with a light microscope are imported into a scanning electron microscope (SEM) running Maps, a simple alignment procedure is used to correlate the data for future automated mapping.

NOTE	When upgrading from a 1.x version of Maps, a new license key is required to use Maps 2.x.
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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
- **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 98](#) section on Calibration.
- **Correlation:** An analysis technique involving the superposition of two sets of data
- **EM:** Electron microscope
- **HD View:** A Microsoft Research image data format used for gigapixel-resolution image data
- **Live Acquisition:** The microscope control software for FEI’s light microscopes (CorrSight™, iMIC, etc.)
- **LM:** Light microscope
- **Maps Offline Viewer:** A version of Maps that has read-only capabilities for Maps projects
- **MIP:** (*LM only*) Maximum intensity projection. Collapses a Z-Stack of images into a single image using the highest intensity pixels from the stack
- **Nav-Cam:** (*EM only*) An optional optical camera that takes a top down image of the stage that can be used for point and click navigation of the sample
- **Registration:** The computational process Maps uses to determine the position of a single tile within the greater tile set. The Registration process is run during tile set acquisition.
- **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
- **Stitching:** The computational task of combining a set of individual images to form a single image

- **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 electron and small DualBeam microscopes
- **xT UI:** xT software user interface
- **Z-Stack:** *(LM only)* A sequence of images acquired at incrementally changed focus depths
- **Z-Stack Browser:** *(LM only)* The Maps controls that allow you to select and view individual image planes in Z-Stack tile sets

System Preconditions

- 3 GB of physical RAM
- 4.5 GB of memory paging allocated in Windows®
- Dual-core processor
- System software is running
- Platform software (xT or LA) must be running before Maps is launched

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.
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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 Restructure

The project directory for a Maps project has been reorganized to add clarity and ease of navigation. At the root most directory, you will see the following:

LayersData	4/12/2016 6:58 ...
MetaData	4/13/2016 7:08 ...
MapsProject.xml	4/13/2016 7:08 ...
MapsProject.xml.backup	4/13/2016 7:07 ...
Project.mapsxml	4/12/2016 6:58 ...
ProjectLog.log	4/13/2016 7:08 ...

The LayersData folder contains a directory structure that mimics the Layer tree in Maps, making it easier to find the image files manually.

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 FEI representative for licensing information.

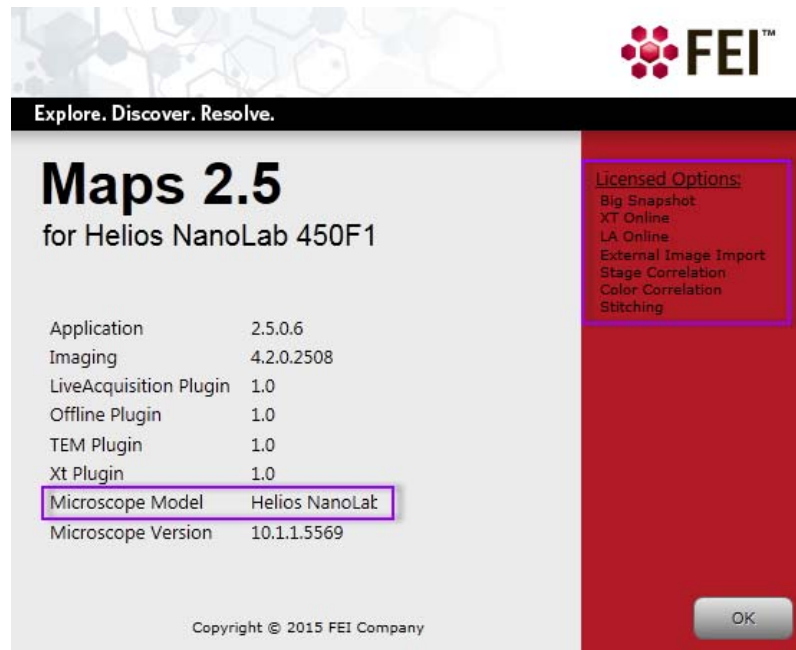



Table 2 Maps Licensed Options (1 of 2)

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, e.g., Helios NanoLab™, Quanta™, Versa, etc.
LA Online	Licensed to run on FEI's light microscopes (CorrSight, iMIC, etc.).
External Image Import	Licensed to allow importing of images from any source, e.g., 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.

Table 2 Maps Licensed Options (2 of 2)

Option	Description
<p>Maps 2.5 Offline Viewer</p>	<p>Offline viewer. Installations of maps Offline Viewer are licensed only for use viewing datasets that have been generated by the full version of Maps.</p> 

2 User Interface

Overview

This document describes the FEI Maps 2.5 application software user interface.

Topics include:

- *“Shortcut Keys” on page 10*
- *“Main Screen” on page 11*
- *“Menus” on page 13*
- *“Tool Box” on page 29*
- *“Layer/Holder Definition Control” on page 32*
- *“Layer Control” on page 41*
- *“Job Queue” on page 53*
- *“Viewer” on page 54*
- *“Save Image to File” on page 62*
- *“Rotational Alignment (EM and Offline Only)” on page 65*
- *“Stage Rotation (EM Only)” on page 67*
- *“Scan Rotation” on page 67*

Shortcut Keys

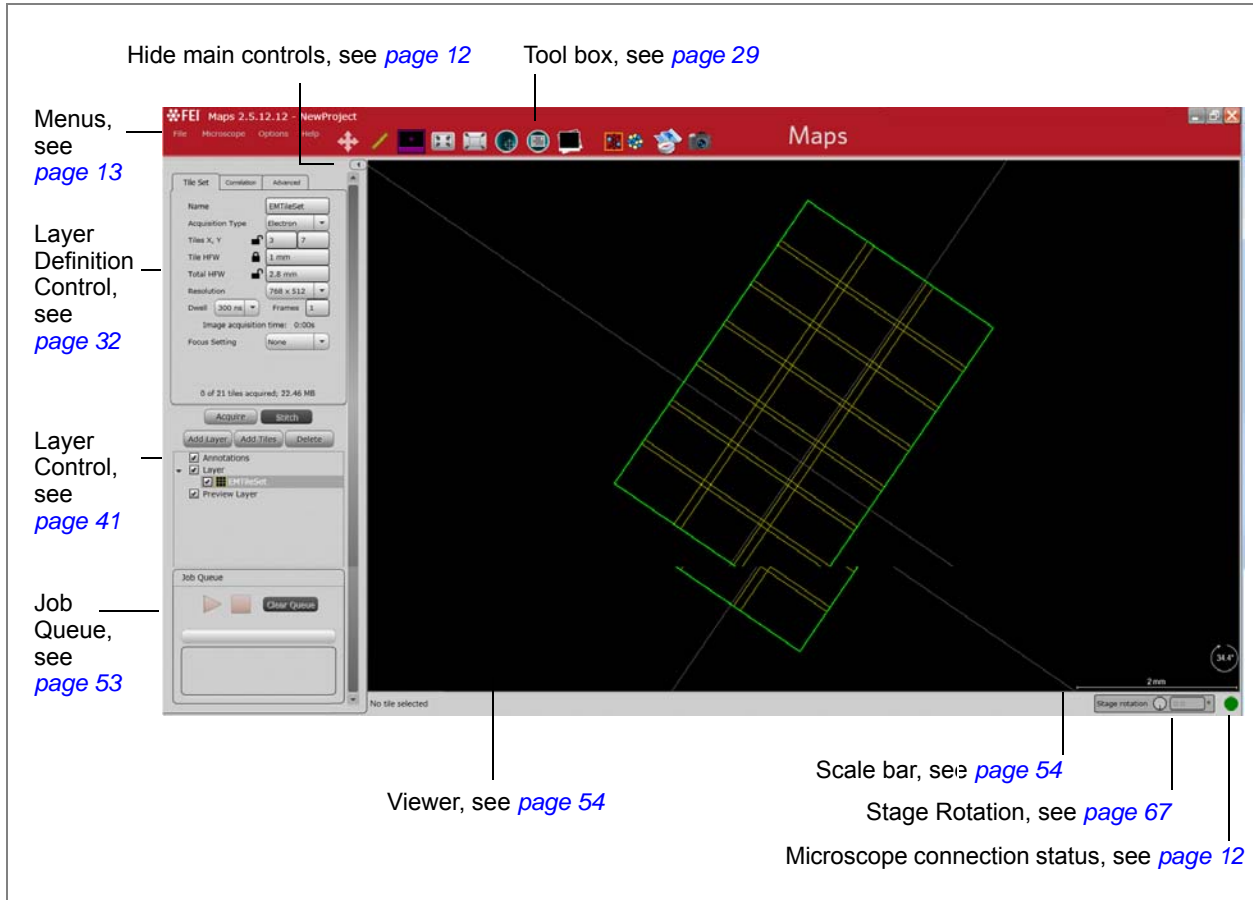
Table 3 Shortcut Keys

Keys	Description
F1	Opens the <i>FEI Maps SW Application User's Guide</i> .
F2	Saves the screenshot with an automatically incremented file name. Same as selecting File > Save Screenshot . See <i>"Save Screenshot" on page 16</i> .
Ctrl + mouse wheel	Zooms in and out.
Shift + mouse wheel	Zooms in and out slowly.
Left-click and drag	Draws a feature of interest rectangle with a number of available options. See <i>"Left-Click and Drag Selected Area" on page 55</i> .
Shift + left-click and drag	Pan (move)
Shift + right-click and drag	Applies digital rotation to the image without moving the stage.
Shift + Ctrl + left-click and drag	Zooms the image to the selected region.
Ctrl+Shift+C	Centers the view on the current layer, same as <i>"Center View on Selection" on page 30</i> and <i>"Center View" on page 48</i> .
Ctrl+G	Shows/hides grid lines for the current layer, same as <i>"Show Grid Lines" on page 48</i> (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 <i>"Hide All Annotations" on page 25</i> .
Ctrl+L	Opens the Log Viewer. Same as selecting Help > Open Logs . See <i>"Open Logs" on page 28</i> .
Ctrl+O	Opens the project folder. Same as selecting File > Open Project Folder . See <i>"Open Project Folder" on page 16</i> .
arrow	Moves selected tile set in small increments.
Shift + arrow	Moves selected tile set in finer increments.
Ctrl + arrow	Selects a tile (manual alignment).

Main Screen

Figure 1 shows the main user interface.

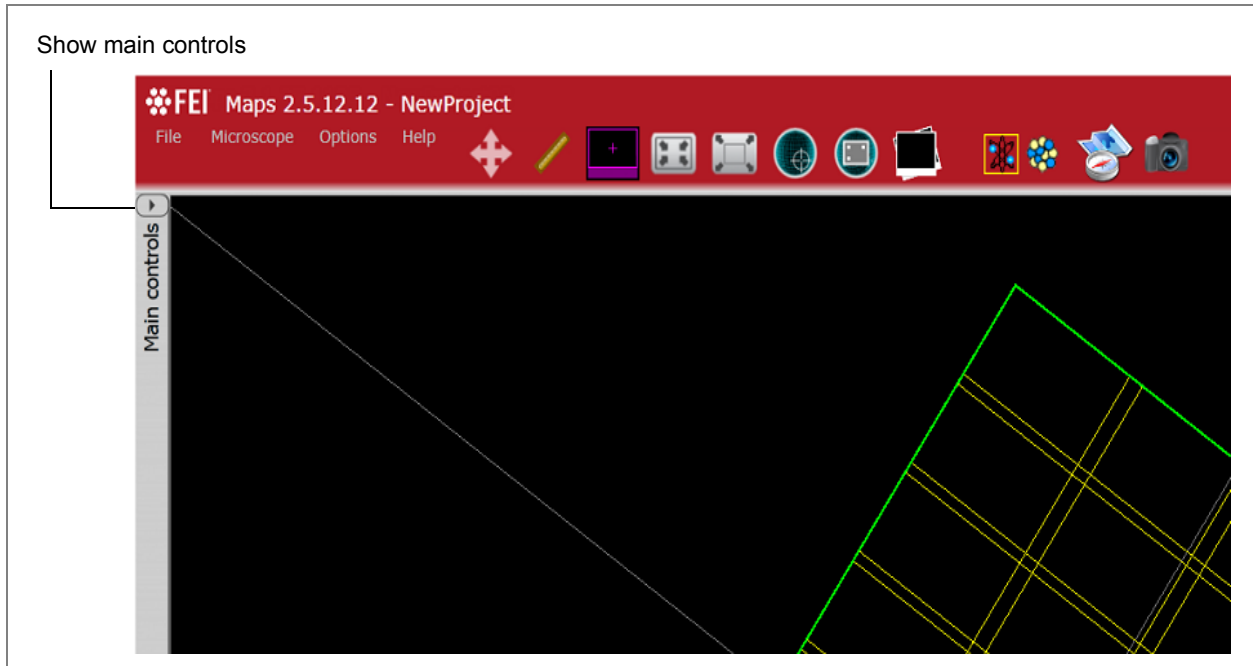
Figure 1 Maps User Interface, Control Panel Showing



Show/Hide Main Controls

Click the left arrow to hide the main controls. It changes to a right arrow. Click the right arrow to show the main controls again.

Figure 2 Maps User Interface, Main Controls Hidden

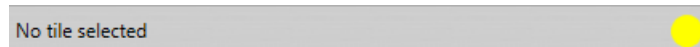


Microscope Connection Status

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 (xT) is running and restart Maps.

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



Interface Button States

- Enabled = Black text on light background
- Disabled = White text on dark background



Menus

Topics include:

- [“File” on page 13](#)
- [“Microscope” on page 19](#)
- [“Options” on page 19](#)
- [“Help” on page 26](#)

File

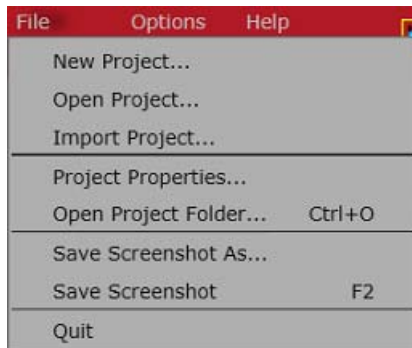


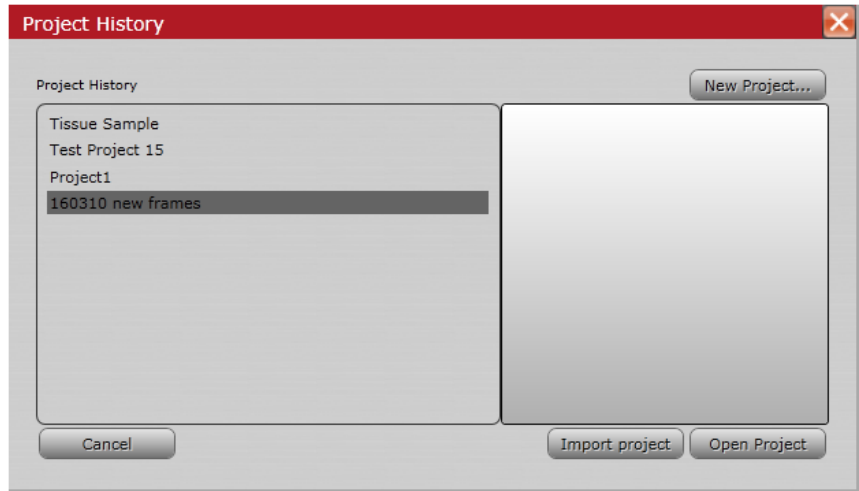
Table 4 Maps File Menu Overview (1 of 4)

Menu Selection	Description
New Project	<p>Displays the New Project window. Define a new Project Name and Description and click Create.</p>

See [“Creating a Project” on page 71](#).

Table 4 Maps File Menu Overview (2 of 4)

Menu Selection	Description
Open Project	<p>Displays the Project History window showing all previous projects.</p> <ul style="list-style-type: none"> • 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.



See ["Creating a Project" on page 71](#).

Table 4 Maps File Menu Overview (3 of 4)

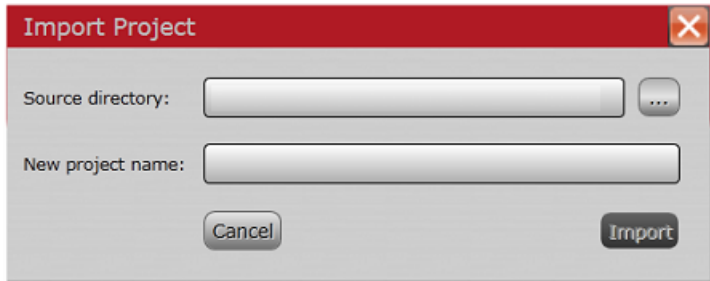
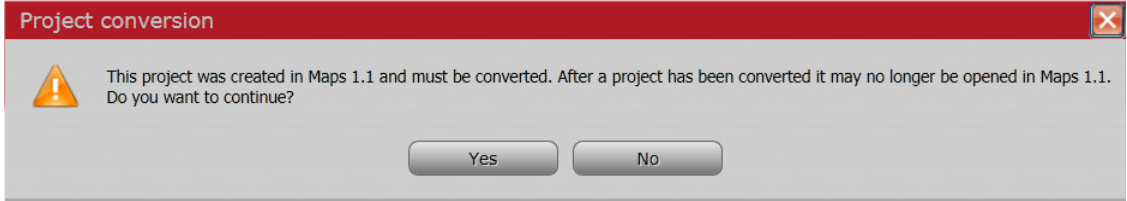
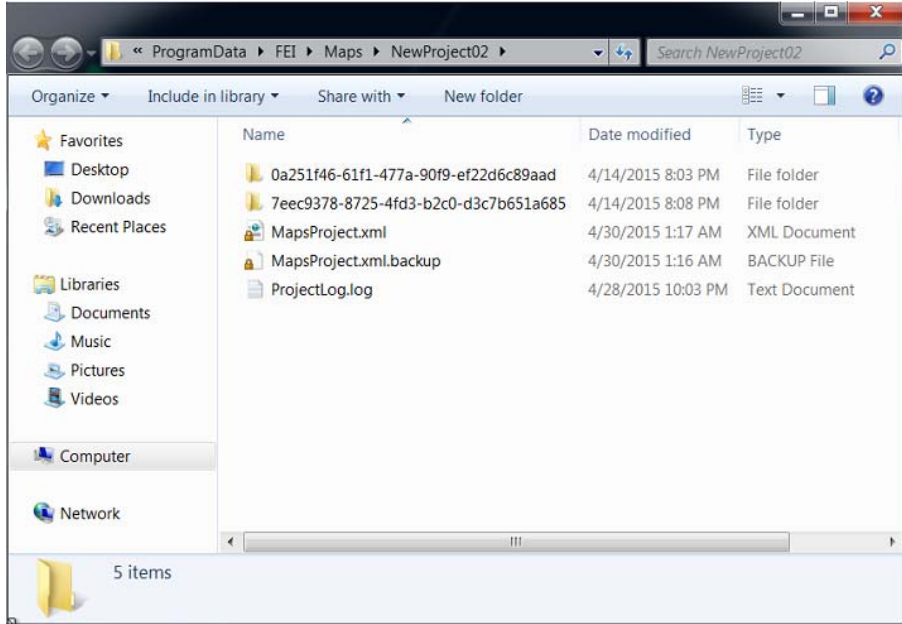

Menu Selection	Description
Import Project	<p data-bbox="521 338 1377 464">Displays the Import Project window. Browse to a Source directory and select a project, rename it if appropriate, and then click Import. The imported project name will appear in the Project History list. This can be used for passing projects between computers.</p> <div data-bbox="527 480 1232 758" style="border: 1px solid gray; padding: 5px;">  </div> <p data-bbox="521 772 1325 831">Note: <i>The import process is only one-way. Older software projects must be converted.</i></p> <p data-bbox="521 842 1395 936">When a project is imported from Maps v1.1, a warning is displayed telling the user that the import is one-way and the project will no longer be compatible with v1.1.</p> <div data-bbox="306 947 1425 1146" style="border: 1px solid gray; padding: 5px;">  </div>
Project Properties	<p data-bbox="521 1171 1333 1230">Displays the Project Properties window for selecting the Project Path, Sample Holder, and a project description.</p> <p data-bbox="521 1241 911 1268">See “Project Properties” on page 17</p>

Table 4 Maps 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 on 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 here.

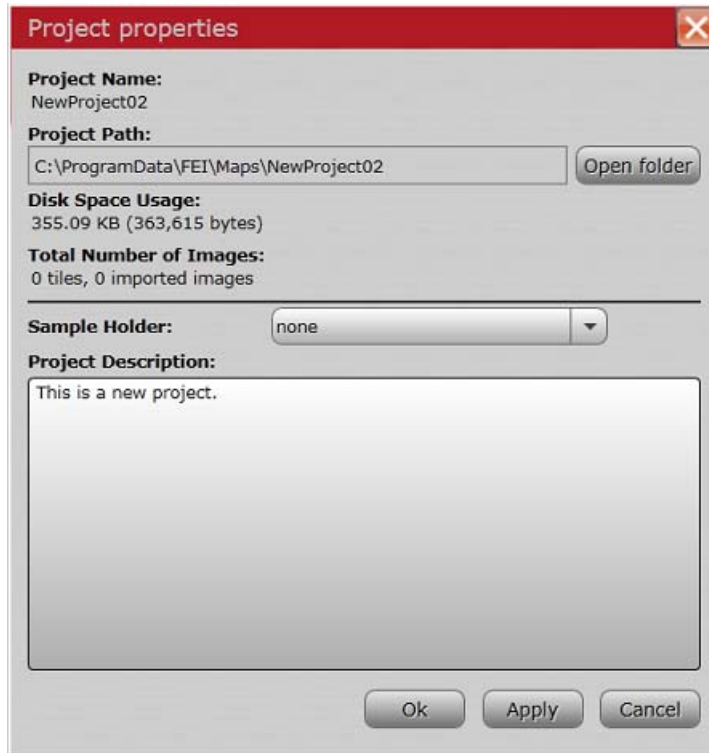



Table 5 Project Properties Overview (1 of 2)

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.

Table 5 Project Properties Overview (2 of 2)

Interface Item	Description
Sample Holder	<p>Displays a dropdown list of possible sample holders that support automated alignment.</p>  <p>Make a selection and click Apply to see the holder outline displayed on the main screen.</p> <ul style="list-style-type: none"> • 3mm Grid holder • Cryo Shuttle • Single 22 mm coverslip holder • Multiwell Slide • Slide 75mm x 25mm • Double 22 mm coverslip holder <p>See <i>"Automated Holder Alignment"</i> on page 98 for more information.</p>
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 red on the viewer.
- The name, drawing, version, etc. display on the Holder tab.
- A newLayer 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.

Microscope

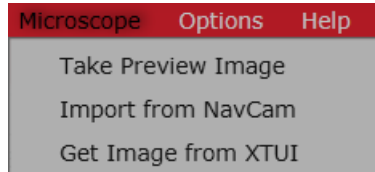


Table 6 Maps Microscope Menu Overview

Menu Selection	Description
Take Preview Image	Acquires a SEM image at the current stage position and HFW and places it on the Maps viewer as a preview image. The HFW can be changed from the Tile right-click menu selection: “Preview Here” on page 57 .
Import from NavCam (EM with Nav-Cam version only)	<i>Disabled only while under vacuum if the door mounted Nav-Cam is installed. See “Nav-Cam Prompt” on page 69.</i> 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 79 .
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 preview image.

Options

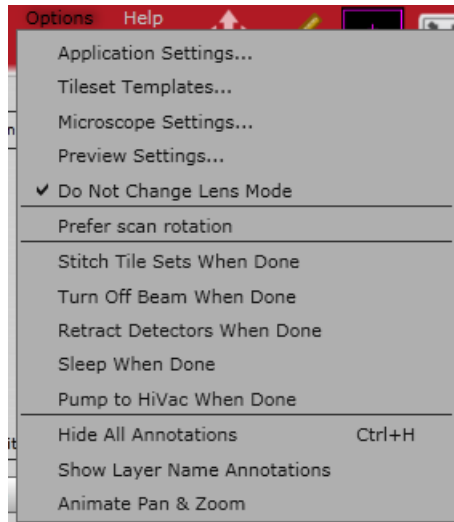


Table 7 Maps Options Menu Overview (1 of 6)

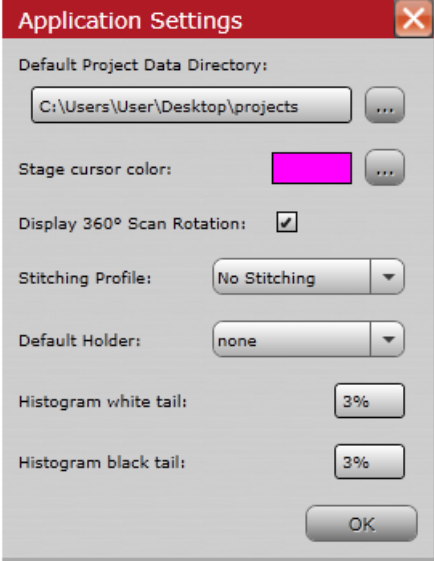
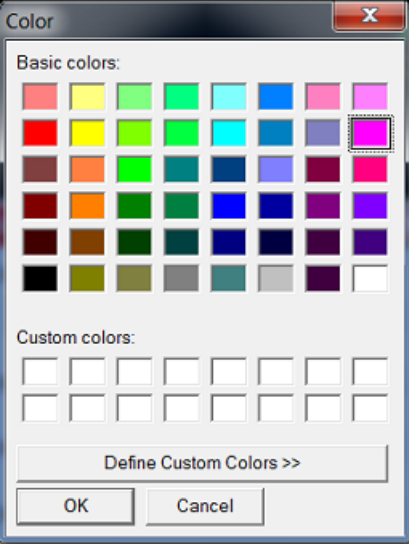
Menu Selection	Description
Application Settings:	Displays the Options window for changing defaults for all new projects.
	
Default Project Data Directory	Displays the default path with a browse button to allow you to choose a different path.
Stage cursor color	Click the browse button to access the Color picker to optionally change the stage cursor color.
	
Display 360° Scan Rotation	<ul style="list-style-type: none"> • When selected, displays 360° scan rotation. • When not selected, displays -180° to 180° scan rotation.

Table 7 Maps Options Menu Overview (2 of 6)

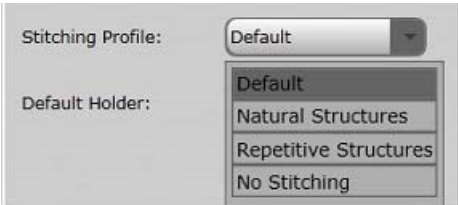
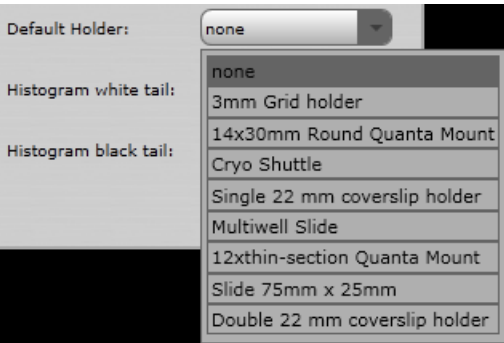
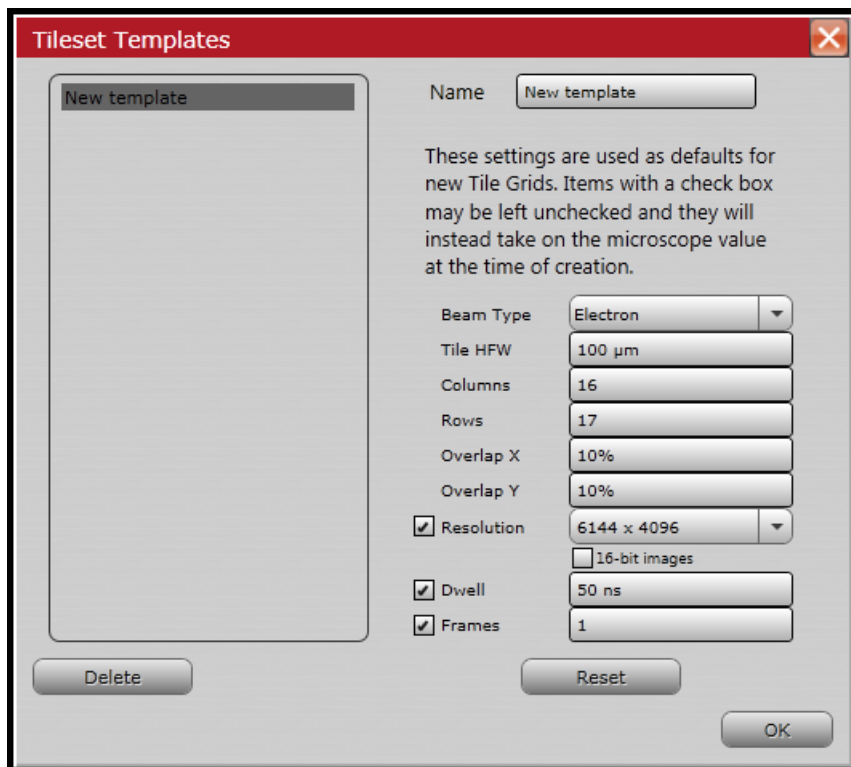
Menu Selection	Description
Stitching Profile	<p data-bbox="540 338 1076 369">Displays a dropdown list of stitching choices.</p>  <p data-bbox="540 600 686 632">Choices are:</p> <ul data-bbox="540 642 1406 1171" style="list-style-type: none"> • Default: The settings in this profile are intended for stitching 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. <p data-bbox="540 1178 1076 1209">See “Stitching Profile Best Practices” on page 25.</p>
Default Holder	<p data-bbox="540 1230 1406 1325">Displays a pulldown list of possible sample holders that support automated holder alignment. Select a holder to be used as a default for all new Maps projects.</p> 
Histogram white tail	<p data-bbox="540 1692 1219 1728">Trims a percentage of histogram pixels to eliminate noise.</p>
Histogram black tail	<p data-bbox="540 1749 1219 1785">Trims a percentage of histogram pixels to eliminate noise.</p>

Table 7 Maps Options Menu Overview (3 of 6)

Menu Selection	Description
Tileset Templates:	Displays a dialog that shows a collection of templates and their respective settings.

EM



LM

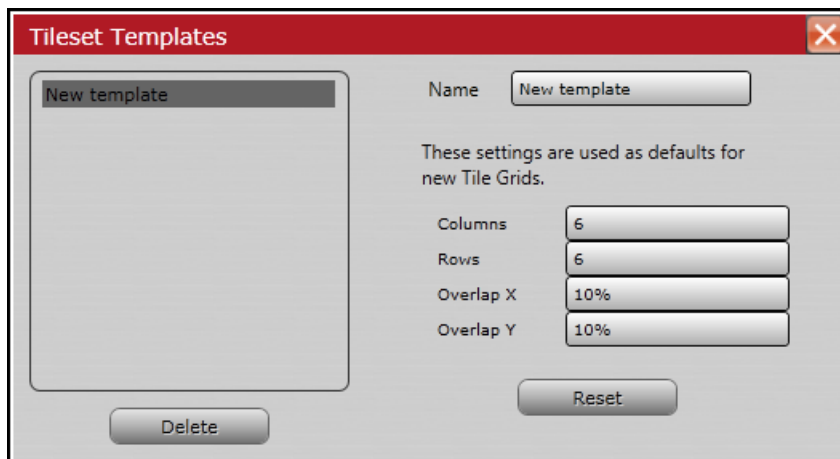


Table 7 Maps Options Menu Overview (4 of 6)

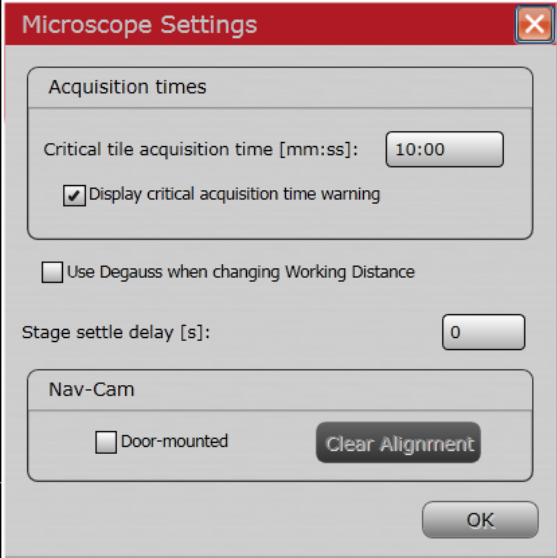
Menu Selection	Description
Microscope Settings:	<p data-bbox="537 333 1414 401">Displays the Options window for changing hardware-related behavior that is not specific to a particular project.</p> 
Critical tile acquisition time	Maps will display a warning whenever the acquisition parameters selected for a tile set, result in a per-tile acquisition duration larger than this threshold.
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.
Nav-Cam: Door Mounted	<p data-bbox="537 1451 1414 1549">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.</p> <p data-bbox="537 1556 1414 1654">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.</p>
Nav-Cam: Clear Alignment	<p data-bbox="537 1669 1414 1738">Clears the saved default Nav-Cam alignment that is applied to all acquired Nav-Cam images. See "Nav-Cam Alignment" on page 79.</p> <p data-bbox="537 1745 1414 1810">To clear the Nav-Cam alignment for a single image, see "Clear Alignment" on page 52.</p>

Table 7 Maps Options Menu Overview (5 of 6)

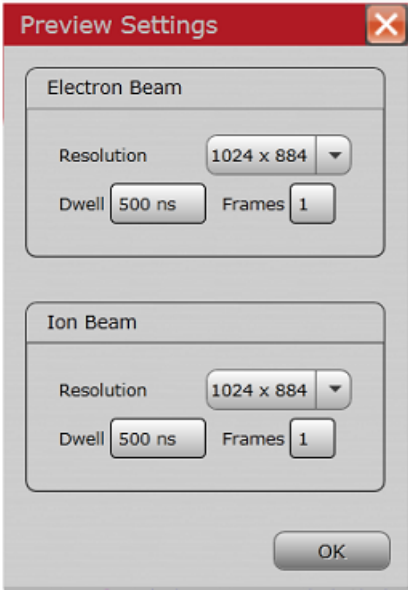
Menu Selection	Description		
Preview Tile Settings	<p><i>Disabled for offline</i></p> <p>Displays the Tile Preview Settings window and the scan settings that will be used to acquire preview images.</p>		
			
Do Not Change Lens Mode	<p>When this option is enabled, it will prevent the Maps application 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.</p>		
<table border="1" style="width: 100%;"> <tr> <td style="background-color: #f4a460; color: white; text-align: center; vertical-align: middle; width: 30px;">WARNING!</td> <td> <p>The Do Not Change Lens Mode feature exists to lock-out 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 disable this feature 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.</p> </td> </tr> </table>		WARNING!	<p>The Do Not Change Lens Mode feature exists to lock-out 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 disable this feature 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.</p>
WARNING!	<p>The Do Not Change Lens Mode feature exists to lock-out 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 disable this feature 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.</p>		
Prefer scan rotation	<p>If there is viewer rotation when creating a new tile set, the rotation will either be applied as stage rotation or scan rotation.</p> <ul style="list-style-type: none"> • When selected: Uses scan rotation. • When not selected: Uses stage rotation. 		
Stitch Tile Sets When Done	<p><i>Disabled for LM and EM offline.</i></p> <p>Automatically adds a stitching job to the end of the job queue for each completed acquisition job.</p>		

Table 7 Maps Options Menu Overview (6 of 6)

Menu Selection	Description
Turn Off Beam When Done	<i>Disabled for LM and EM offline.</i> Turns off the beam after any queued acquisition and stitching jobs are completed.
Retract Detectors when Done	<i>Disabled for LM and EM offline.</i> Retracts any insertable detectors after any queued acquisition and stitching jobs are completed.
Sleep When Done	<i>Disabled for LM and EM offline.</i> 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.
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. <i>Note that this is a slower process and takes more memory.</i> <ul style="list-style-type: none"> • Pan: To make a sweeping movement • Zoom: To simulate movement rapidly away from or toward a subject using a zoom lens

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.
- 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.
- Consider using larger dwell times as higher image quality may reveal small features that facilitate image recognition.

- 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.
- Recommended overlap for systems with a mechanical stage (e.g., Quanta FEG):
 - HFWD > 50 μm overlap: 10%
 - HFWD 20–50 μm overlap: 15%
 - HFWD < 20 μm overlap: 20%

If stitching is still unsuccessful, consider increasing the overlap even more.

Help

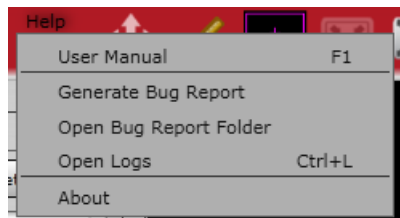
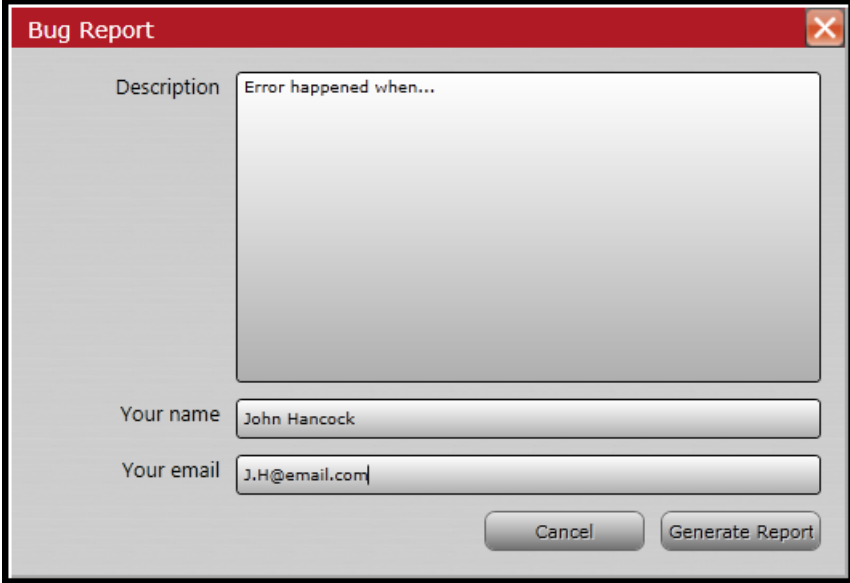


Table 8 Help Menu Overview (1 of 3)

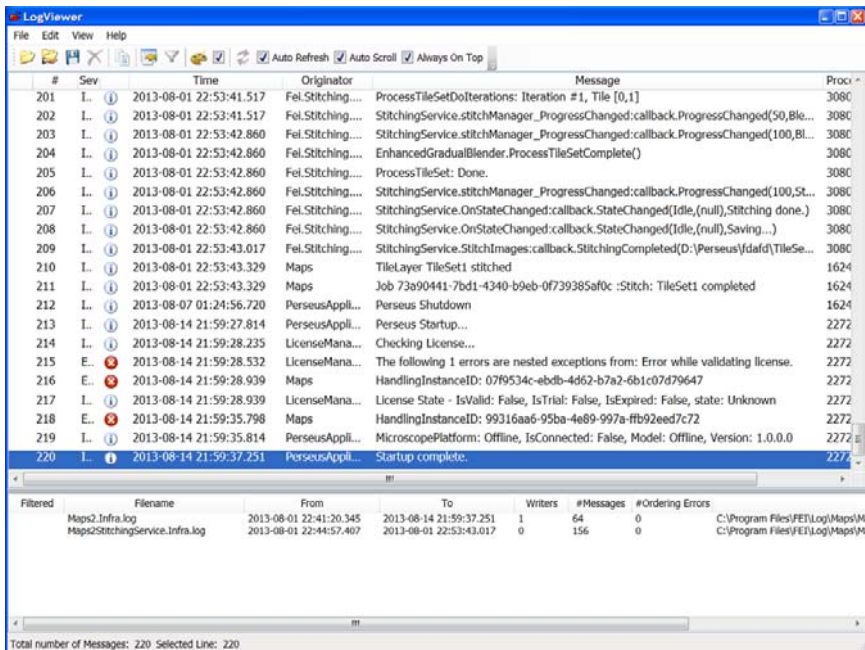
Menu Selection	Description
User Manual (F1)	Displays a PDF of this user’s guide.

Table 8 Help Menu Overview (2 of 3)

Menu Selection	Description
Generate Bug Report	Displays the Bug Report dialog used to generate a bug report. 
Open Bug Report Folder	Displays the folder where the generated bug reports are stored.

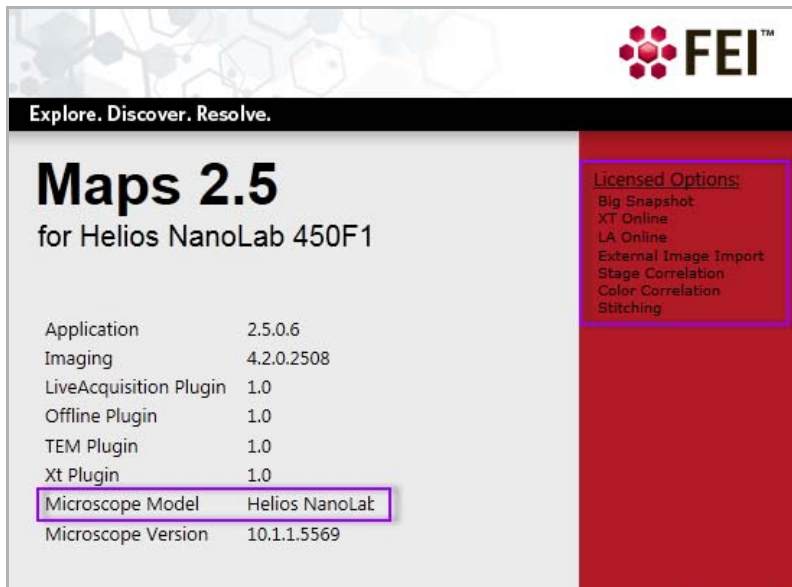
Enter a description of the issue and your name and email address. The Maps development team will contact you if more details are needed.

Table 8 Help Menu Overview (3 of 3)

Menu Selection	Description
Open Logs (Ctrl+L)	<p>Displays the Log Viewer.</p> 

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 Box

Figure 3 Tool Box, Electron Microscope

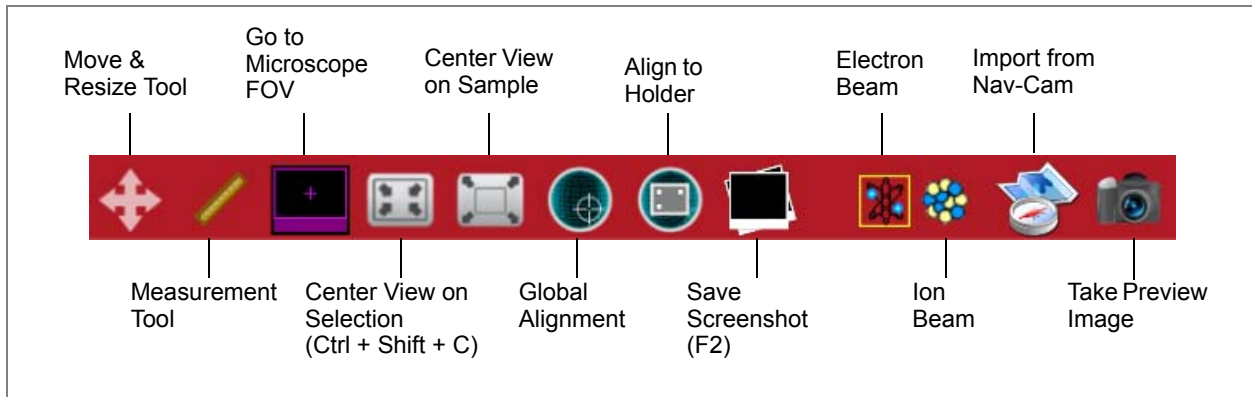


Figure 4 Tool Box, Light Microscope

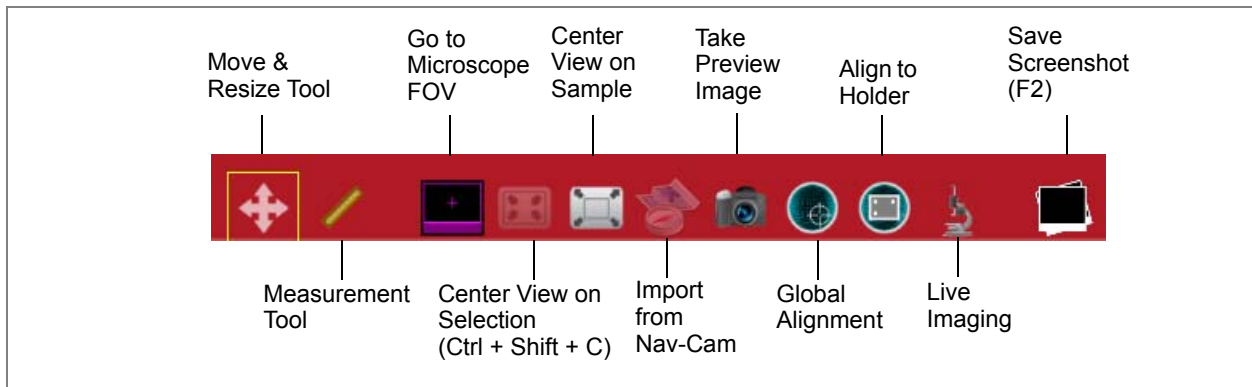


Table 9 Tool Box Overview (1 of 3)

Tool	Description
Electron Beam (SEM) <i>(EM, Online version only)</i>	Selects the Electron beam for imaging.
Ion Beam (FIB) <i>(EM, Online version only)</i>	Selects the Ion beam for imaging.
Move & Resize Tool	Moves or resizes the tile grid with click and drag.

Table 9 Tool Box Overview (2 of 3)

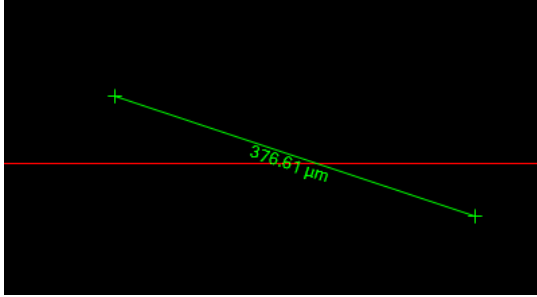
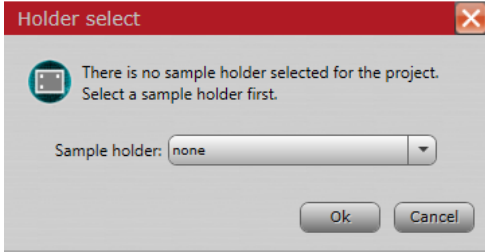
Tool	Description
Measurement Tool	Draws a line and displays the length as a callout.
	
Go to Microscope FOV	Pans and zooms the view to match the microscope (electron beam) field of view (FOV).
Center View on Selection (Ctrl + Shift + C)	<i>Disabled until a project is open.</i> Pans & zooms the viewer so that the currently selected layer item is centered.
Center View on Sample	Zooms out to the full sample view.
Import from Nav-Cam (EM with Nav-Cam version only)	<i>Disabled only while under vacuum if the door mounted Nav-Cam is installed. See “Nav-Cam Prompt” on page 69.</i> 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 79.
Take Preview Image	Acquires a SEM image at the current stage position and HFW and places it on the Maps viewer as a preview image. The HFW can be changed from the Tile right-click menu selection: “Preview Here” on page 57.
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 159.

Table 9 Tool Box Overview (3 of 3)

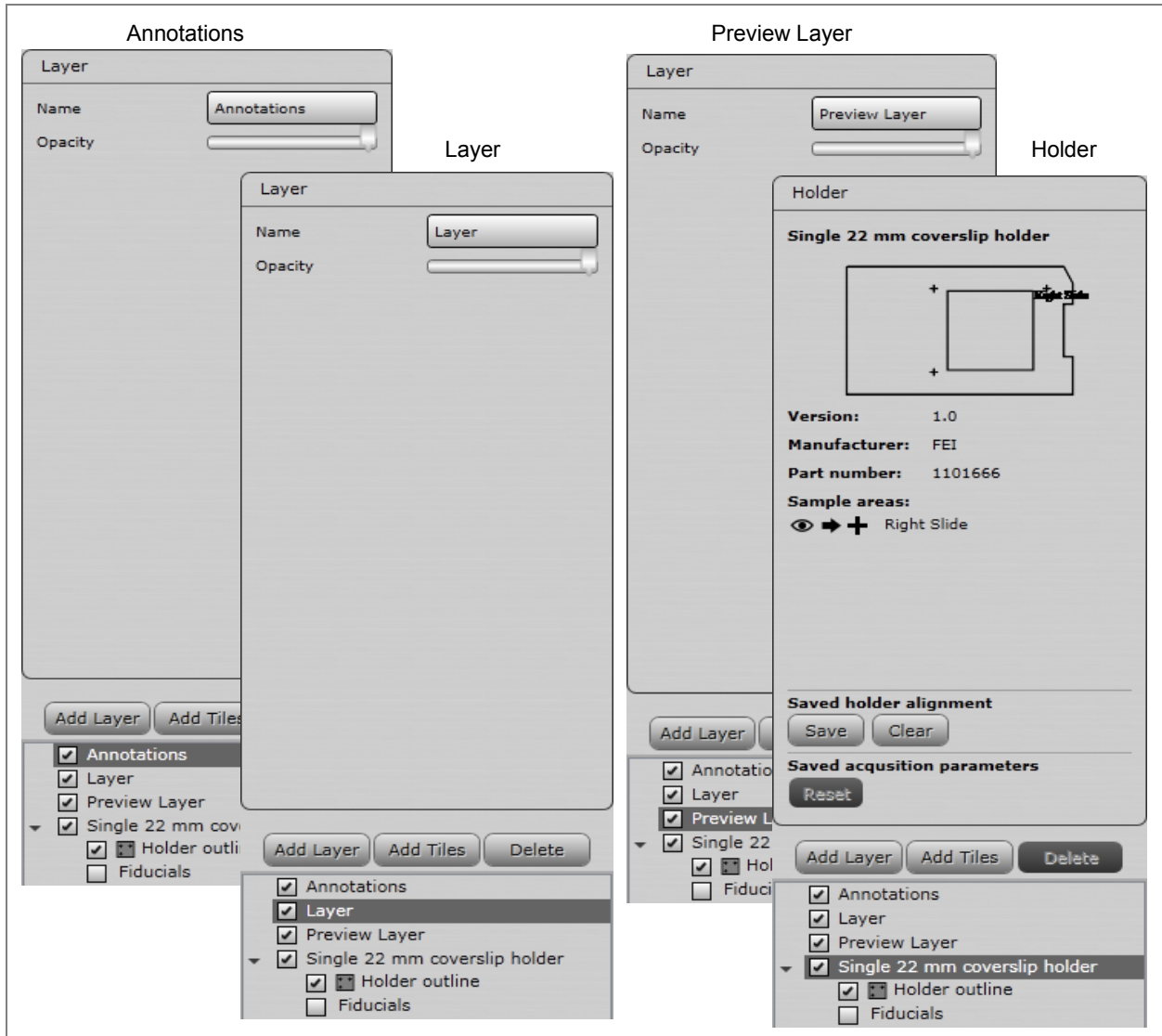
Tool	Description
Align to Holder	<p>Performs an Automated Holder Alignment; requires a specific holder hardware.</p> <p>If no sample holder has been selected for the project, a dialog box displays to prompt you to select a holder.</p> 
	<p>See <i>“Automated Holder Alignment”</i> on page 98.</p>
Live Imaging (LM version only)	<p>Toggles live imaging on and off. See <i>“Live Imaging”</i> on page 139.</p>
Save Screenshot (F2)	<p>Briefly displays a dialog box with the saved (automatically incremented) file name.</p> <p>Also available from File > Save Screenshot.</p>

Layer/Holder Definition Control

Displays controls/information for the selection in the Layer control: Annotations, Layer, Preview Layer, or Sample Holder.

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

Figure 5 Layer/Holder Definition Control



Tile Set Tab

When you create a new tile set, the parameters for basic tile acquisition are populated with defaults.

Figure 6 Tile Set Tab, EM and LM Views

EM View

LM View

Lock methodology: The three Tile controls in the EM view 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.

Table 10 Tile Set Tab Overview (1 of 3)

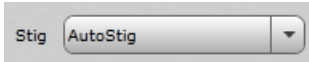
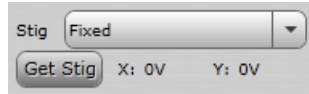
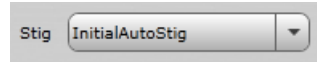

Control	Description
Name	Displays the name of the current tile set.
Acquisition Type (EM View only)	Specifies the beam to be used for imaging. Choices are: Electron or Ion.
Tiles X, Y	Sets the number of tiles in the X and Y directions.
Tile HFW	<ul style="list-style-type: none"> For EM: Specifies the horizontal field width (HFW) for each tile. For LM: Not changeable. This is determined by the selected objective lens
Total HFW	Shows the total HFW for all of the tiles.
Resolution	Specifies the image resolution. Choices will be specific to each system type. This is a read-only field for the LM.
Dwell (EM View only)	Specifies the amount of time the beam dwells on each pixel when images are acquired.
Stigmation Options: (EM View only)	<p>Specifies the stigmation options:</p> <ul style="list-style-type: none"> 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. 
Frames (EM View only)	Sets the number of image frames.

Table 10 Tile Set Tab Overview (2 of 3)


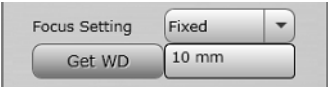

Control	Description
Focus Setting	<p>Specifies the focus setting. Choices are:</p> <ul style="list-style-type: none"> • None: The application will not touch the focus at all. You are free to change the focus via the xT UI during the run. • Autofocus: Runs the autofocus routine at each tile before acquisition. This can result in better focus per tile, but adds considerably more time to acquisition.  <ul style="list-style-type: none"> • Fixed: Uses the same working distance for every tile.  <ul style="list-style-type: none"> • 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.  <p>Procedure: Navigate to the first location. Manually focus the image. When image is in focus, click Set WD1 button. The current working distance will be remembered for that point and the button will now be checked. Repeat for the other two Set WD buttons. Once all three are checked, tiles are ready to be acquired.</p> <p>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.</p> <p>Click Reset to clear all three WD settings.</p> <ul style="list-style-type: none"> • 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.
Get WD (EM View only)	Reads the current working distance from the system.
Pixel Size (LM View only)	Displays the physical size of a single pixel at the current acquisition settings. This is based on Tile HFW and Resolution.
Overlap X, Y (LM View only)	Displays the percentage of overlap between tiles in the tile set as well as the pixels.
Stitching Channel (LM View only)	Selects the channel to be used for stitching reference. It is best to choose the channel that will have the most image data.

Table 10 Tile Set Tab Overview (3 of 3)

Control	Description
Channels (LM View only)	Lists the channels that are selected for acquisition.
Z-Stack Option (LM View only)	Displays the Z-stack option selected, if any. See “Z-Stack Browser” on page 144 .
Focus Option (LM View only)	Displays the Focus option selected, if any.
Estimated Time	Displays the estimated time for completing the image acquisition of the total tile set.
Time Series (LM View only)	Allows a tile set acquisition to be acquired multiple times at a specified time interval to collect images of a sample that is changing with time.
Shading Correction (LM View only)	Uses a blank reference image to correct (remove) shading effects in all images acquired inside the tile set.
Acquire	Immediately acquires an image of the current tile in the tile set using the specified parameters. Acquisition will stop if the beam goes off.
Stitch	Enabled after tile set(s) are acquired. Begins stitching process.

Advanced Tab

EM View

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

Figure 7 Advanced Tab, EM View

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.

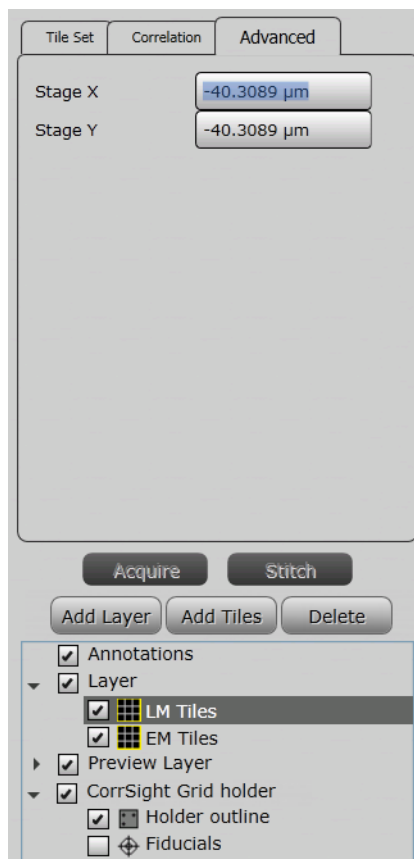
Table 11 Advanced Tab EM View Overview (1 of 2)

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.
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.
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: <ul style="list-style-type: none"> • Raster: Left to right, then top to bottom. • Serpentine: Alternating left to right pattern per row, running top to bottom. • Spiral: 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.
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.

Table 11 Advanced Tab EM View Overview (2 of 2)

Control	Description
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.
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.

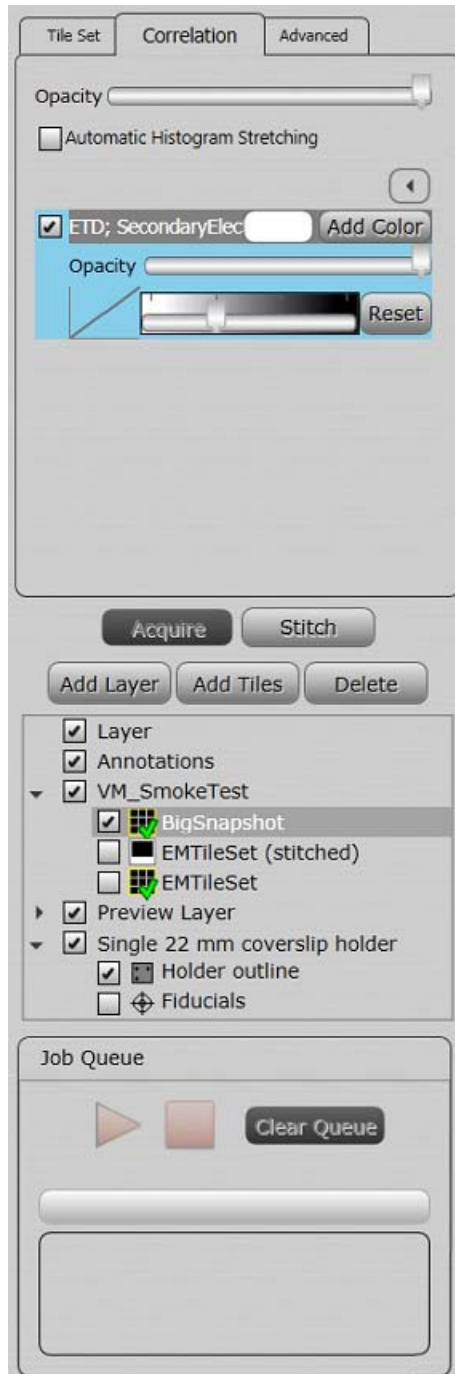
LM View



Only Stage X and Stage Y display for LM view. They set the X and Y stage locations of the center of the tile grid.

Correlation Tab

Use the controls on this tab to apply simple coloring to the tile set images when acquired. See [“Correlation and Alignment” on page 148](#) for descriptions of the controls and histogram.



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.

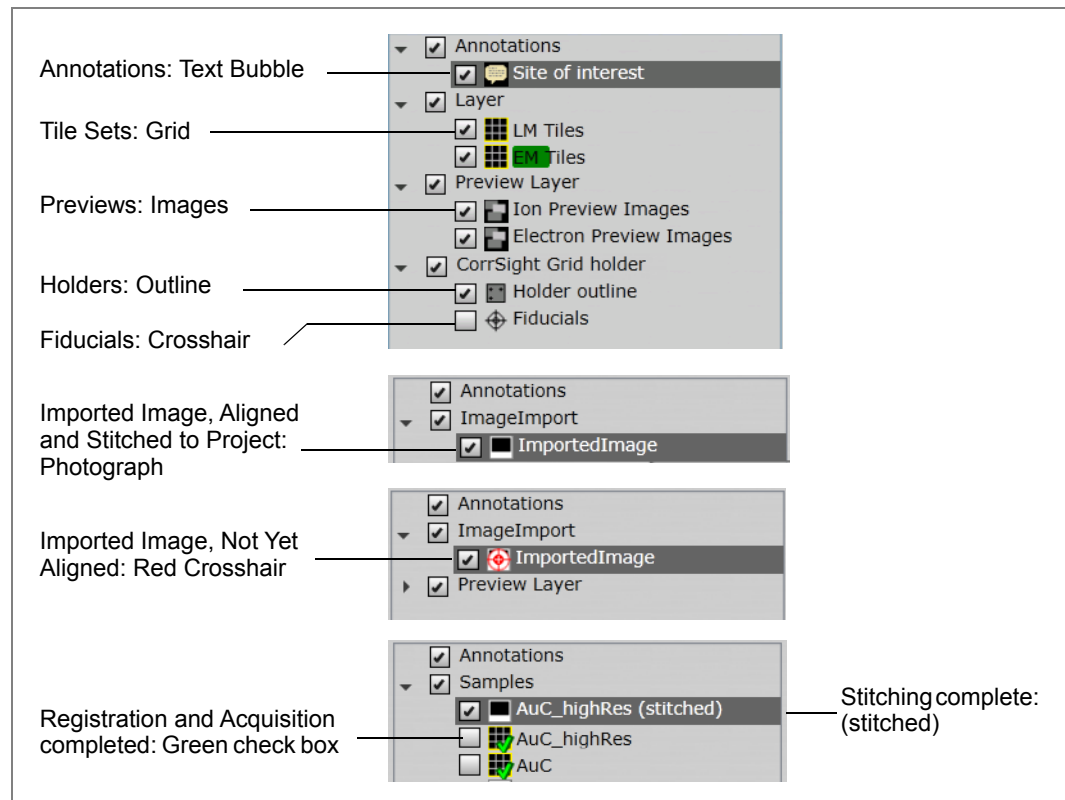
Double-click on an item in the Layer control to center it in the viewer.



Icons

Each created layer item has a unique icon.

Figure 8 Layer Control Icons



Progress Bar

The tile set in the Layer control has a double progress bar behind it.

- The dark green represents the progress of Registration.
- The light green represents the progress of Acquisition.
- When both Registration and Acquisition have completed, the bar disappears and the green check mark appears in the Tile set icon.

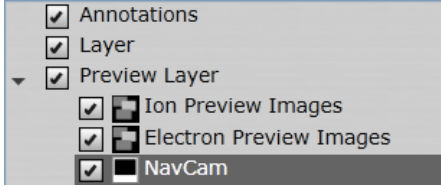


Controls

Table 12 Layer Control Overview (1 of 2)

Control	Description
Add Layer	Adds another Layer to the tree. When you have multiple layers, the selected layer will be green and the nonselected layer(s) will be yellow.
Add Tiles	Displays the New Tile Layer window. Select either LM Tile Set or EM Tile Set in the Layer Type field. <div data-bbox="518 972 909 1138" data-label="Image"> </div> <p>Enter a name and click OK. The new name is added.</p> <div data-bbox="519 1184 1187 1457" data-label="Image"> </div> <p>Select the template to be used. It defaults to the microscope default.</p>
Delete	Prompts for confirmation, and then deletes the highlighted item in the tree.
Annotations	Shows/hides the annotation layer(s). Annotations can be areas of interest (AOI) or sites of interest (SOI). See "Add area of interest" on page 56 and "Add Site of Interest" on page 59 .
Layer	Displays a list of existing tile sets or imported images for each layer.

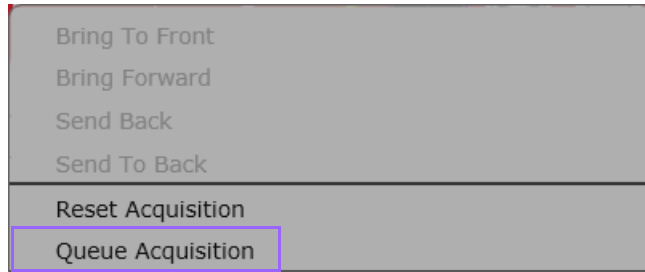
Table 12 Layer Control Overview (2 of 2)

Control	Description
Preview Layer	<p>Displays the individual Preview layers in the Layer control. The Nav-Cam image will also appear in the Layer control.</p>  <p>See <i>“Nav-Cam Alignment” on page 79</i>. After saving the alignment, the same alignment is applied to each a new Nav-Cam image.</p>
Sample Holder	Displays the name of the selected default sample holder

Tile Set Multi Select

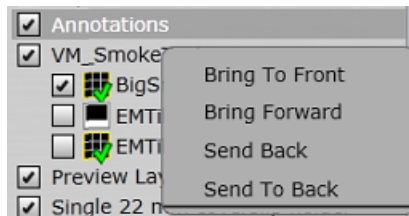
To quickly line up multiple tile sets for the Job Queue:

1. Hold down **Ctrl** while left-clicking on Layer items.
2. Right-click (with **Ctrl** still held down) to access the context menu.
3. Select **Queue Acquisition**.



Annotations Right-Click Menu

Right-click on Annotations to access a context menu for moving the annotation layer forward or backward.



Right-click on a created annotation to access additional choices in the context menu.

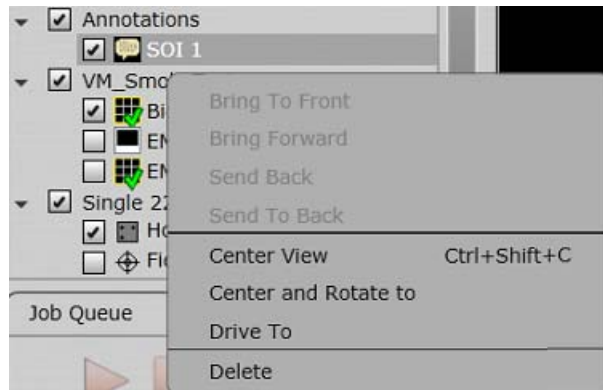
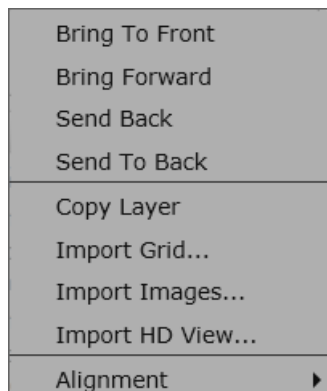


Table 13 Annotation Right-Click Menu Overview

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.
Center View (Ctrl + Shift + C)	Centers the view on the created annotation.
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.

**Table 14 Layer Right-Click Menu Overview (1 of 3)**

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.
Copy Layer	Copies all tile sets in the layer to a new layer.

Table 14 Layer Right-Click Menu Overview (2 of 3)

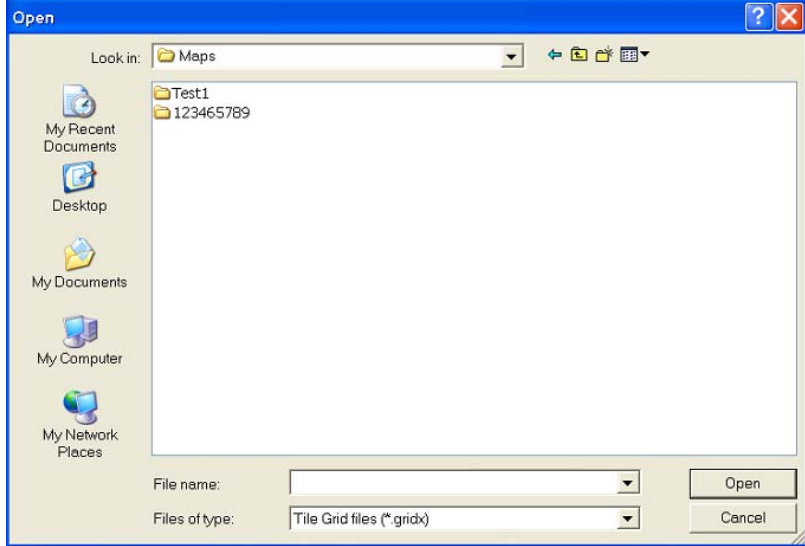
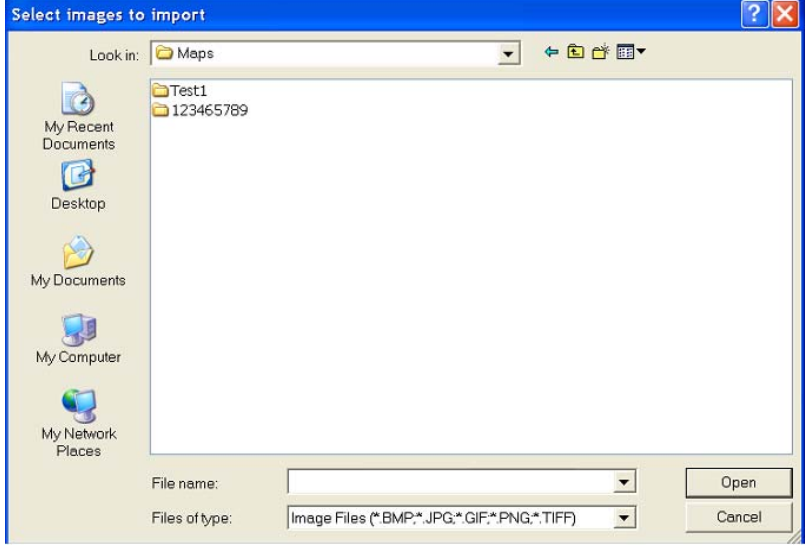
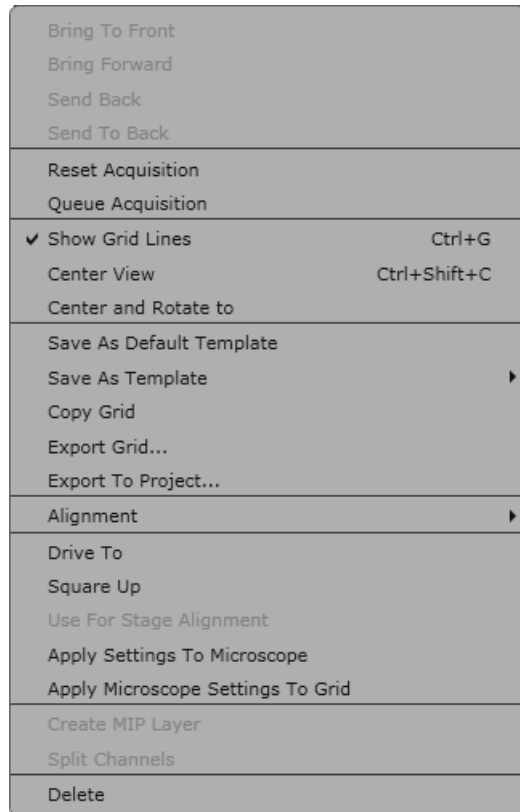
Menu Selection	Description
Import Grid	Displays a standard Open window for browsing to a saved tile grid file.
	
Import Image	Displays a standard Open window for browsing to a saved image file from another source, e.g., an optical microscope.
	
<p>These images, once aligned to the stage, can provide a navigation guide for the user to find areas of interest, like the FEI Nav-Cam does.</p> <p>See “Importing Images from Other Sources” on page 87.</p>	
Import HD View	Displays a standard Browse window for selecting an HD View image to import into Maps.

Table 14 Layer Right-Click Menu Overview (3 of 3)

Menu Selection	Description
Align Layer	Aligns an entire layer, including all tile sets and images, at once. Similar to Global Alignment, but for a subset of data. See <i>"Alignment" on page 159</i> .

Tile Set Right-Click Menu

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

**Table 15 Tile Set Right-Click Menu Overview (1 of 4)**

Menu Selection	Description
Bring to Front	Moves the tile set layer to the front (topmost) layer.
Bring Forward	Moves the tile set layer forward one layer.
Send Back	Moves the tile set layer back one layer.
Send to Back	Moves the tile set layer to the lowest layer.
Reset Acquisition	Clears all acquired image data in the selected tile set and unlocks the parameter fields in the Basic tab for editing.

Table 15 Tile Set Right-Click Menu Overview (2 of 4)

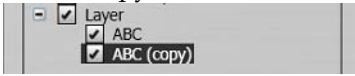
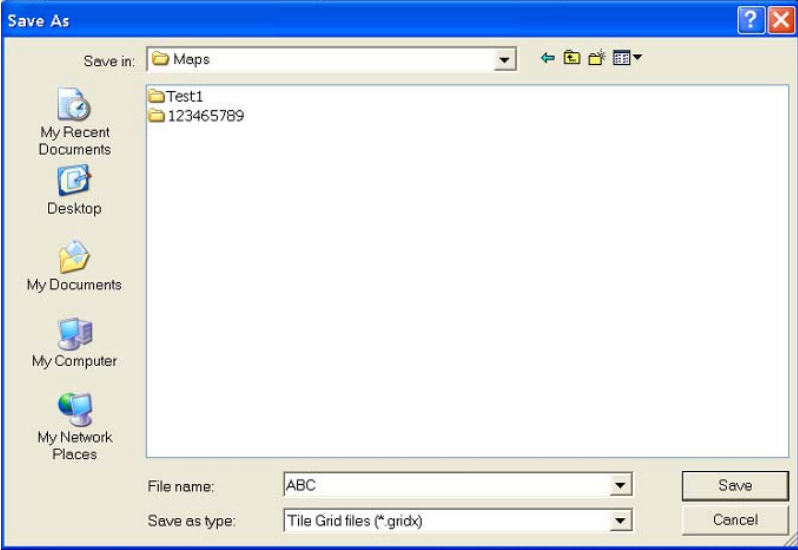
Menu Selection	Description
Queue Acquisition	Moves the selected tile set into the job queue for acquisition. See <i>"Tile Set Multi Select"</i> on page 44.
Show Grid Lines	Toggles to show/hide the tile grid lines onscreen. Shortcut = Ctrl + G .
Center View	Pan & zooms the viewer so that the currently selected tile grid is centered. Shortcut keys = Ctrl + Shift + C . <i>(This is the same functionality as the Center View on Selection, see page 30.)</i>
Center and Rotate to	Pan, zooms, and digitally rotates the viewer so that the currently selected tile grid is centered and shown at its natural rotation.
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	Adds a copy of the selected tile set to the list beneath Layer. 
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.

Table 15 Tile Set Right-Click Menu Overview (3 of 4)


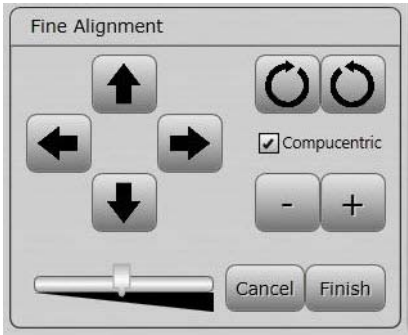
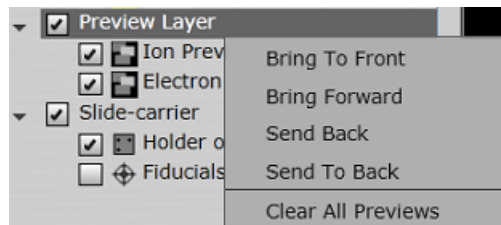
Menu Selection	Description
Alignment:	
Align	Aligns a single tile set or image. Similar to Global Alignment, but for a single item. See <i>"1-, 2-, or 3-Point (Coarse) Alignment"</i> on page 159.
Fine Alignment	<p>Use Fine Alignment to fine-tune the alignment of layers and tile sets visually.</p> <p>The Fine Alignment controls only display after clicking Next after a 1-, 2-, or 3-point alignment.</p> 
	<p>Up, Down, Left, Right Arrows: Moves the image in the direction of the arrow.</p>
	<p>Rotate Right, Rotate Left: Rotates the image in the direction of the circular arrows.</p>
	<p>Compucentric check box:</p> <ul style="list-style-type: none"> • 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.
	<p>-/+: Zooms in and out on the image.</p>
	<p>Sensitivity Slider: Sets the sensitivity for all of the fine alignment controls: Left = fine; Right = coarse.</p>
	<p>Back: Returns to the selection of alignment points in the coarse alignment procedure. From there, click Cancel if you want to undo the coarse alignment.</p>
	<p>Finish: Closes the Fine Alignment control area.</p>
Clear Alignment	Clears any tile set alignment that has been applied in Maps.
Drive To	Drives to the selected tile set.

Table 15 Tile Set Right-Click Menu Overview (4 of 4)

Menu Selection	Description
Square Up	Rotates the stage to match the orientation of the tile set.
Use for Stage Alignment (EM only)	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.
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.
Split Channels	Divides the individual channels of a tile set into separate tile sets. <i>Note: This operation is permanent and cannot be undone.</i>
Delete	Deletes the selected tile set.

Preview Layer Right-Click Menu

Right-click on Preview Layer to access a context menu.



Right-click on a single preview image to see a menu selection for clearing only the selected image.

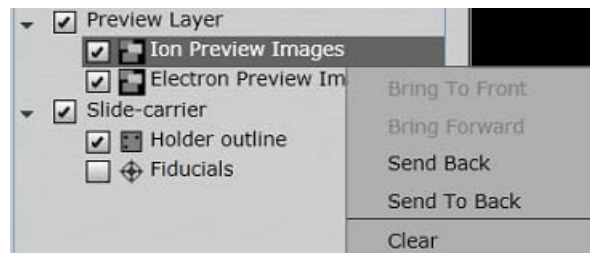


Table 16 Preview Layer Right-Click Menu Overview

Menu Selection	Description
Bring to Front	Moves the preview layer to the front (topmost) layer.
Bring Forward	Moves the preview layer forward one layer.
Send Back	Moves the preview layer back one layer.
Send to Back	Moves the preview layer to the lowest layer.
Clear All Previews	Clears all preview images.
Clear	Clears previews from the selected preview layer only.

Preview Layer, Nav-Cam Right-Click Menu

Right-click on Nav-Cam in the Preview Layer to access a context menu. Nav-Cam has some unique selections. See [“Tile Set Right-Click Menu” on page 47](#) for the common selections.

Table 17 Preview Layer, Nav-Cam Right-Click Menu Overview (1 of 2)

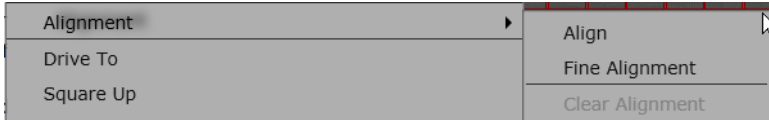
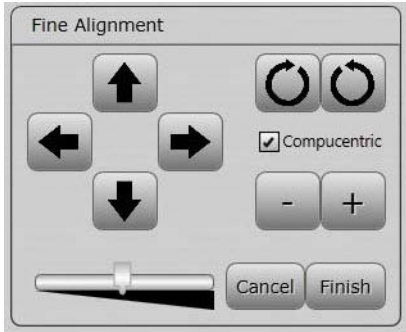
Menu Selection	Description
Alignment:	
Align	Aligns a single tile set or image. Similar to Global Alignment, but for a single item. See “1-, 2-, or 3-Point (Coarse) Alignment” on page 159 .

Table 17 Preview Layer, Nav-Cam Right-Click Menu Overview (2 of 2)

Menu Selection	Description
<p>Fine Alignment</p>	<p>Use Fine Alignment to fine-tune the alignment of layers and tile sets visually.</p> <p>The Fine Alignment controls only display after clicking Next after a 1-, 2-, or 3-point alignment.</p>  <p>Up, Down, Left, Right Arrows: Moves the image in the direction of the arrow.</p> <p>Rotate Right, Rotate Left: Rotates the image in the direction of the circular arrows.</p> <p>Compucentric check box:</p> <ul style="list-style-type: none"> • 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. <p>-/+: Zooms in and out on the image.</p> <p>Sensitivity Slider: Sets the sensitivity for all of the fine alignment controls: Left = fine; Right = coarse.</p> <p>Back: Returns to the selection of alignment points in the coarse alignment procedure. From there, click Cancel if you want to undo the coarse alignment.</p> <p>Finish: Closes the Fine Alignment control area.</p>
<p>Clear Alignment</p>	<p>Clears the Nav-Cam alignment for the individual Nav-Cam image. For clearing the default alignment for all Nav-Cam images, see “Nav-Cam: Clear Alignment” on page 23.</p>
<p>Save Nav-Cam Alignment</p>	<p>After saving, all subsequent Nav-Cam acquisitions using Maps will use this alignment.</p>

Job Queue

Contains process control buttons, the remaining time for the process to complete, a progress bar, a list of tile sets that are lined up for acquisition, and a **Clear Queue** button.

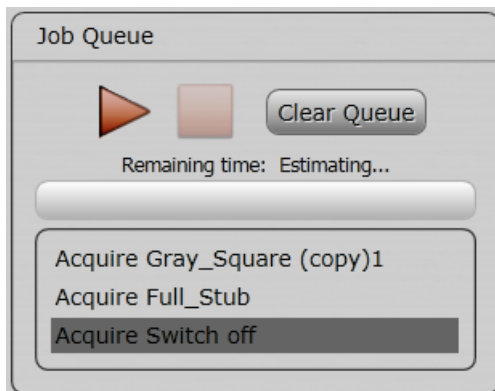




Table 18 Job Queue Overview

Control	Description
Start 	<i>Button is enabled after Stop is clicked.</i> Restarts the current acquisition or stitching process.
Stop 	<i>Button is enabled after acquisition or stitching is started.</i> Stops the acquisition or stitching process. Click Start to resume the process.
Status Area	Displays the progress of the acquisition or stitching process.
Queue	Displays the list of selected tile set(s) for acquisition or stitching.
Clear Queue	Clears the list of selected tile sets for processing.

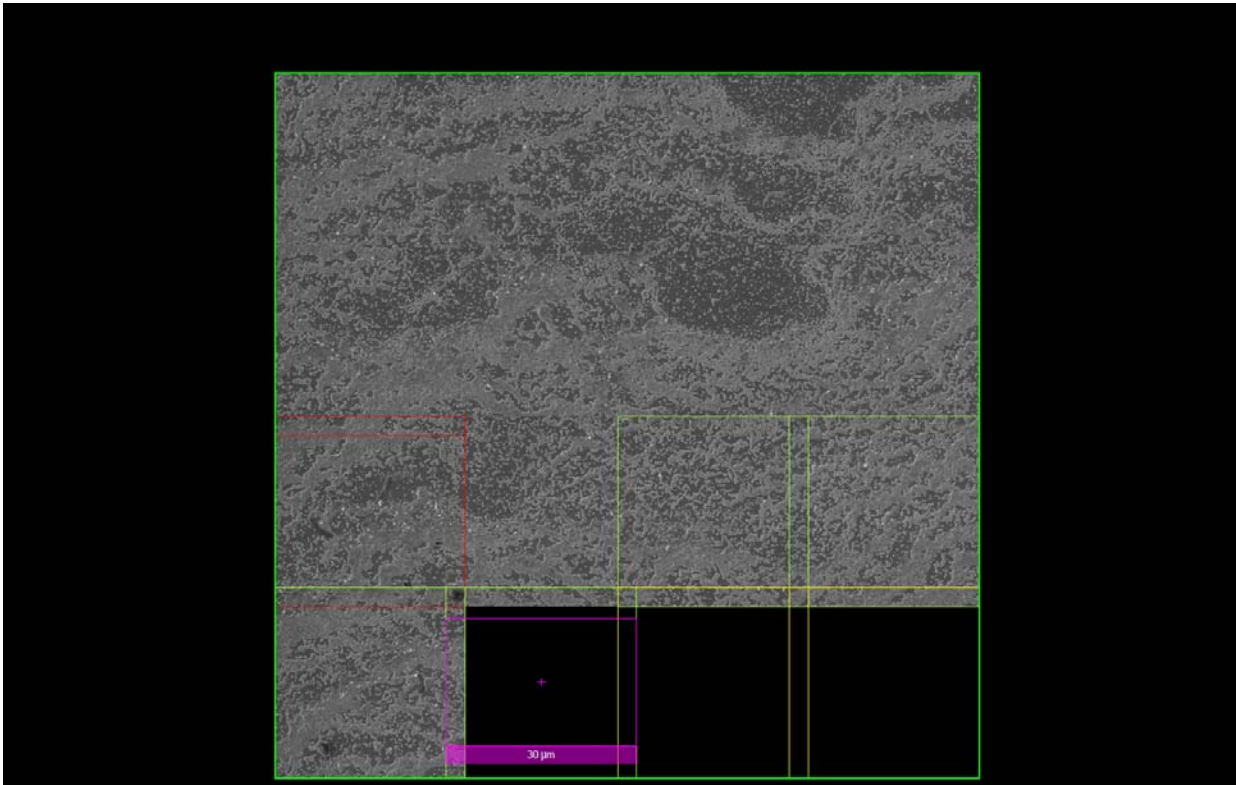
Job Queue, Right-Click Menu

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

Viewer

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).
- 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.



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 91](#))

- **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 91*) will allow previously red tiles to become successful.

Left-Click and Drag Selected Area

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

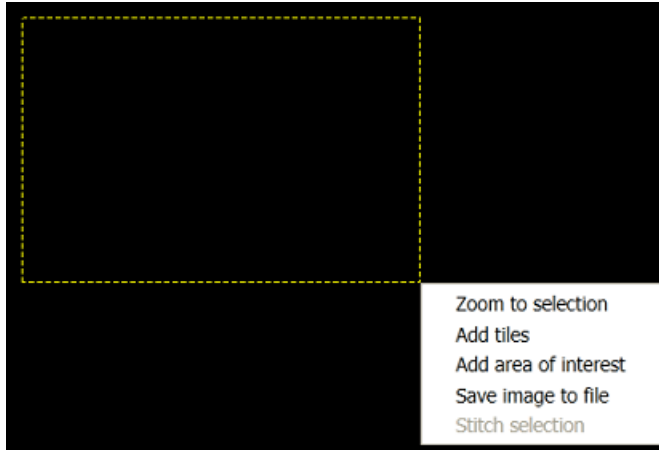
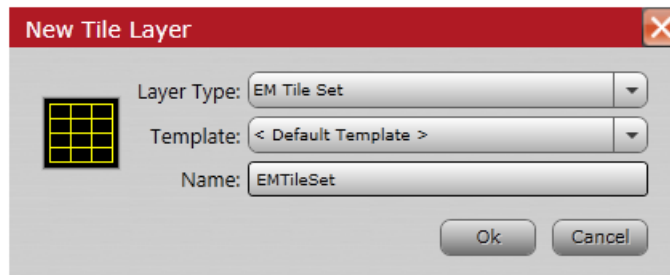


Table 19 Viewer Selected Area Overview

Menu Selection	Description
Zoom to selection	Zooms the field of view to the selected area.

Add tiles Displays a New Tile Layer window for naming a new tile set.



Select a tile set template, enter a name, and then click **OK**. The new tile set will match the settings saved in the tile set template and will be placed in the area selected.

See *“Defining a Tile Set” on page 84*.

Table 19 Viewer Selected Area Overview

Menu Selection	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 62.
Stitch selection	Stitches a subset of tiles, as selected by the rectangle. See “Stitching” on page 89.

Viewer Tile Right-Click Menu

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

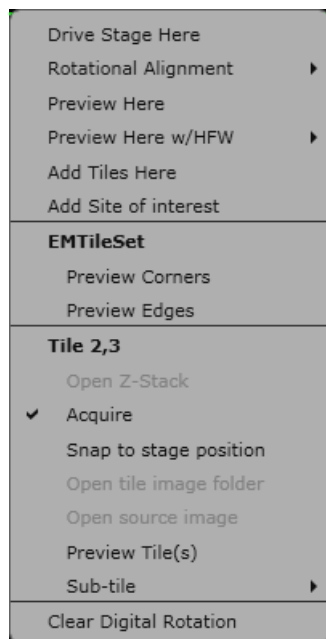


Table 20 Viewer Tile Right-Click Menu Overview (1 of 5)

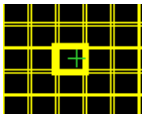
Menu Selection	Description
Drive Stage Here	Moves and centers the stage to the selected tile (see the green cross). 
Rotational Alignment	Allows rotation of the stage to Place Point 1 , Place Point 2 , or Cancel . See <i>“Rotational Alignment (EM and Offline Only)”</i> on page 65.
Preview Here	Acquires a preview SEM image at the selected stage coordinate and current HFW.

Table 20 Viewer Tile Right-Click Menu Overview (2 of 5)

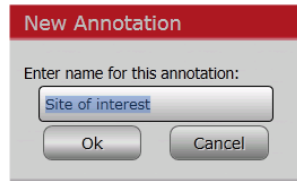
Menu Selection	Description
Preview Here with HFW	<p data-bbox="581 340 1409 401">Acquires a preview SEM image at the selected stage coordinate and at a selected HFW, independent of the tile grid.</p> <p data-bbox="581 407 797 436">Submenu choices:</p> <div data-bbox="587 449 782 722" style="border: 1px solid gray; background-color: #f0f0f0; padding: 5px; margin: 5px 0;"> <p data-bbox="630 464 688 489">2 μm</p> <p data-bbox="630 499 699 525">10 μm</p> <p data-bbox="630 535 699 560">50 μm</p> <p data-bbox="630 571 711 596">100 μm</p> <p data-bbox="630 606 711 632">200 μm</p> <p data-bbox="630 642 711 667">500 μm</p> <p data-bbox="630 678 688 703">1 mm</p> </div> <p data-bbox="581 737 1386 798">This is the same functionality as using the Take Preview Image tool box button. See <i>"Take Preview Image"</i> on page 30.</p>
Add Tiles Here	<p data-bbox="581 821 1224 846">Adds a new Tile Grid centered on the clicked location.</p>

Table 20 Viewer Tile Right-Click Menu Overview (3 of 5)

Menu Selection	Description
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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.



Note: This is different from the New Annotation dialog box for creating an Area of Interest, which marks an area of the stage.

The new SOI becomes a new Annotation layer in the Layer control. Add notes and assign a color to display in the viewer.

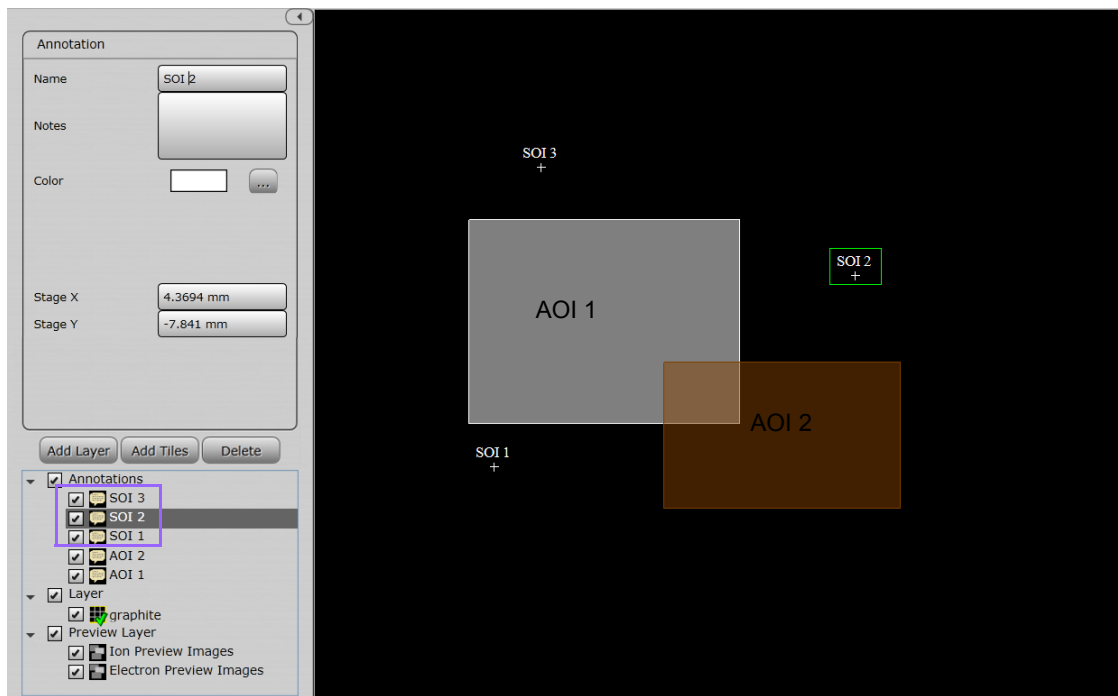


Table 20 Viewer Tile Right-Click Menu Overview (4 of 5)

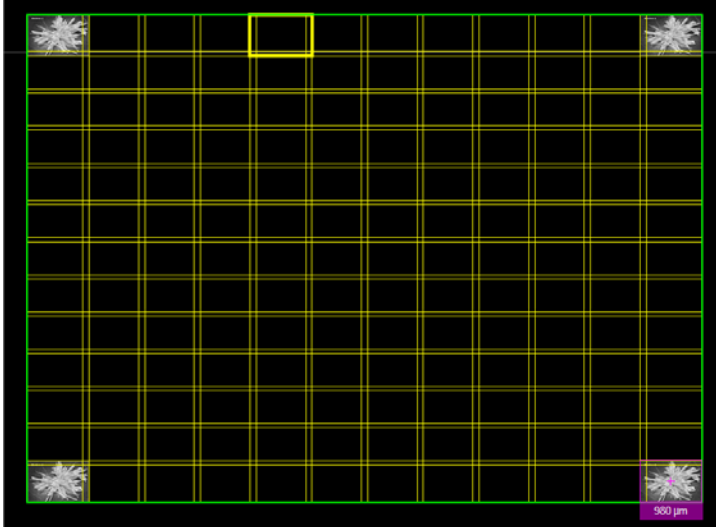
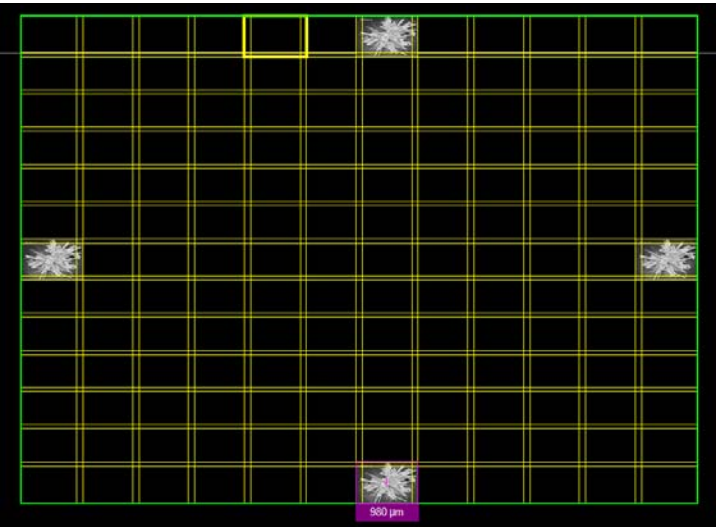
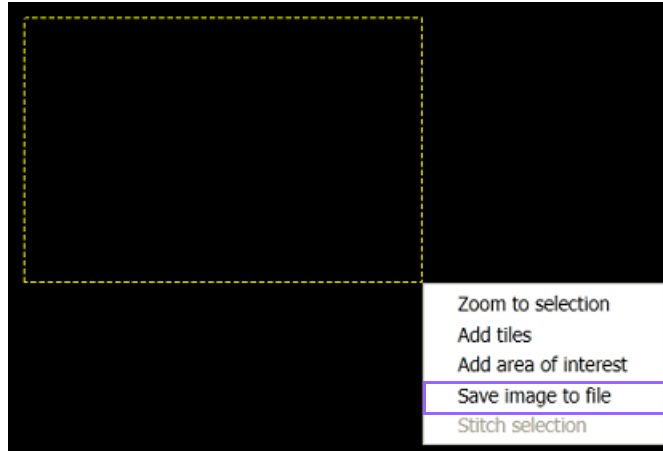
Menu Selection	Description
Sample (EM tiles in this example)	Displays the name of the selected tile set. The submenu has choices to: Preview Corners or Preview Edges.
Preview Corners	After running Preview Corners, preview images appear in the corners of the tile set.
	
Preview Edges	After running Preview 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” on page 144 .

Table 20 Viewer Tile Right-Click Menu Overview (5 of 5)

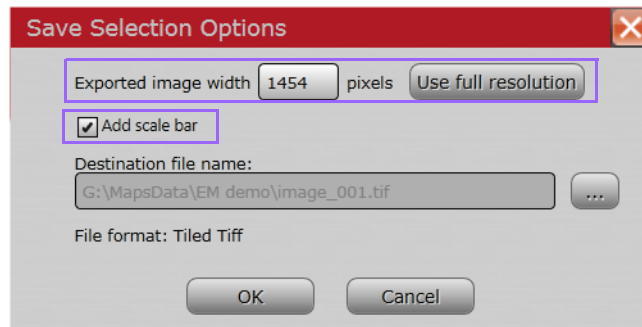
Menu Selection	Description
Acquire/Re-acquire	<ul style="list-style-type: none"> • Acquire: Displays if the tile has not been acquired. • Re-acquire: Displays if the tile has already been acquired.
Snap to Stage Position	Moves the tile to the current stage position.
Open Tile Image Folder	Opens explorer 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)	Displays the image for that tile. Any tiles without pertinent image data can be disabled from image acquisition.
Sub-tile	<p>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.</p> <div data-bbox="586 810 802 1087" style="border: 1px solid black; background-color: #cccccc; padding: 5px; margin: 10px 0;"> <p style="text-align: center;">2 x 2 3 x 3 5 x 5 10 x 10 25 x 25 50 x 50 100 x 100</p> </div> <p>The subtile names are automatically added to the Layers control (TileSet (3,4)).</p>
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.

Save Image to File

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



1. Select **Save Image to File**. The Save Selection Options dialog box appears.

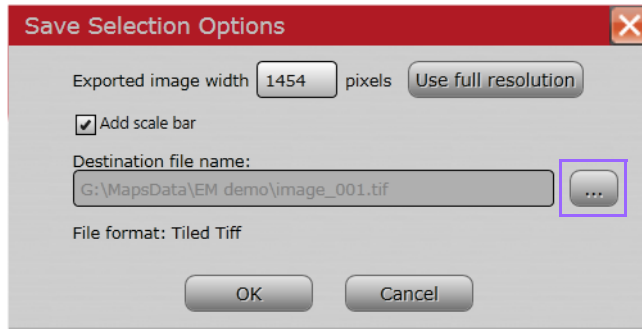


2. Enter a desired resolution (**Export Image Width** in pixels) or click **Use Full Resolution** to set the exported image width to the native pixel size.

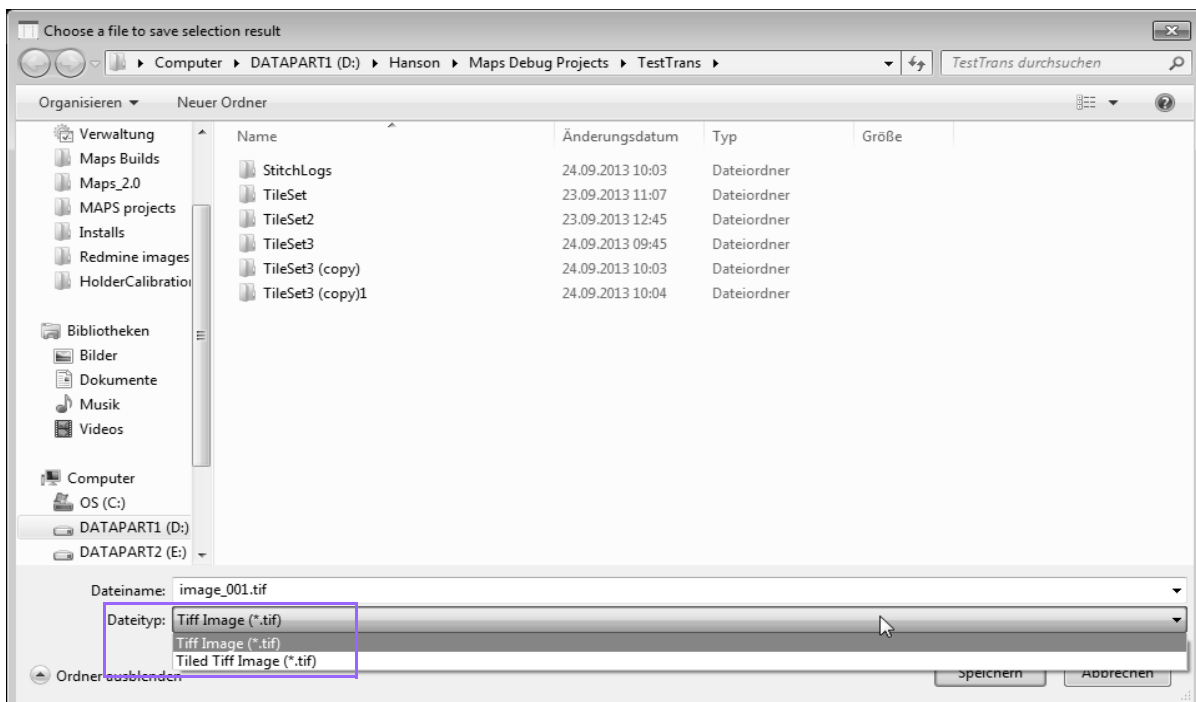
NOTE	Full resolution exports for large selections of high-resolution data are not available for all image types.
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3. Enable **Add Scale Bar** to place the viewer scale bar on the exported image.

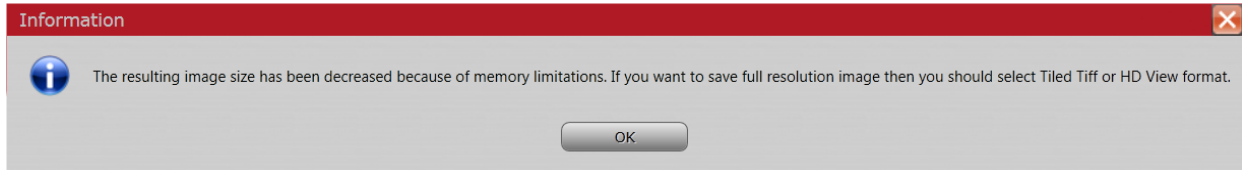
- Click the browse (...) button to display the browser window.



- File type options include Tiff Image and Tiled Tiff Image. Select a file type, file name, and location and then click **Save**.



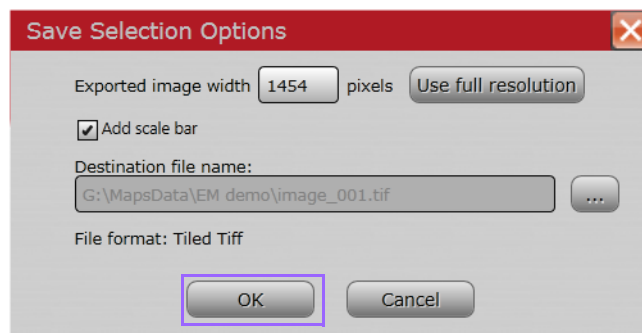
There are limitations for the TIFF file type. If TIFF is desired, but the resolution of the selected area is too large for TIFF export, a warning appears and the exported image width in pixels will be automatically reduced to the maximum for TIFF export.



To save large resolution images, the Tiled TIFF format should be used.

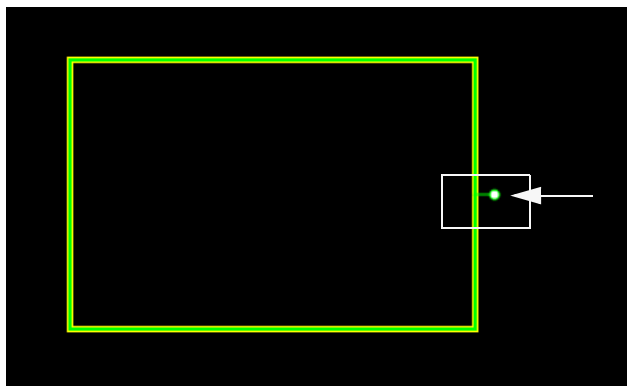
NOTE	Not all image processing software supports the Tiled Tiff format. It is recommended to use the TIFF format to ensure compatibility with external image processing packages.
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- The Save Selection Options window is still open. Click **OK** to close it.



Rotation Control in the Viewer

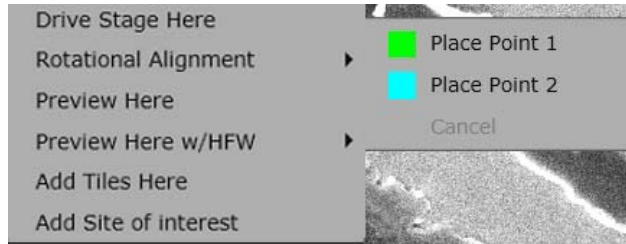
Grab the lollipop looking control with the mouse to easily rotate tile layers and annotation layers.



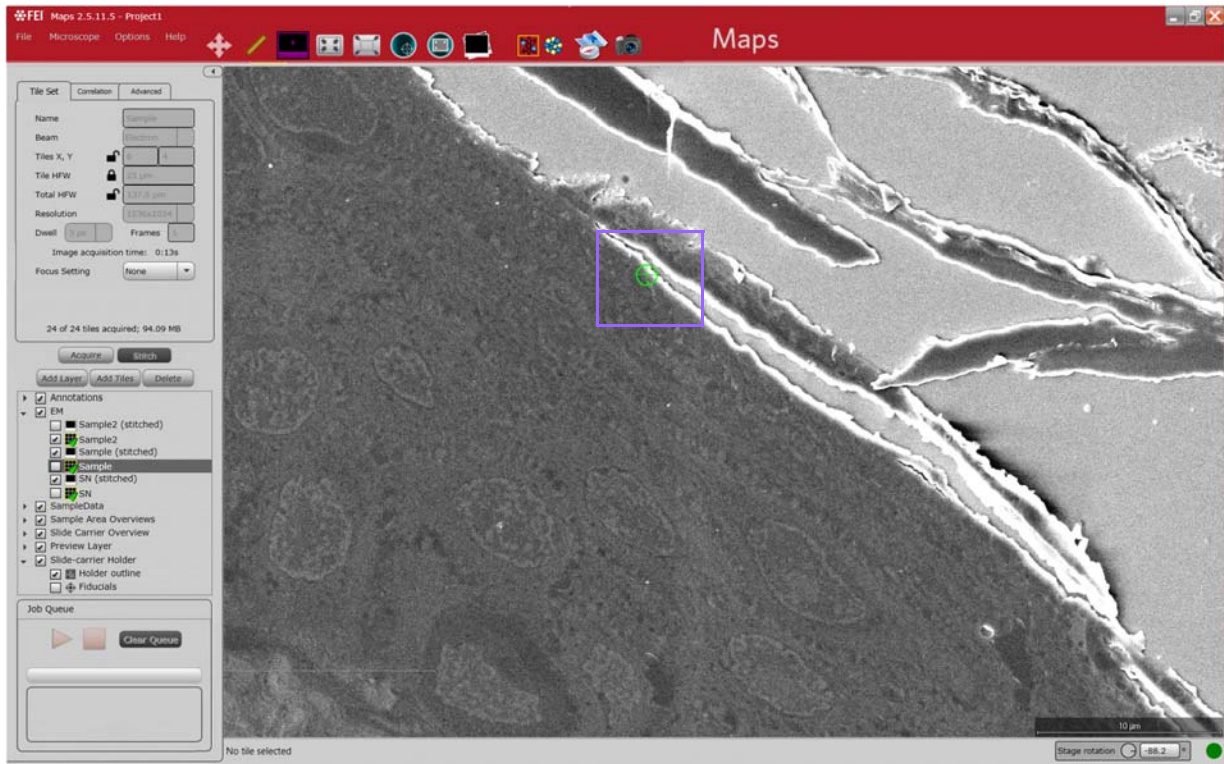
Rotational Alignment (EM and Offline Only)

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.
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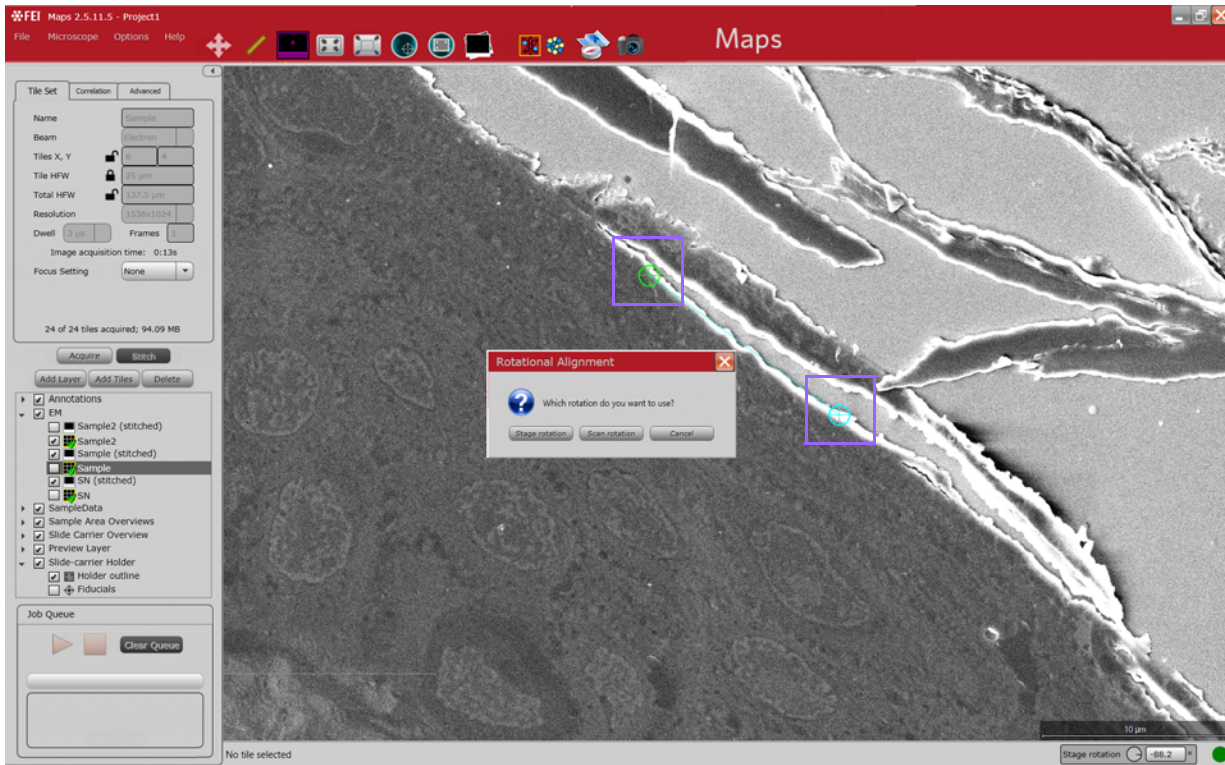
1. Select **Rotational Alignment** from the viewer tile right-click menu to rotate the stage. Click **Place Point 1**.



2. Click and drag the green target that appears to a feature on the image for Point 1.

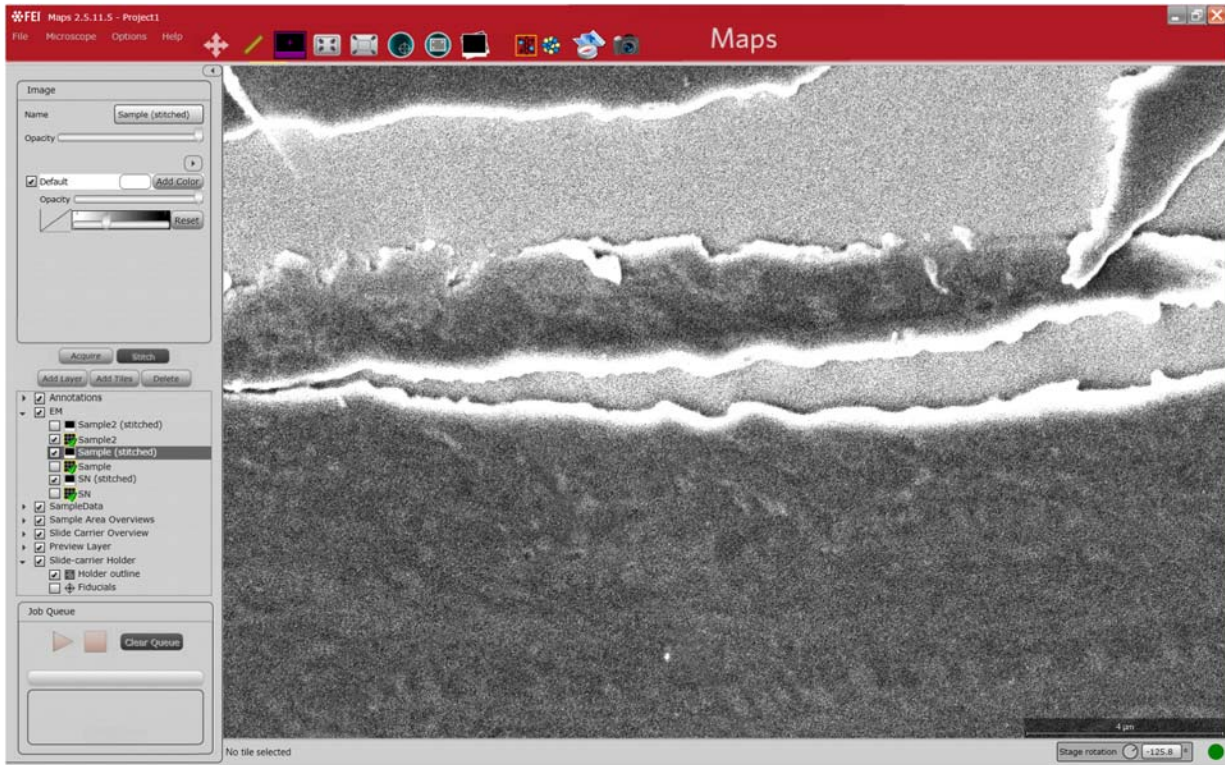


3. Place **Point 2**. A message appears asking which rotation you want to use.

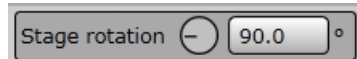


- **Stage Rotation:** Uses stage rotation to align the image.
- **Scan Rotation:** Uses scan rotation to adjust the image.

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

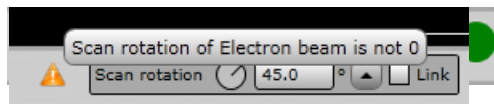


Stage Rotation (EM Only)



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

Scan Rotation



The tooltip indicates that the scan rotation is not at 0 degrees.

3 Basic Operations

Overview

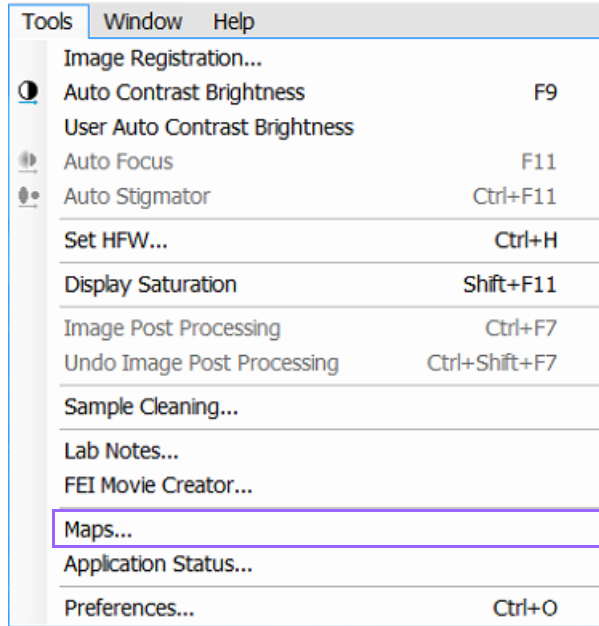
This chapter describes the basic process flow for Maps.

Topics include:

- *“Launching the Application” on page 69*
- *“Creating a Project” on page 71*
- *“Setting Options” on page 75*
- *“Nav-Cam Alignment” on page 79*
- *“Running the Automated Holder Alignment” on page 83*
- *“Defining a Tile Set” on page 84*
- *“Acquiring a Tile Set” on page 86*
- *“Importing Images from Other Sources” on page 87*
- *“Stage Navigation” on page 89*
- *“Stitching” on page 89*

Launching the Application

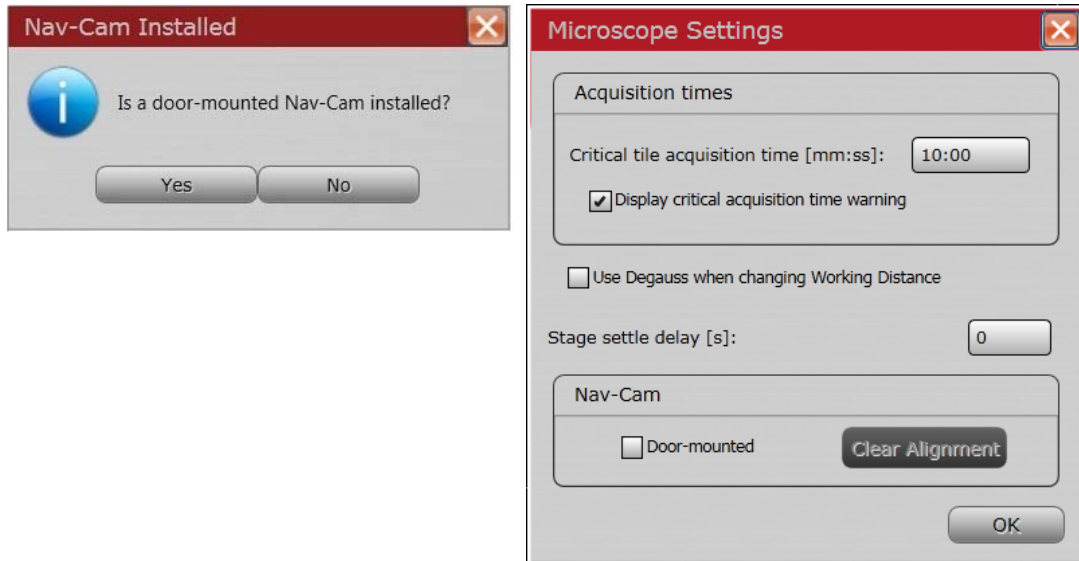
From within the xT user interface, select **Tools > Maps** to launch the Maps software.



From within CorrSight, click the desktop icon to launch Maps.

Nav-Cam Prompt

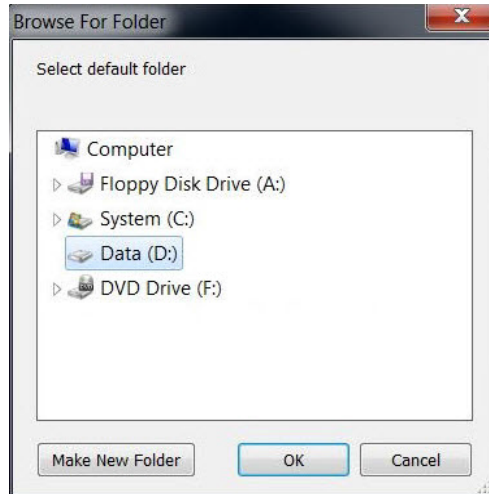
Upon first start, if a Nav-Cam is installed, the application will ask the user if it is a door-mounted Nav-Cam.



- If **No** is chosen, the same check box is not automatically selected, but the user has the option to select it manually.
- If no Nav-Cam is installed, the check box is disabled.

Browse for Directory Prompt

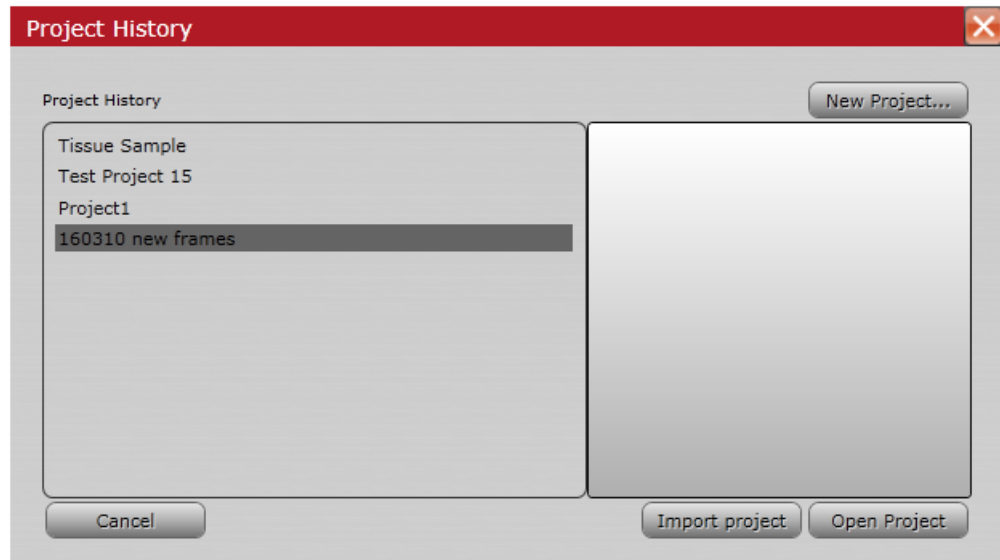
You will be prompted to browse to a default location for storing project data.



It is recommended that you 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.

Application Window

The application window displays with the Project History window open.



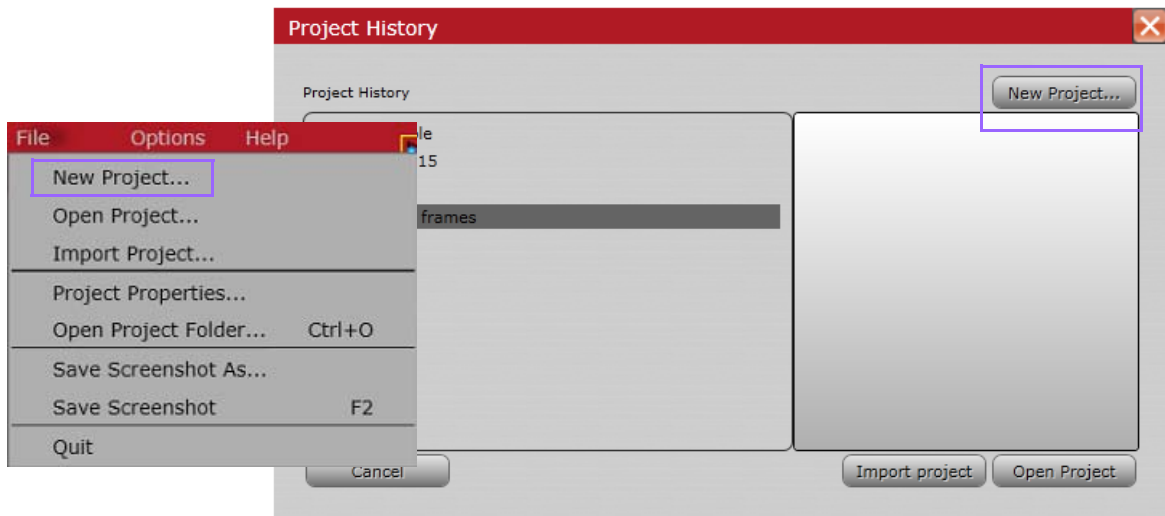
- If this is your first project, the list will be empty and the **Open Project** button will be disabled. Click **New Project** and proceed to *“New Project” on page 71*.
- If this is **not** your first project, the list will be populated with existing projects from which to choose. If you want to open an existing project, highlight a project name in the list and click **Open Project** and then proceed to *“Open Project” on page 73*. If you want to create a new project, click **New Project** and proceed to *“New Project” on page 71*
- If you want 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 73*.

Creating a Project

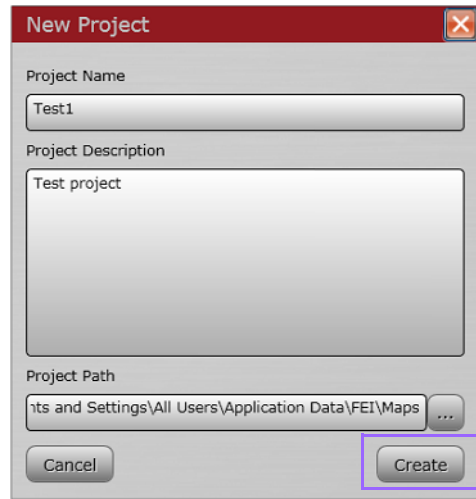
Create a new project, import a project, or open an existing project using File menu selections (*page 13*) or Project History buttons.

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.



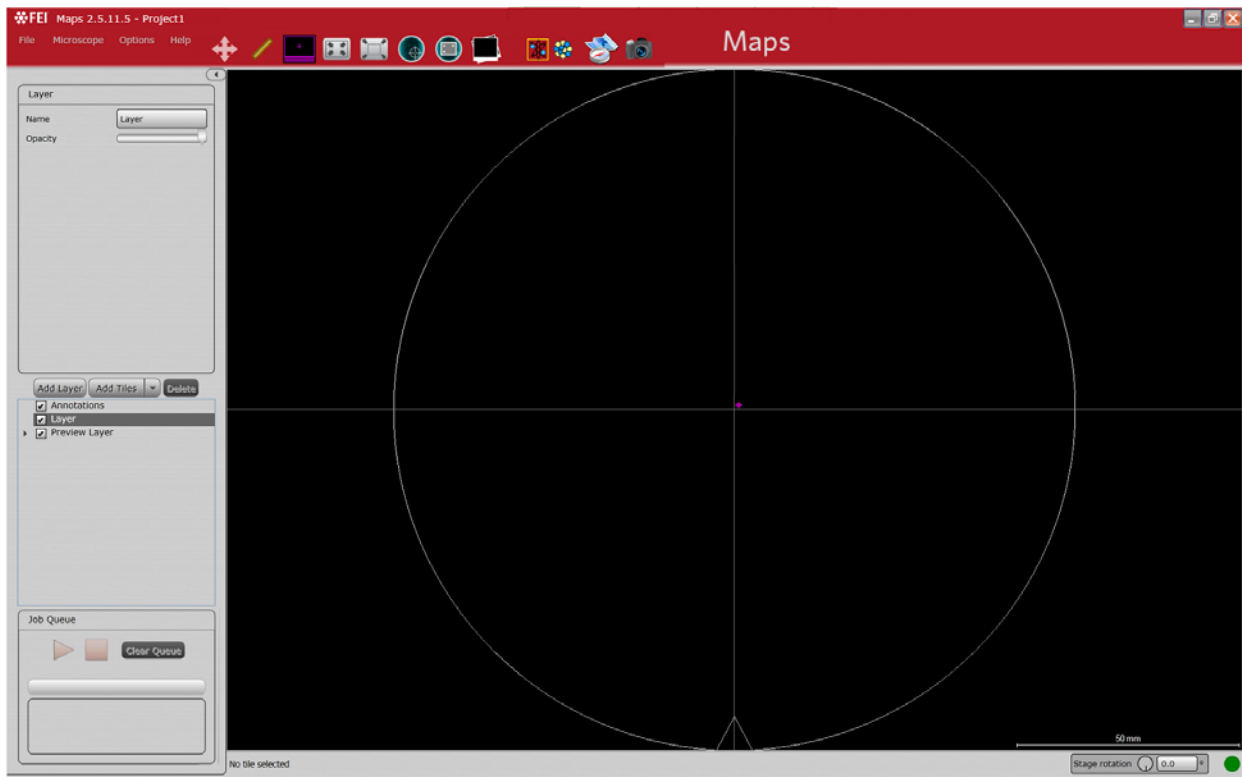
- In the New Project window that appears, define the new Project Name, and enter a brief Project Description. Then click **Create**.



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

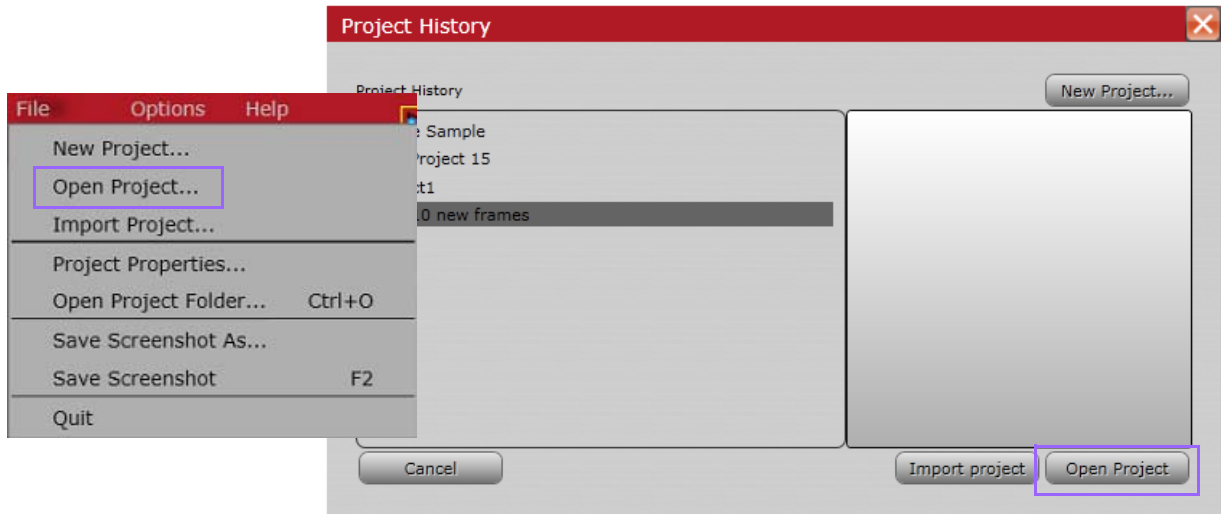
The new project name appears in the application title bar and the Layer control shows a default Preview Layer and Layer.

The purple rectangle represents the HFW and the purple + is the current stage position.



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**.

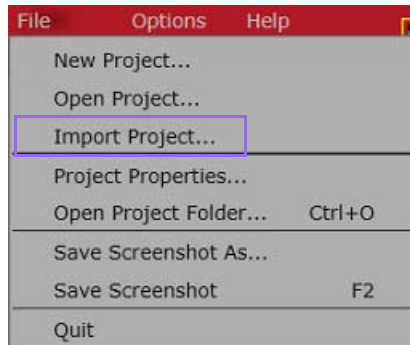


The selected project name appears in the application title bar.

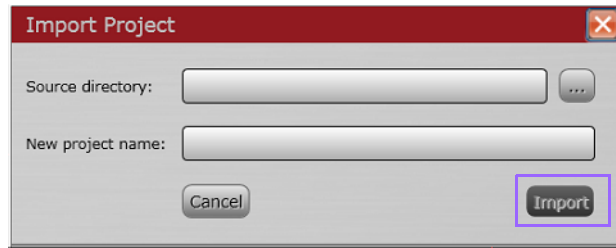
Import Project

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

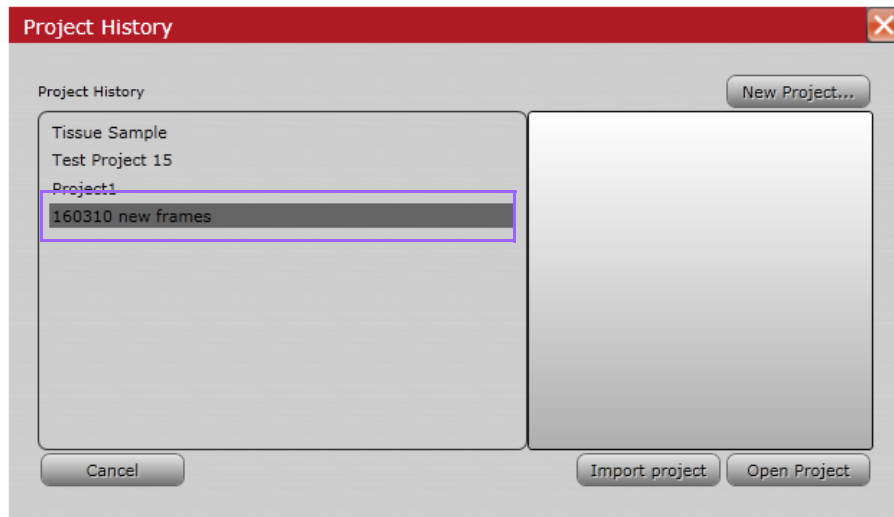
1. To import (save) an existing project, select **File > Import Project**.



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

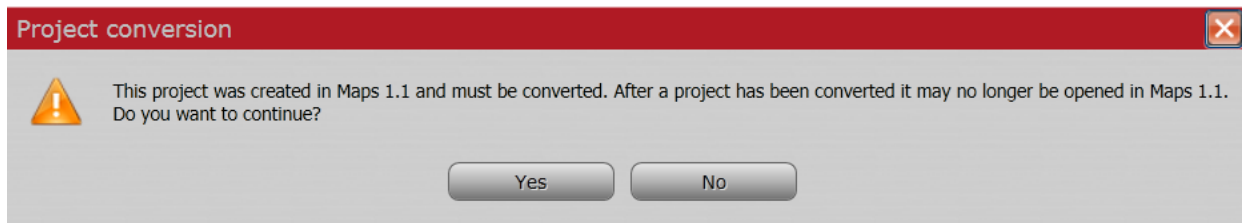


The imported project opens and the file name is added to the project history list.



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.

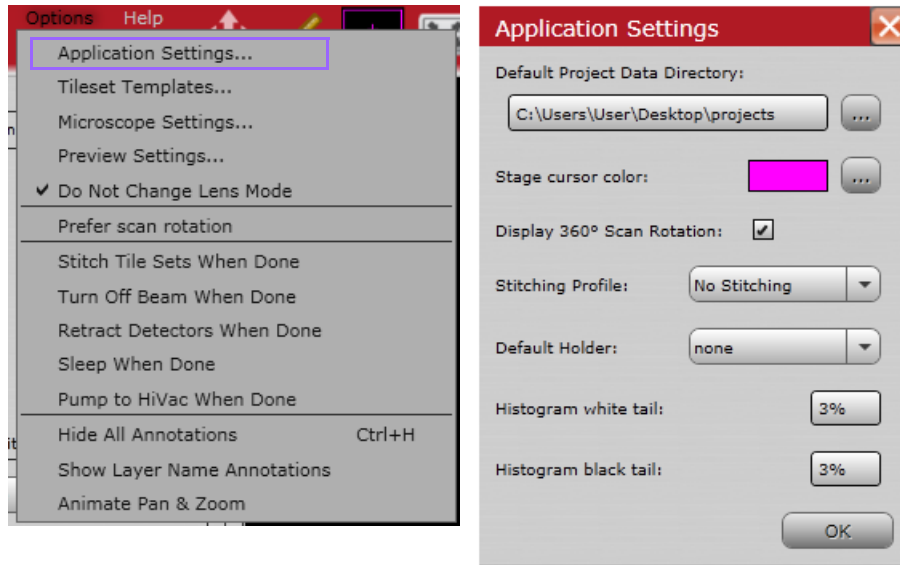


Setting Options

Select new project defaults from the Options menu.

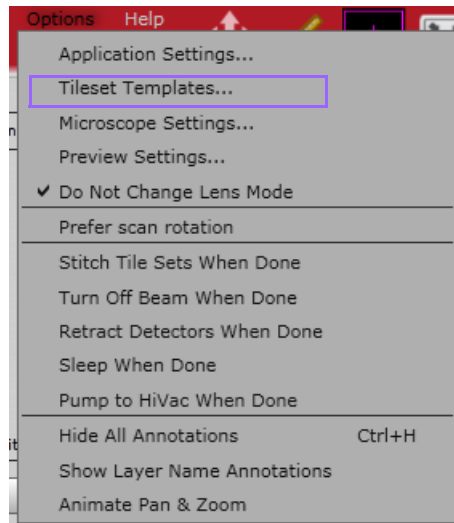
Application Settings

Select **Options > Application Settings** to display a window for setting defaults. See *“Application Settings:” on page 20* for descriptions of the controls.

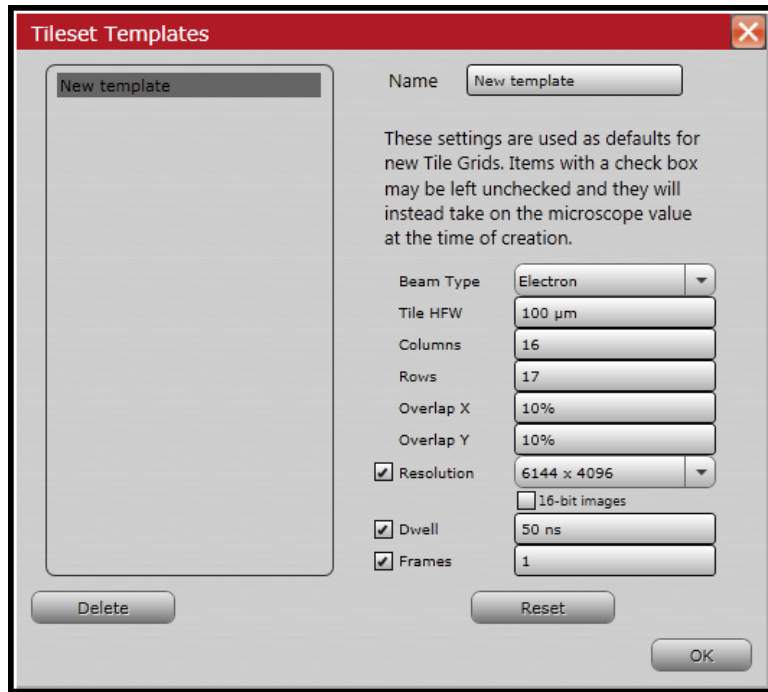


Tileset Templates

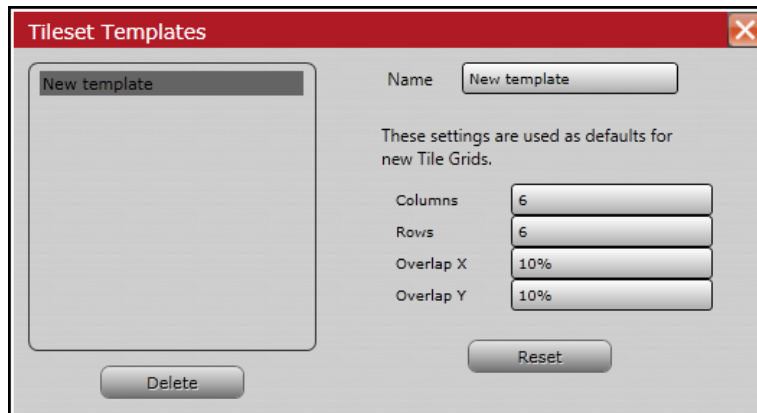
Select **Options > Tileset Templates** to display a dialog that shows a collection of templates and their respective settings.



EM

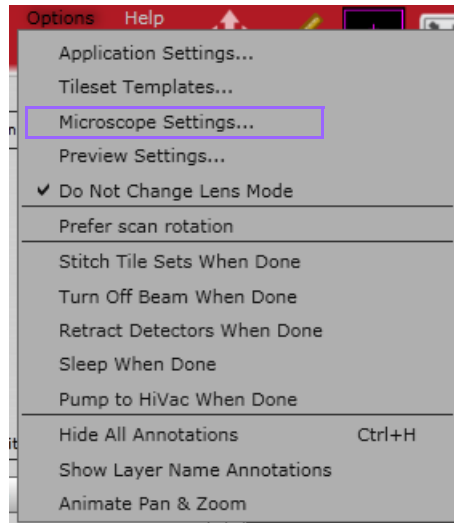


LM

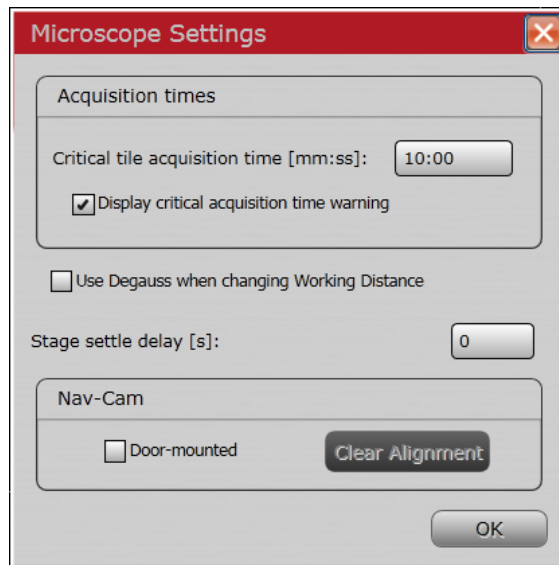


Microscope Settings

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

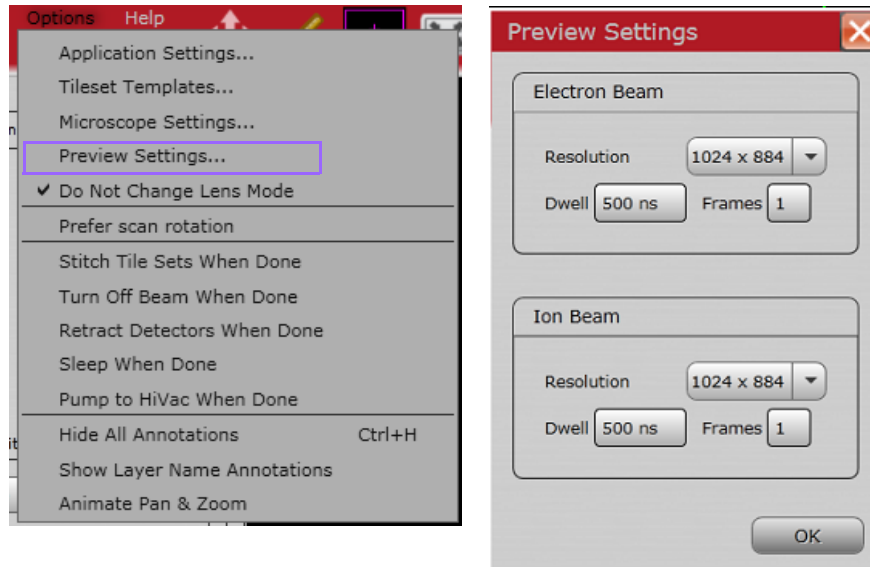


Set defaults here for Acquisition times, Using Degauss when changing working distance, Stage settle delay, and clearing the Nav-Cam alignment.



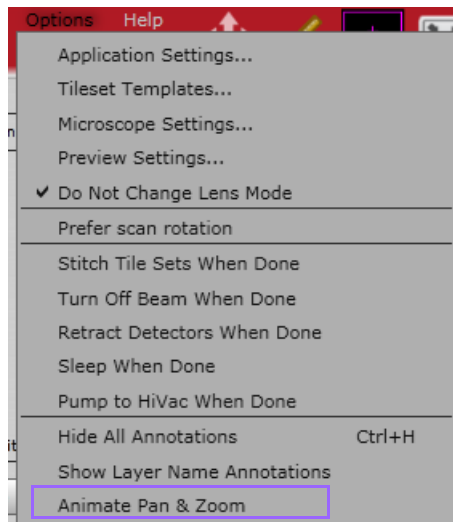
Preview Settings

Select **Options > Preview Settings** to display the Preview Settings window and review or change the scan settings that will be used to acquire preview images.



Animate Pan & Zoom

Select **Options > Animate Pan & Zoom** to allow a smoother transition of panning and zooming. *Note that this is a slower process and takes more memory.*



Nav-Cam Alignment

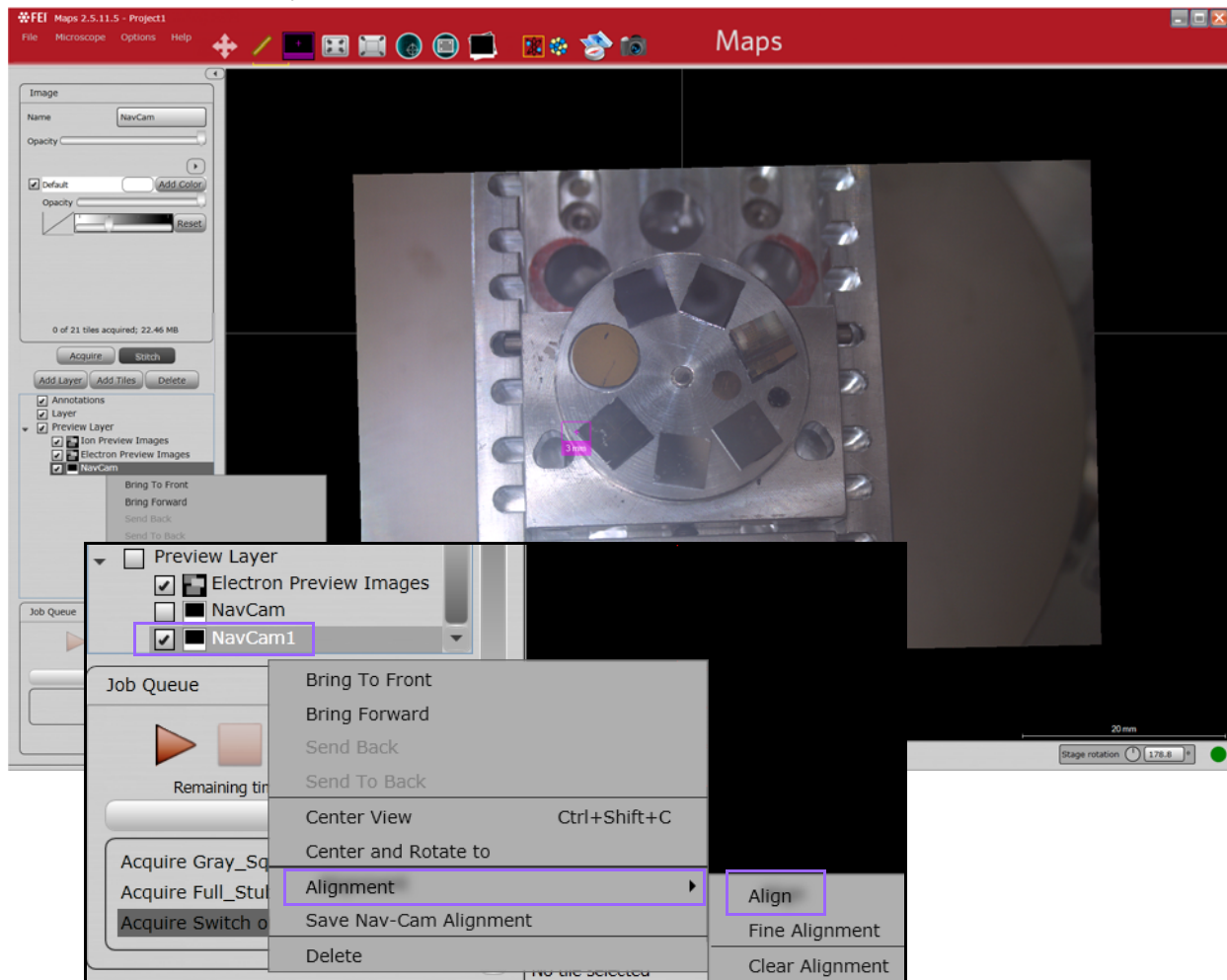
For systems that have the Nav-Cam, Maps is able to acquire Nav-Cam images and align them. Maps is also able to save the Nav-Cam Alignment to be automatically applied to all future Nav-Cam images acquired with Maps.

Procedure for Nav-Cam Alignment:

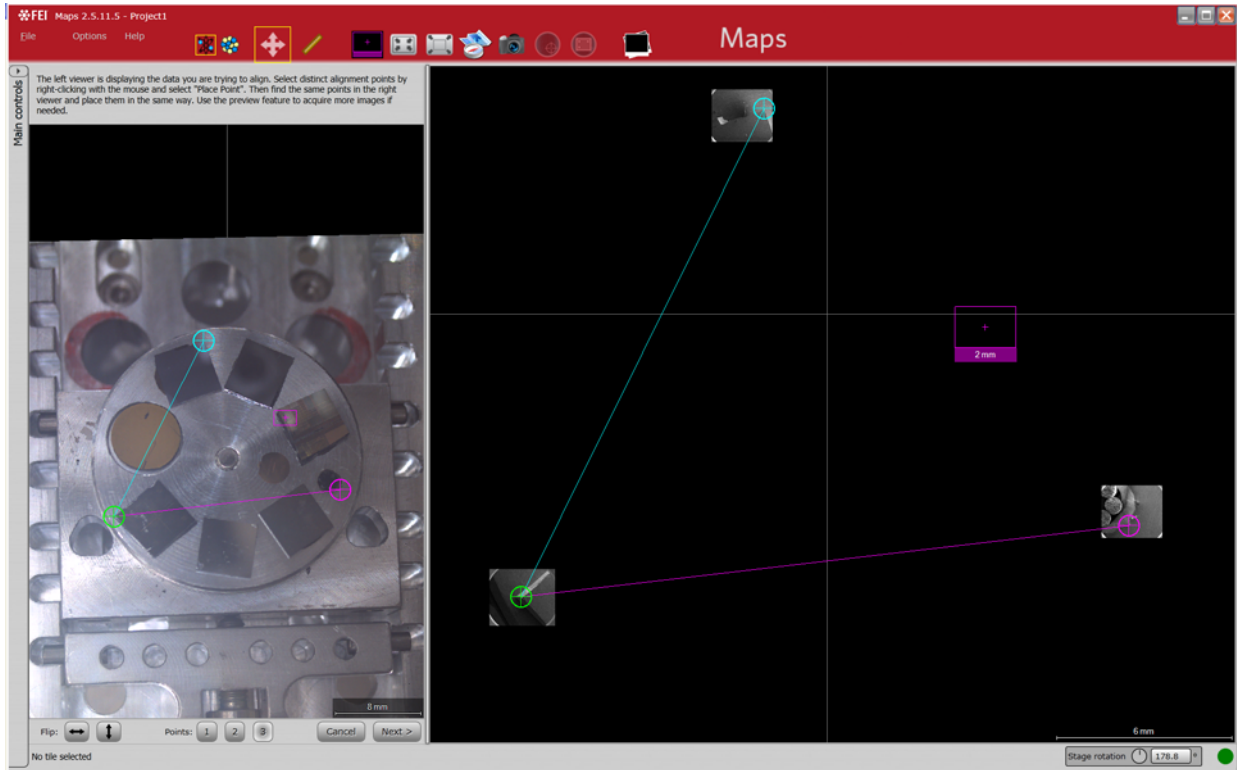
1. Click the **Import from Nav-Cam** tool box icon.



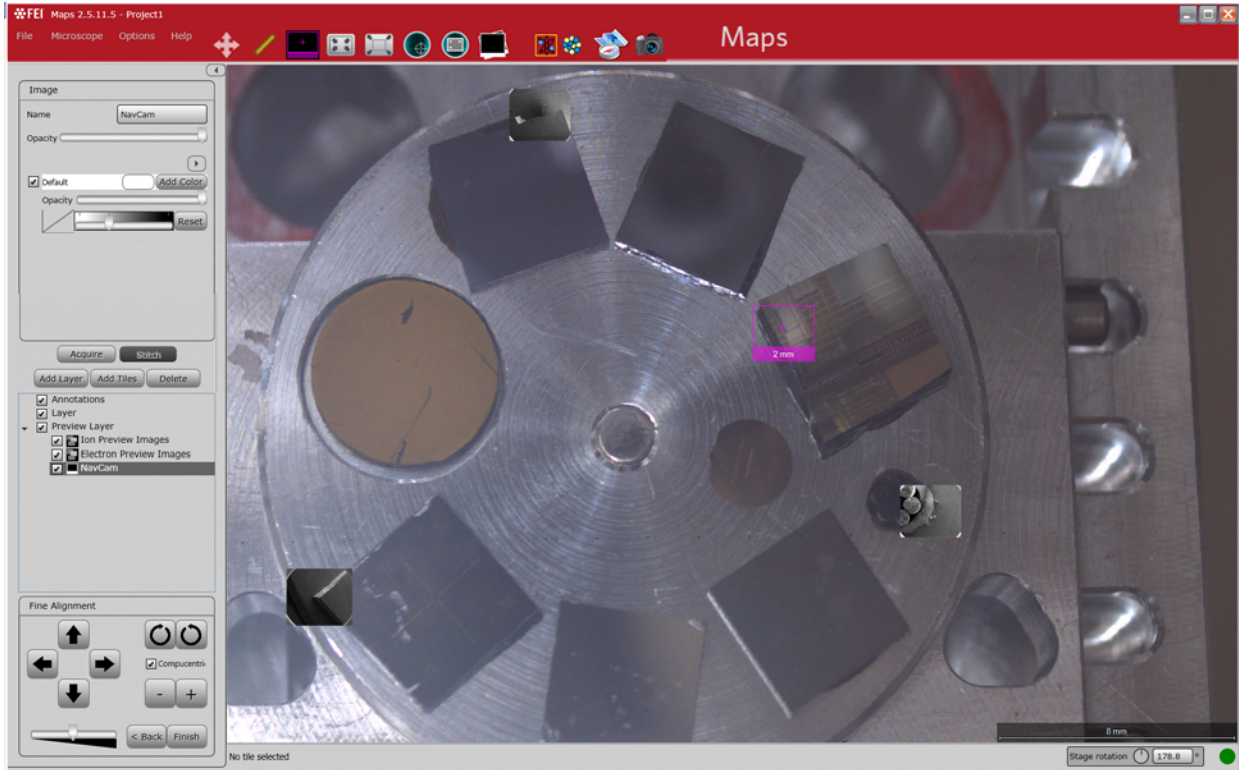
2. When the Nav-Cam image appears in Maps, right-click on its name in the Layer control and click **Align** in the menu that appears.



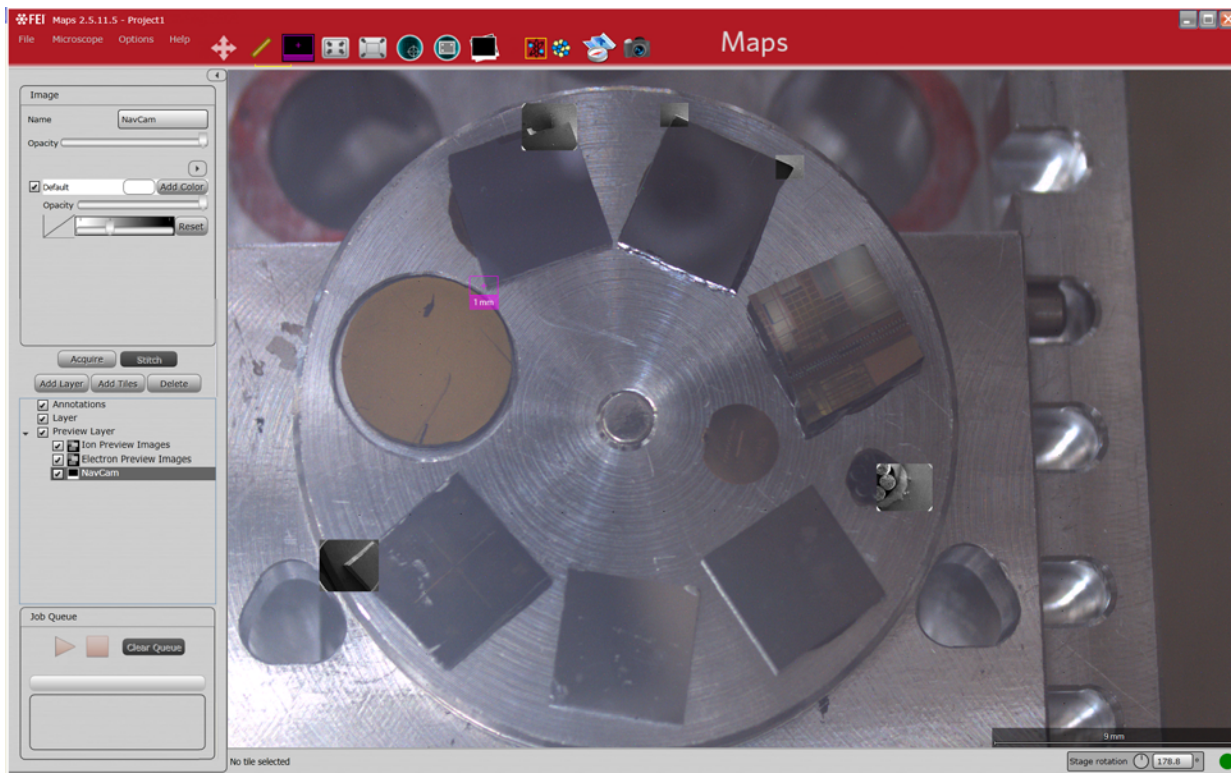
3. Perform a 2 or 3 point alignment as described in *“Alignment” on page 159.*



4. Perform fine alignment adjustments, if needed, as described in *"Fine Alignment"* on page 161.



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 **Save Nav-Cam Alignment** in the menu that appears.

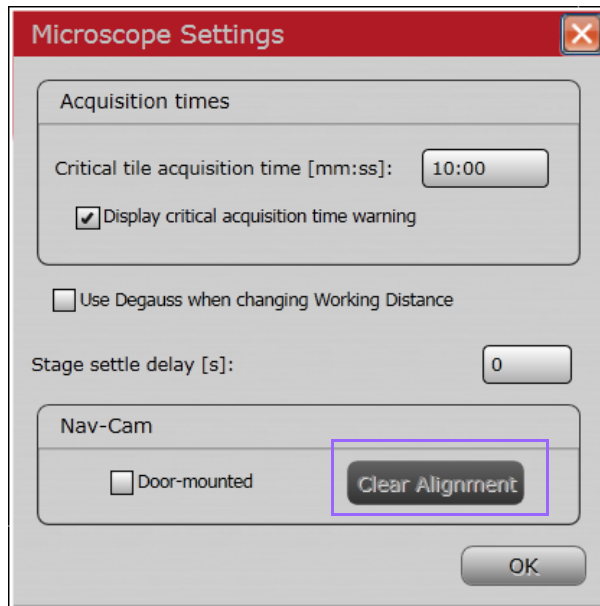


All subsequent Nav-Cam acquisitions using Maps will use this alignment.

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**.



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



Running the Automated Holder Alignment

See [“Automated Holder Alignment” on page 98](#) for information.

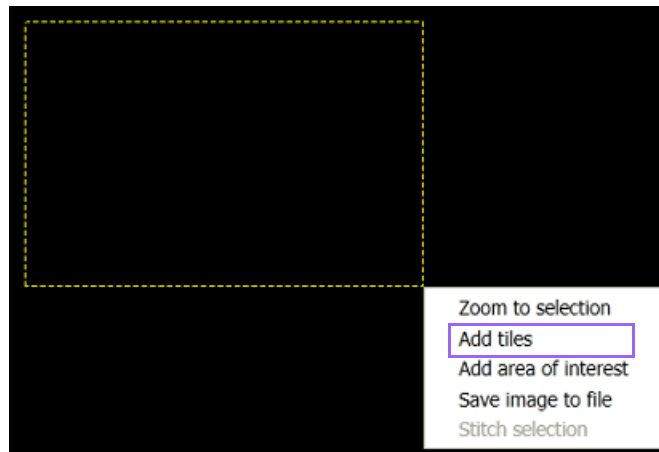
Defining a Tile Set

Define the tile set for your project using **Add Tiles** in the Layer control or **Add Tiles** from the left-click and drag selected area menu.

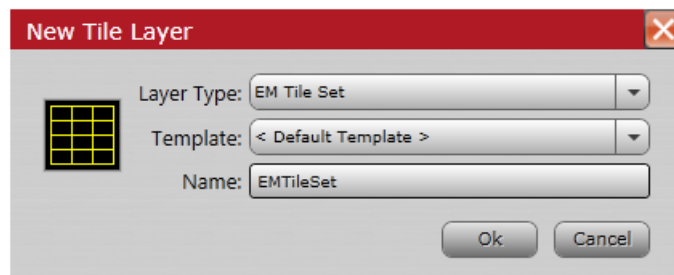
1. Click the **Add Tiles** button on the Layer control.



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

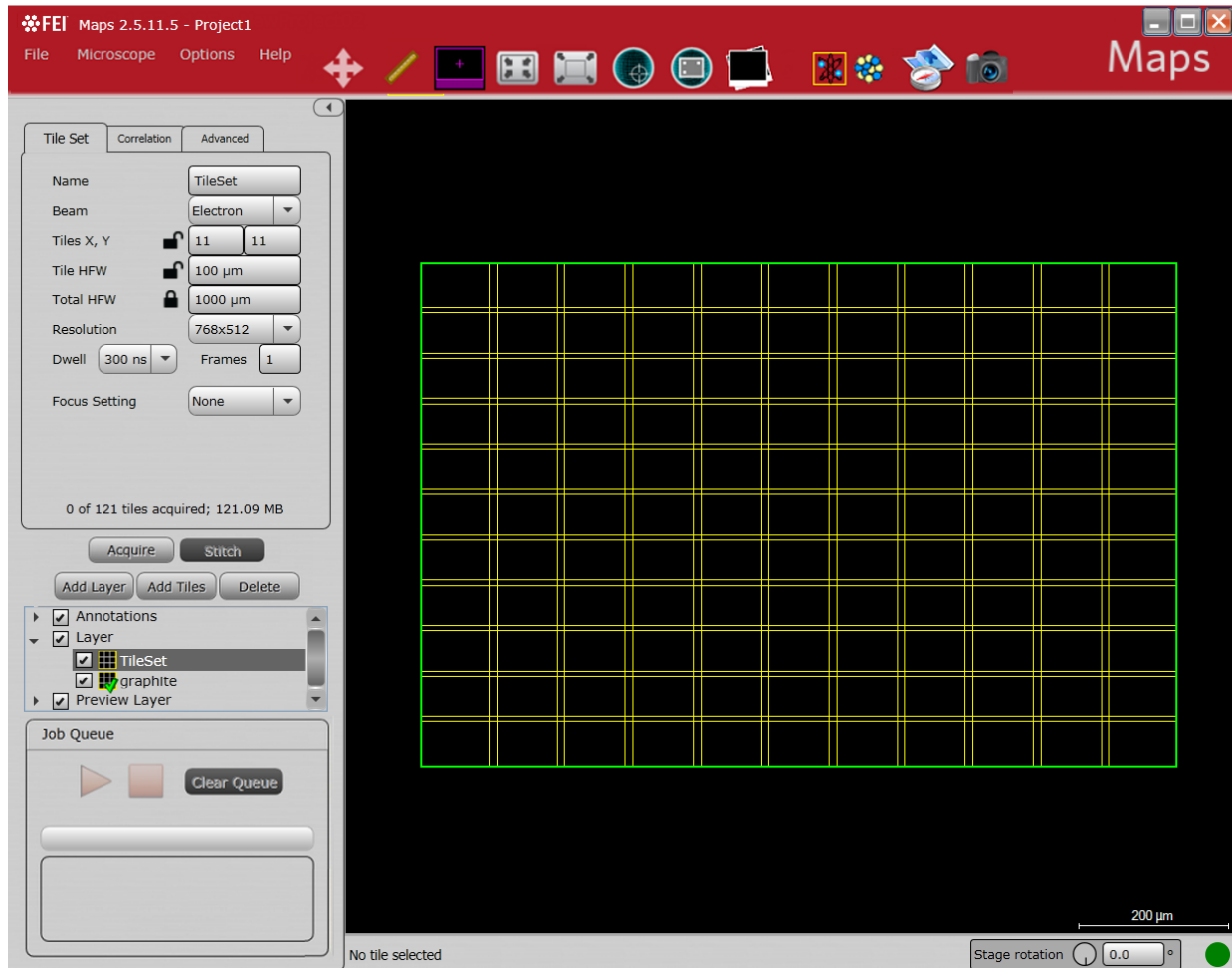


2. In the New Tile Layer window that appears, enter a name and click **OK**.



Select a tile set template, enter a name, and then click **OK**. The new tile set will match the settings saved in the tile set template and will be placed in the area selected.

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.



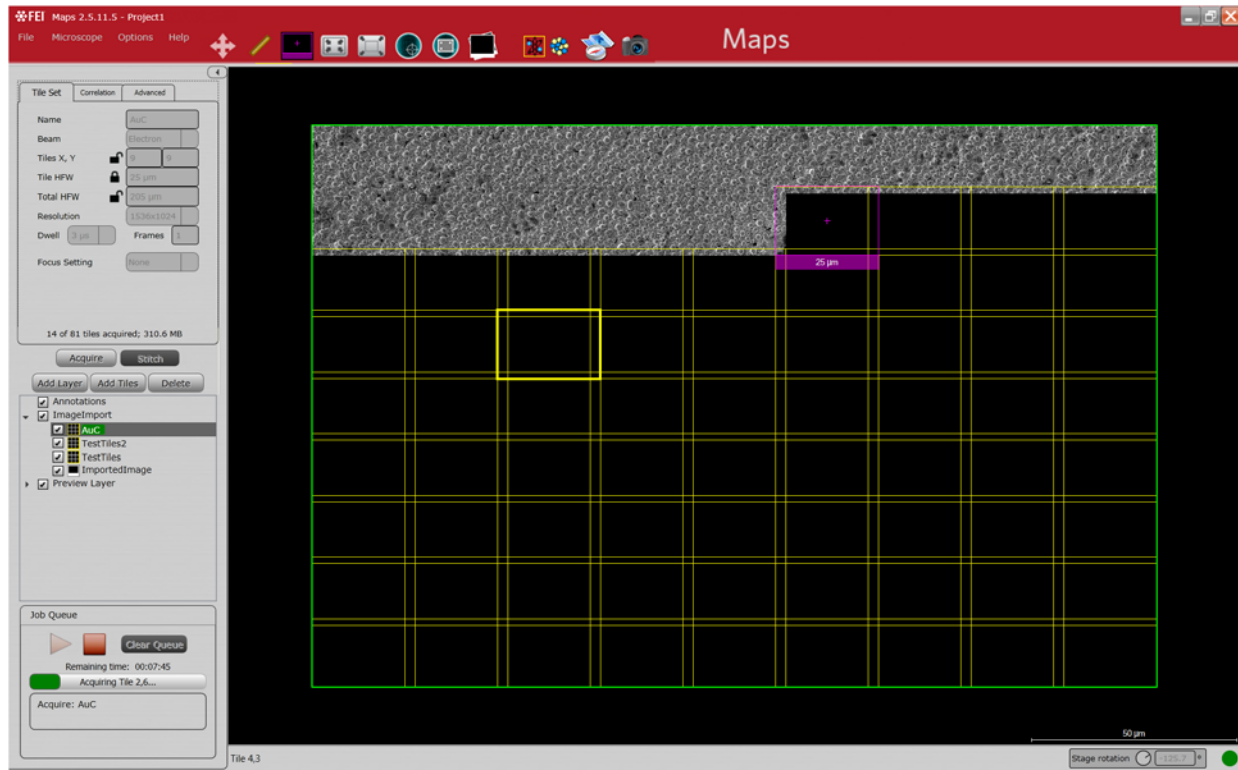
Configure Tiling Parameters

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

Select a tile set or multiple tile sets in the Layer control and click **Acquire**. Acquisition begins automatically on the tile set selected first and then moves through the remaining tile sets in order of selection. The remaining time for acquisition displays above the progress bar in the Job Queue.

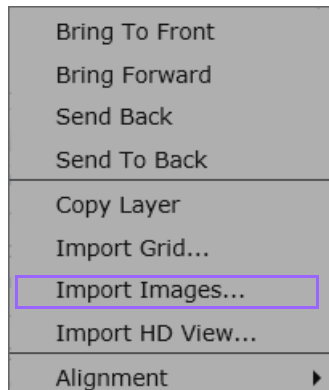
Tile images are registered for stitching as they are acquired and displayed in the grid. The tile sets are colored as they are registered to give immediate feedback on the stitching confidence. See *"Tile Set Grid Color Key"* on page 54.



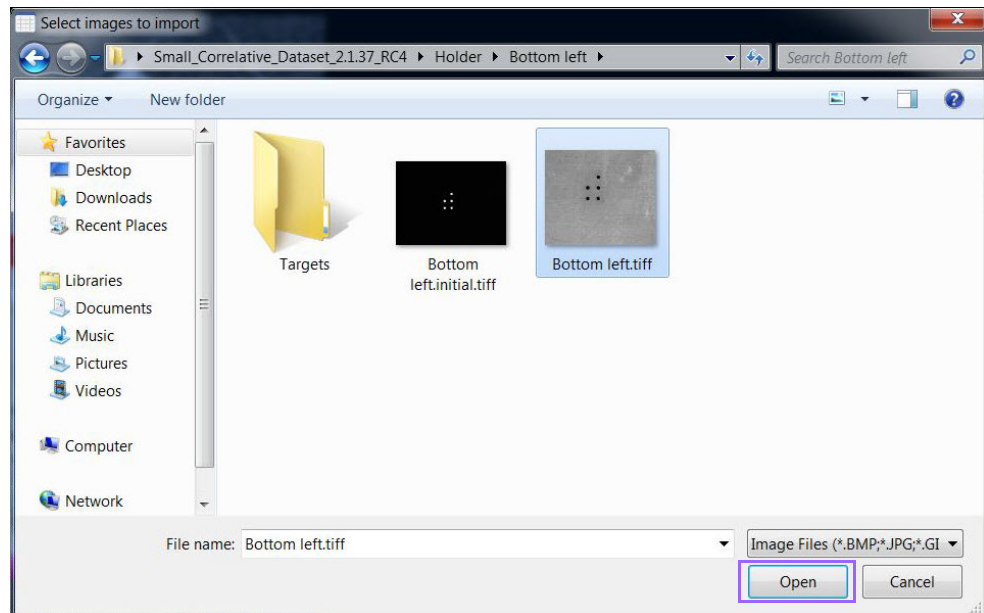
Importing Images from Other Sources

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.

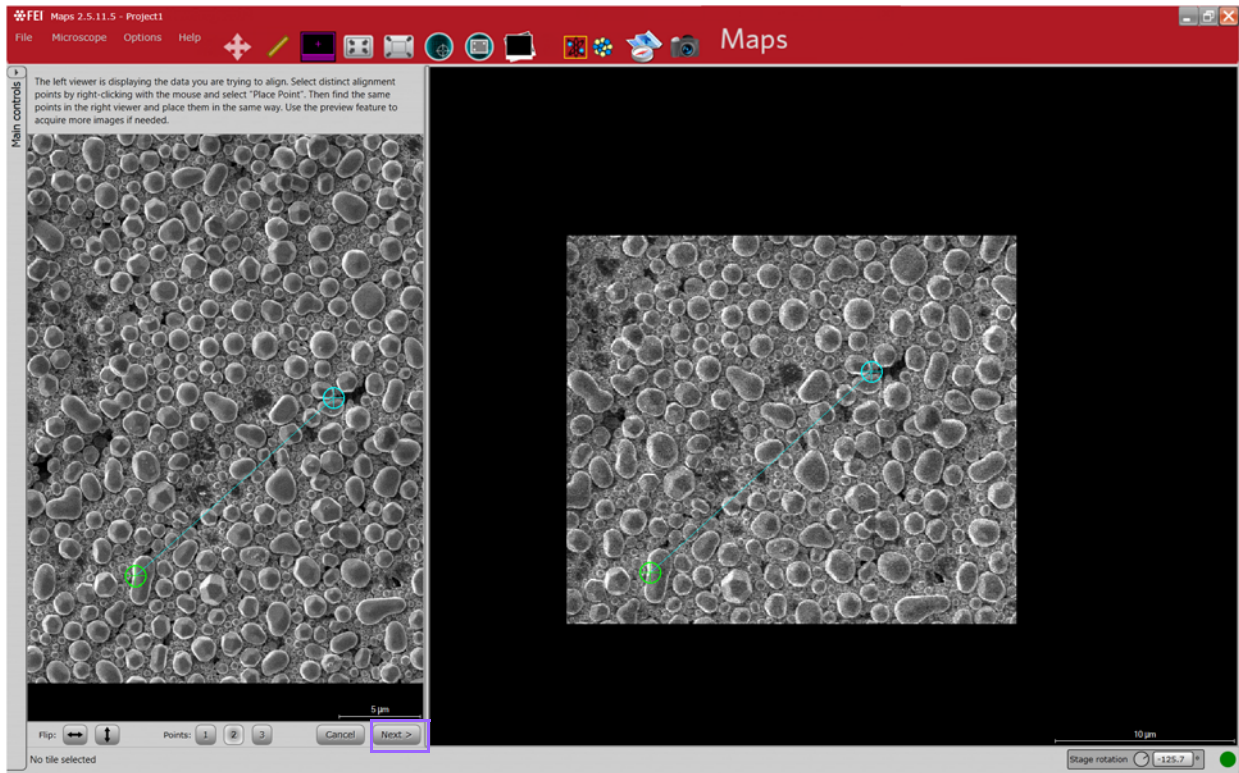


2. In the standard Open window that appears, browse to a saved image and then click **Open**.



Most standard image formats are supported, including but not limited to: BMP, JPG, GIF, PNG, or TIFF images.

The imported image displays to the left of the main viewer.



- 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 159](#) 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 are automatically aligned, so this step can be skipped, although you may want to do an alignment for fine tweaking.

Stage Navigation

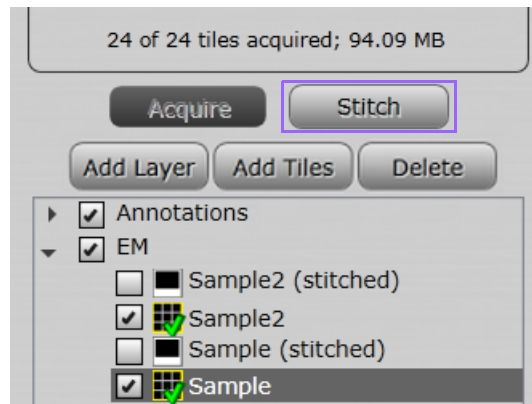
Double-click anywhere inside the large white circle to move the stage to that location.

Stitching

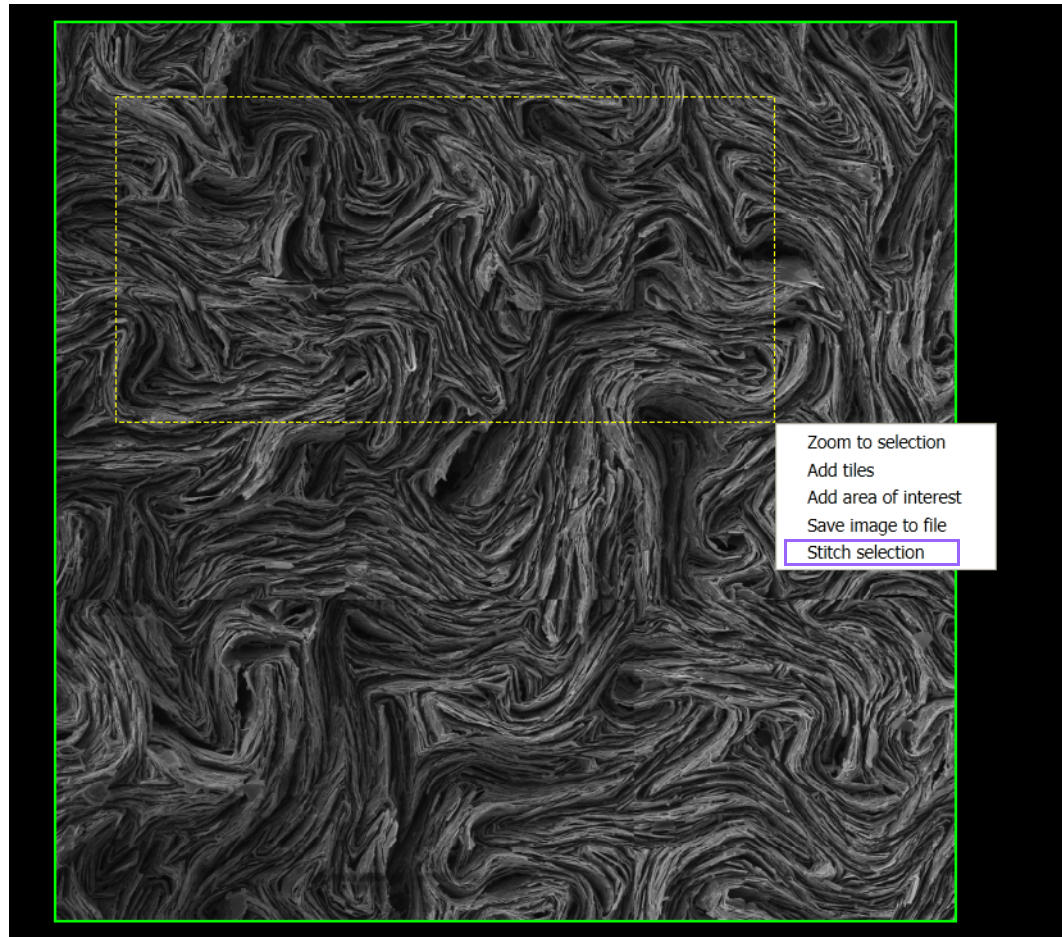
After a tile set has been acquired, the **Stitch** button is enabled.

Initiate Stitching

Select the acquired tile set in the Layer control and click **Stitch**. The manual UI immediately displays 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 [“Left-Click and Drag Selected Area” on page 55](#).

Manual Alignment

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

Please confirm alignment of the tiles.
 To inspect and edit tile alignment, select a tile in the grid below, or use the "Previous" and "Next" buttons to cycle through failed tiles.

HotKeys:

- Ctrl+Arrow: Select a tile
- Arrow: Move selected tile
- Shift+Arrow: Fine move selected tile

Stitching Manual Alignment Histogram

Displays the histogram for the Stitching Channel (see "[Stitching Channel](#)" on page 35).

Figure 9 *Stitching Manual Alignment Histogram*

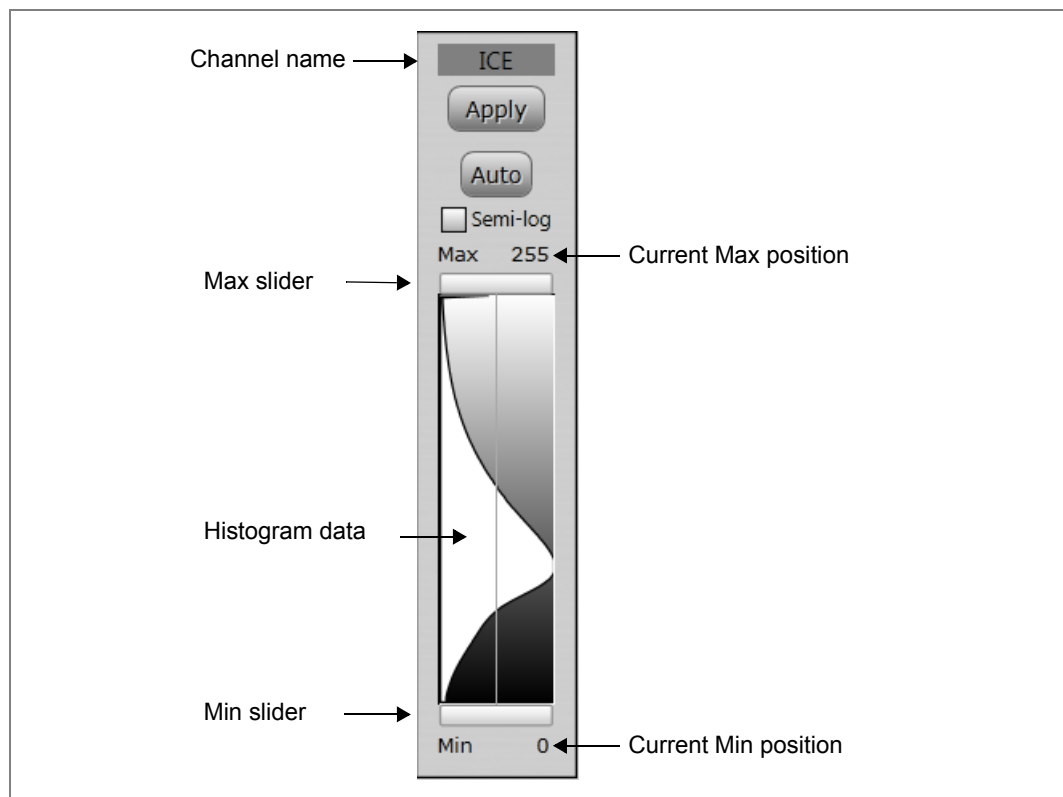
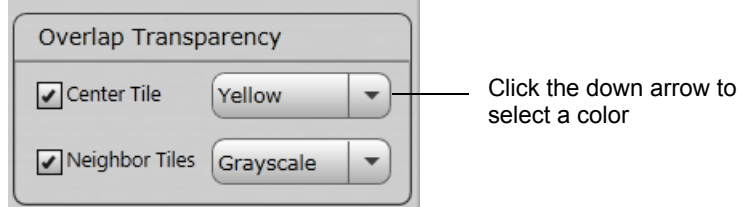


Table 21 **Stitching Manual Alignment Histogram Overview**

Interface Item	Description
Channel name	<ul style="list-style-type: none"> • For EM: Displays the channel name, which is the detector name. • For LM: Displays the individual channel name as determined by the LA 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**.



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

Figure 10 Manual Alignment with Transparency Off

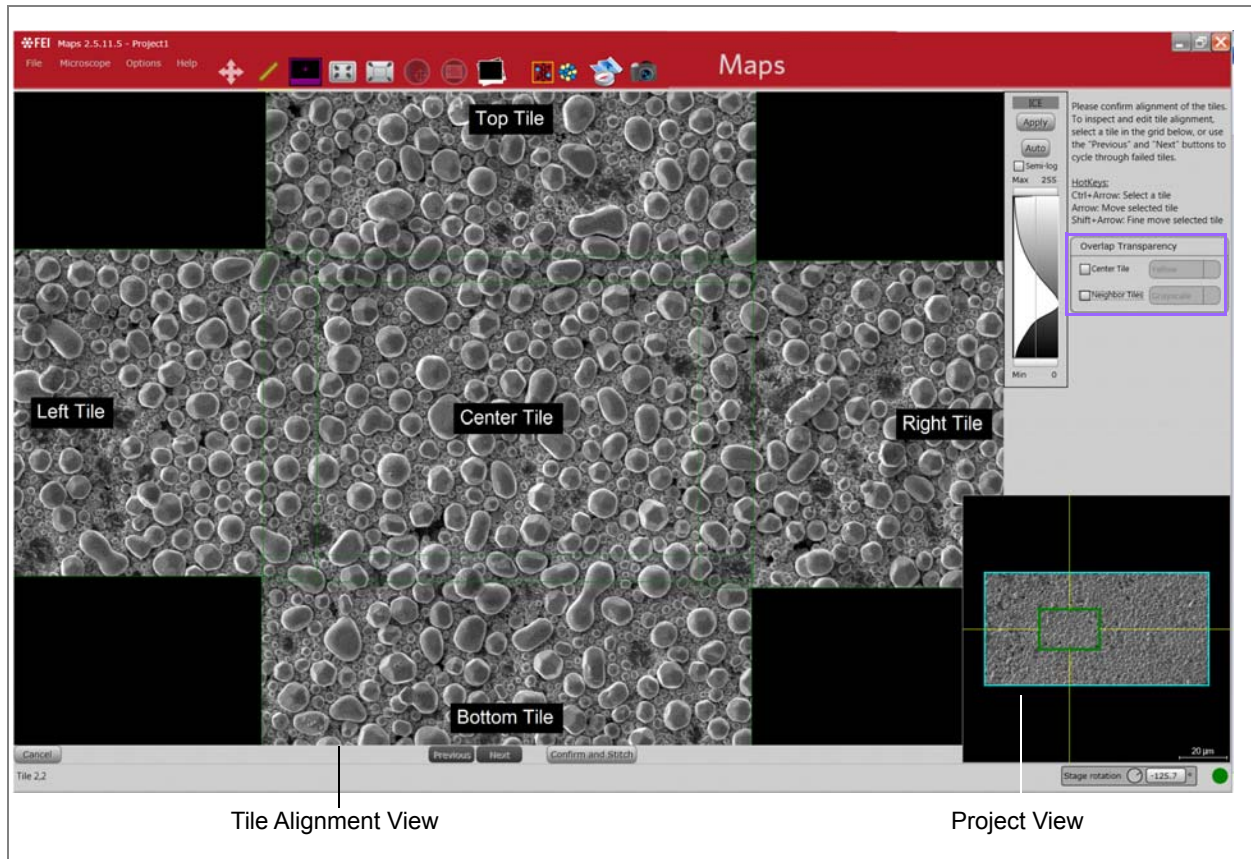
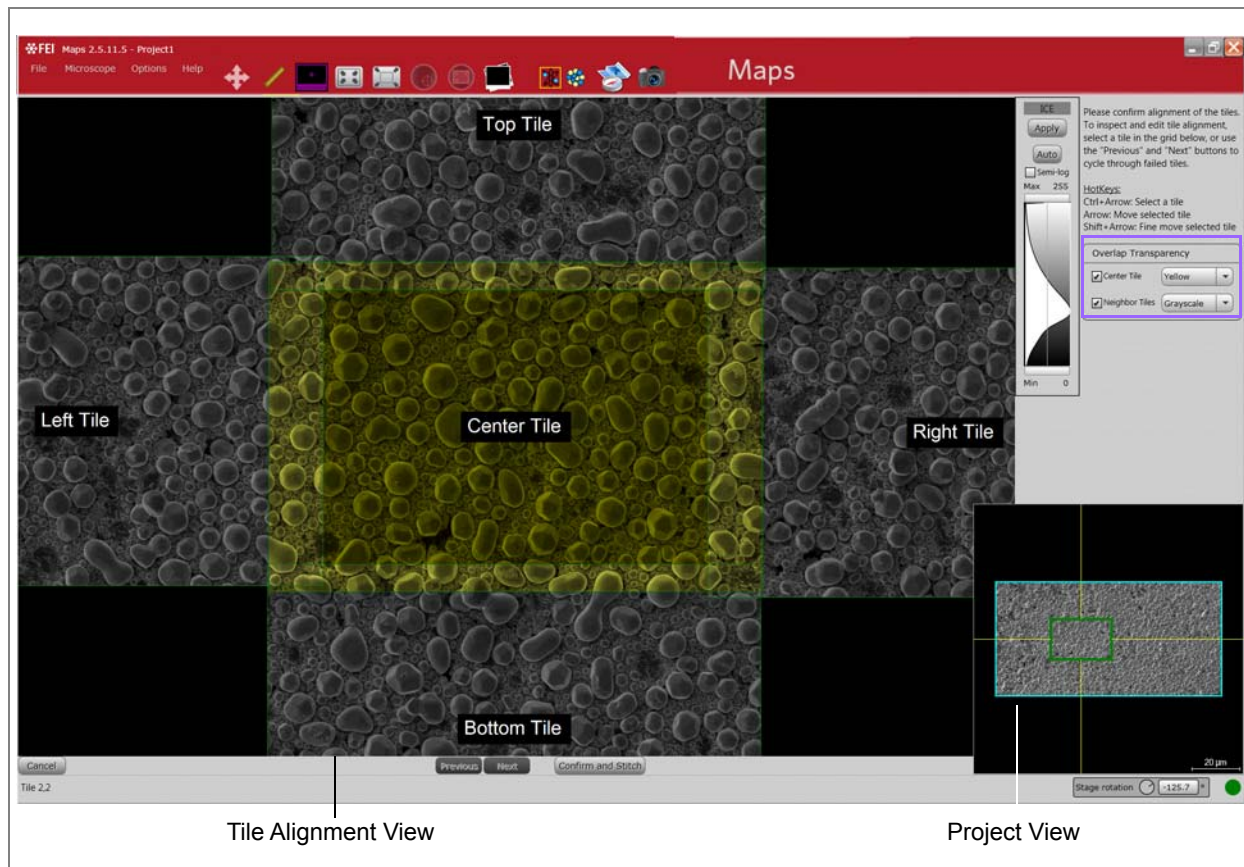


Figure 11 Manual Alignment with Transparency On



Two Views

Manual stitching makes use of two views, see [Figure 10, “Manual Alignment with Transparency Off,” on page 93](#):

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

This view displays the currently selected tile and its top, bottom, left and right neighbors. 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 move its surrounding tiles by using **Alt** + left-mouse drag.

- 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 its neighbors.

Stitching Alignment Status Color Key

Each tile outline is colored to indicate stitching alignment status.

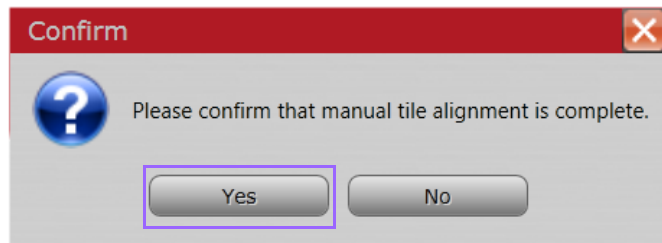
- **Green:** The automatic tile alignment succeeded.
- **Orange:** One side of the tile alignment failed, but another succeeded. These tiles do not require manual alignment.
- **Red:** The tile failed to align to either neighbor. These require manual alignment. After realignment, the outline turns **blue**.

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.

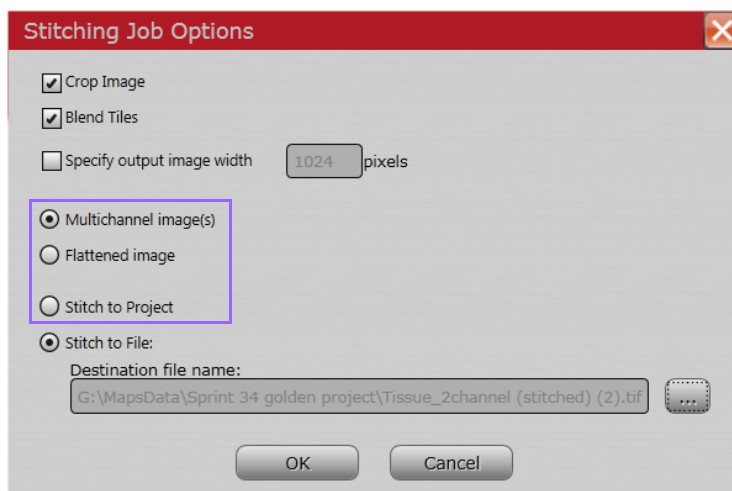



Table 22 Stitching Job Options Overview

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)	<i>Currently enabled for Multichannel TIF data only.</i> Outputs each channel to a separate image file. See “Auto Selection” on page 97.
Flattened image	<i>Currently enabled for Multichannel TIF data only.</i> All channels are collapsed to a single image.
Stitch to Project	<p>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.</p>  <p>When selected, the field for Destination file name is disabled. 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.</p>
Stitch to File	<p>Stitches the tile set into an HD View or single image and exports the file to the specified folder.</p> <p>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.</p> <p><i>Note: You must download HD View™ from the Microsoft web site to open the HDView.htm file to see the stitched image.</i></p>
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 TIF, the default stitching job option is auto selected.

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

4 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 LM and EM systems. It is a guideline for using Maps and the automated holder alignment, and can also be used for LM- or EM-only as well as multi-system correlative Maps projects.

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.

Topics include:

- [“Sample Holders” on page 98](#)
- [“Automated Holder Alignment User Interface” on page 106](#)
- [“LM Holder Calibration” on page 110](#)
- [“EM Holder Calibration” on page 117](#)
- [“Correlating Projects” on page 130](#)
- [“Troubleshooting” on page 131](#)

Sample Holders

Maps automated holder alignment supports the following three holders:

- [“3mm Grid Holder” on page 100](#)
- [“Single 22 mm Coverslip Holder” on page 102](#)
- [“Double 22 mm Coverslip Holder” on page 105](#)

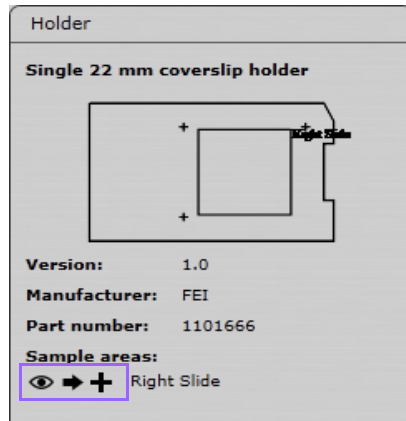
For the following three holders, Maps can draw a graphical overlay to assist with navigation. The graphical overlay can be aligned manually to other image layers, but the automated holder alignment is not supported on these layers:

- [“Cryo Shuttle” on page 101](#)
- [“Multiwell Slide” on page 103](#)
- [“Slide 75mm x 25mm” on page 104](#)

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, 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.
- Sample area quick access buttons appear.

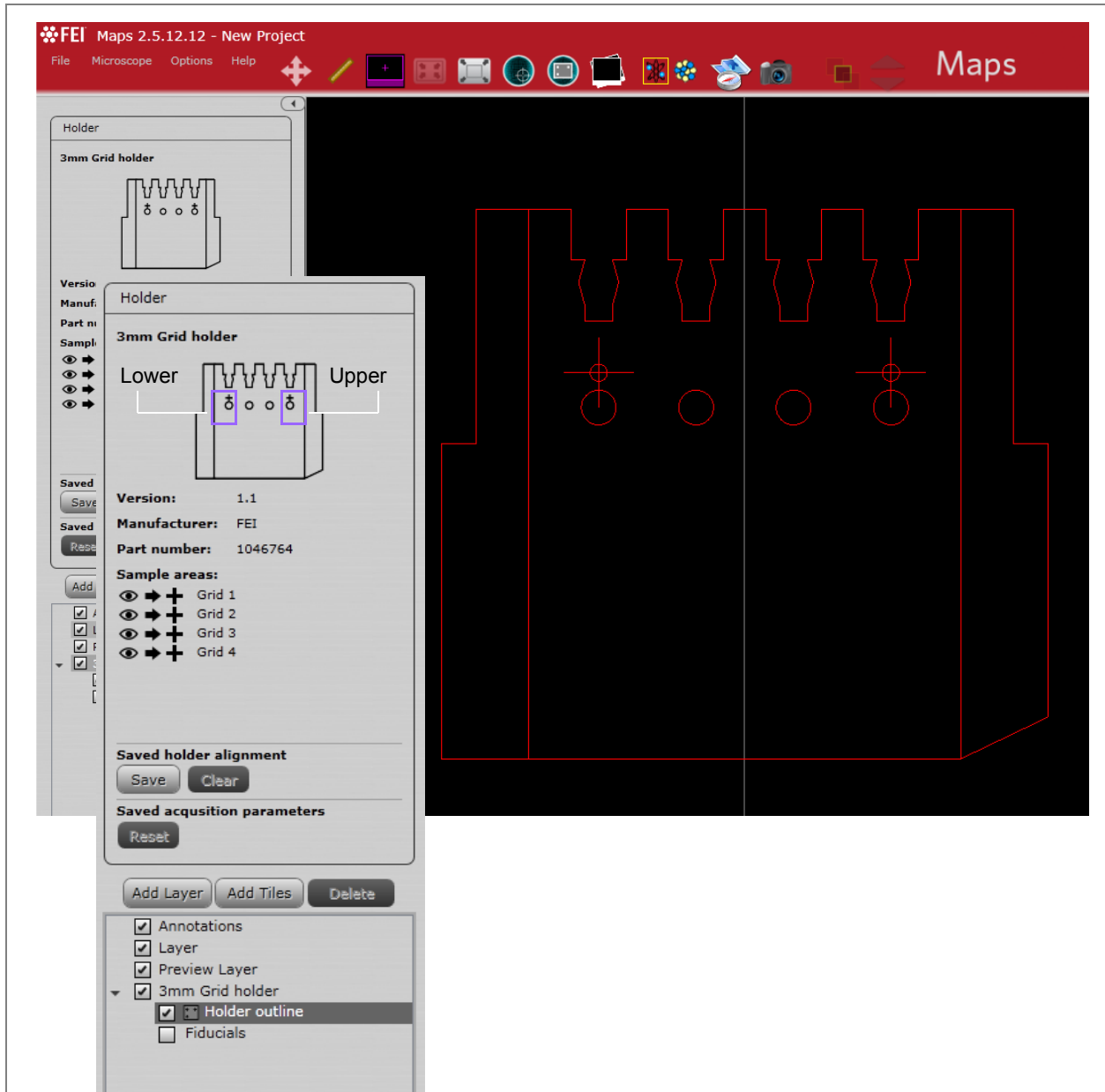


- **Eyeball:** Snaps the view to the sample area.
- **Right arrow:** Drives the stage to the center of the sample area.
- **Plus:** Creates a new tile set that covers the sample area.

3mm Grid Holder

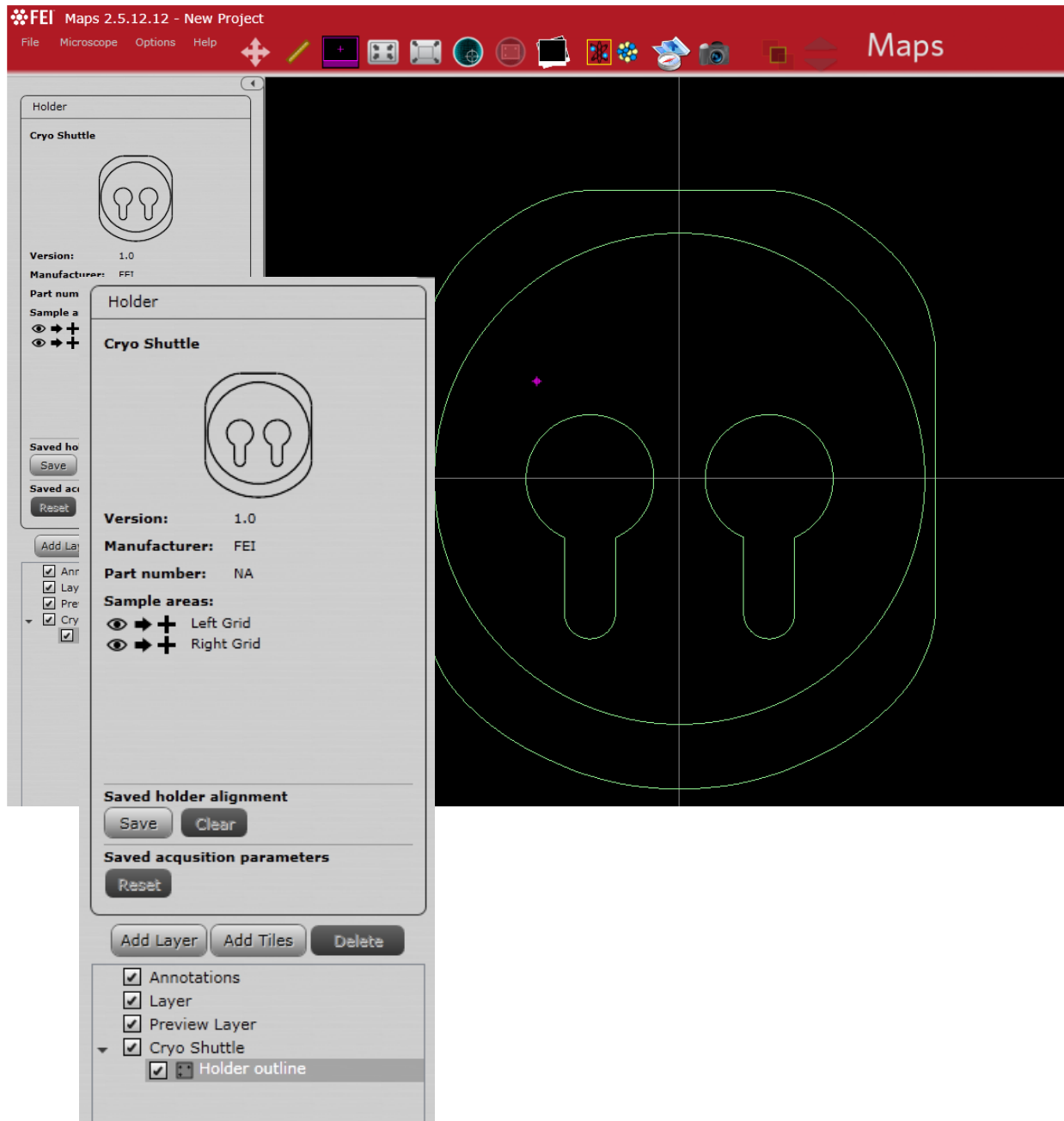
This is a holder for 3 mm diameter grids that can be loaded inside a transmission electron microscope (TEM).

Figure 12 3mm Grid Holder with Upper and Lower Fiducials Marked



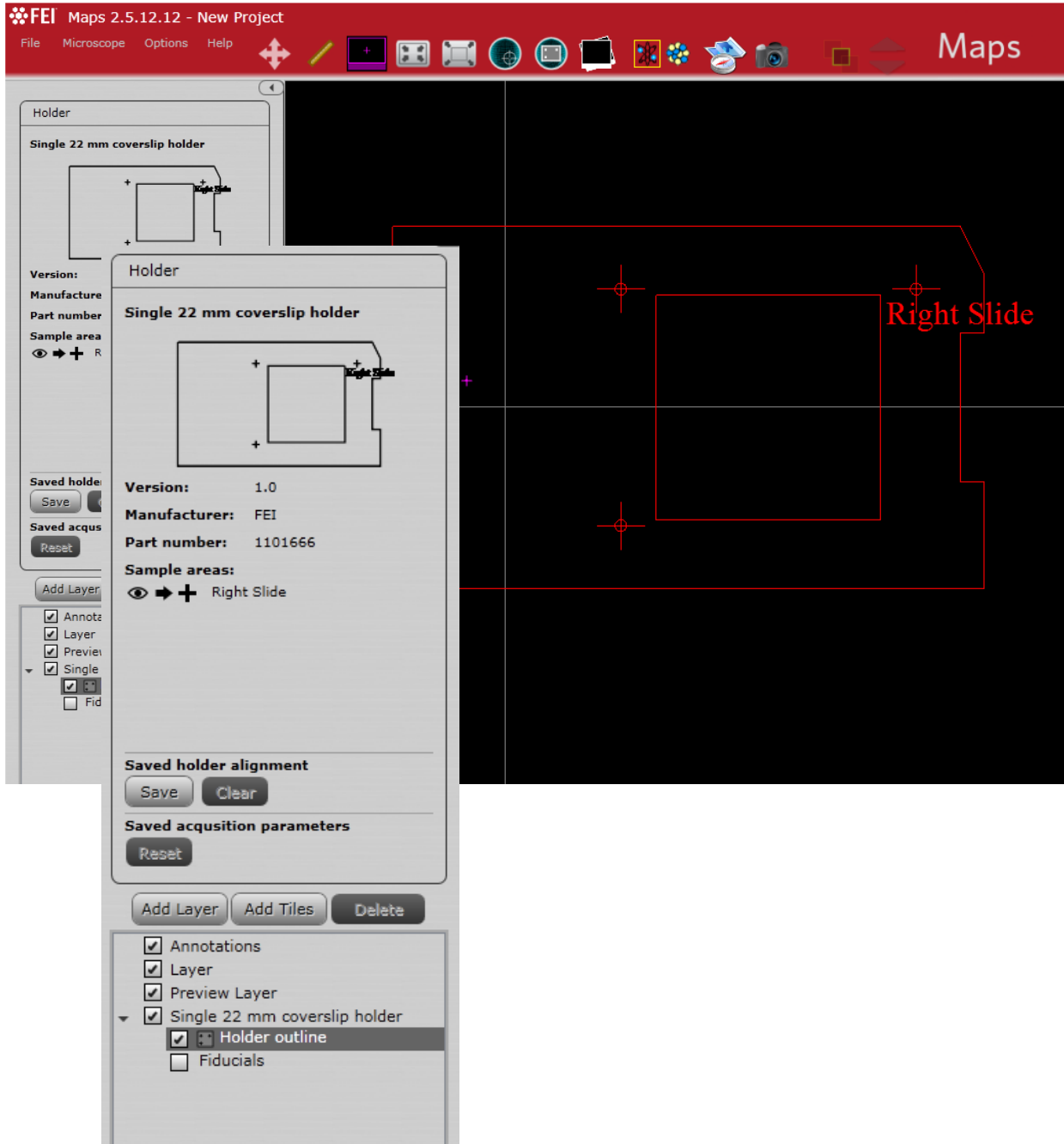
Cryo Shuttle

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



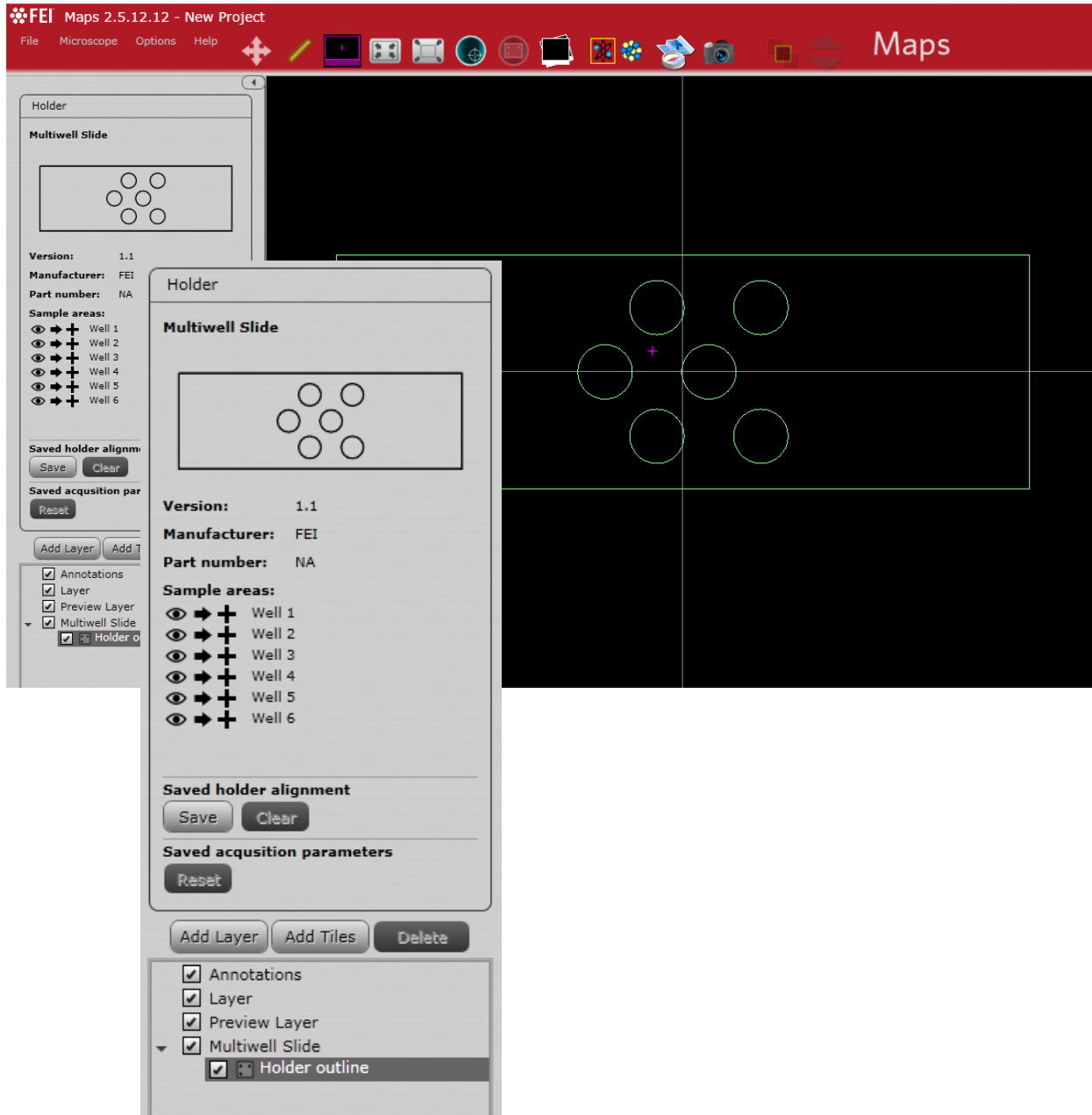
Single 22 mm Coverslip Holder

This is a single 22 mm coverslip holder.



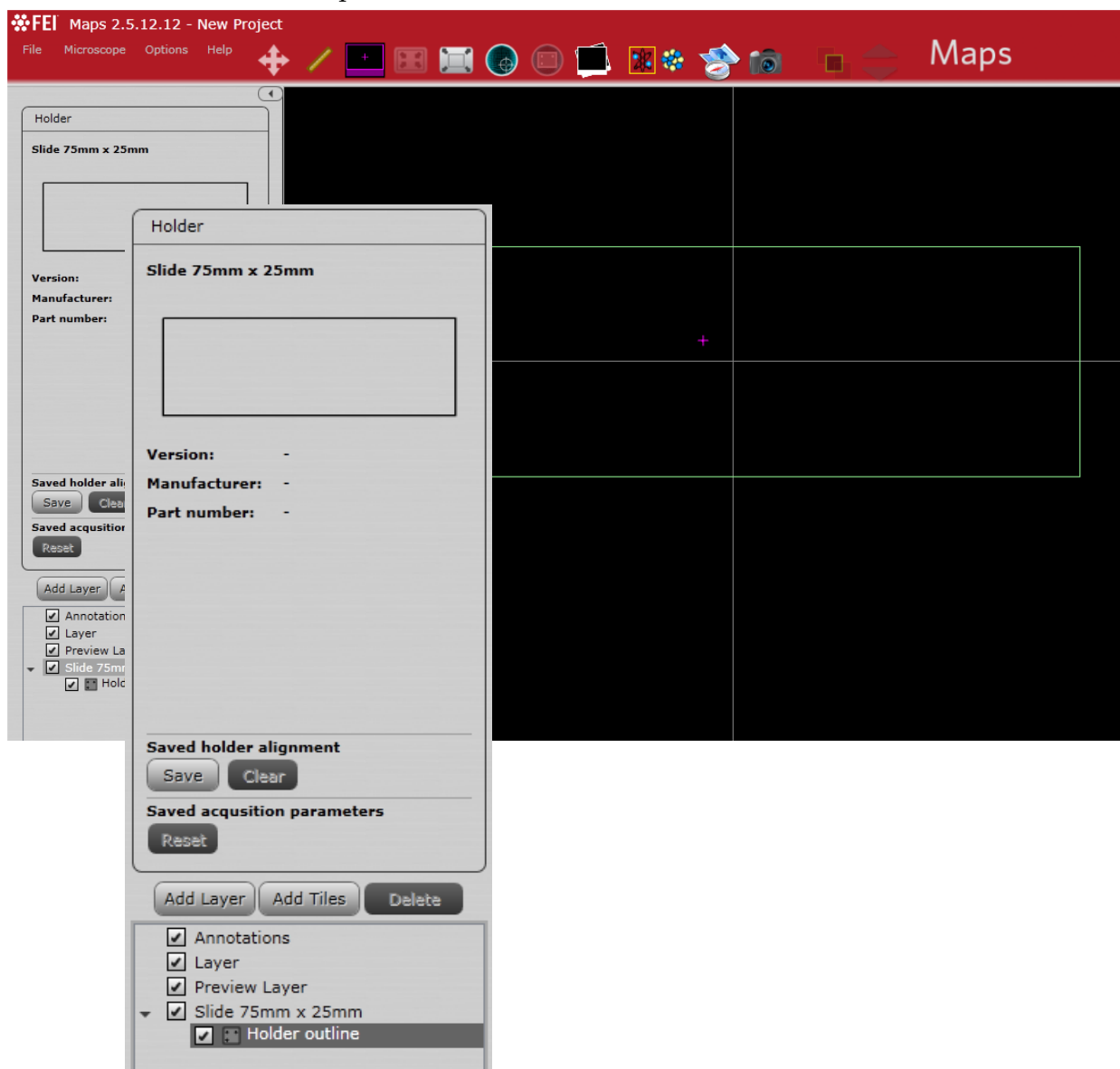
Multiwell Slide

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



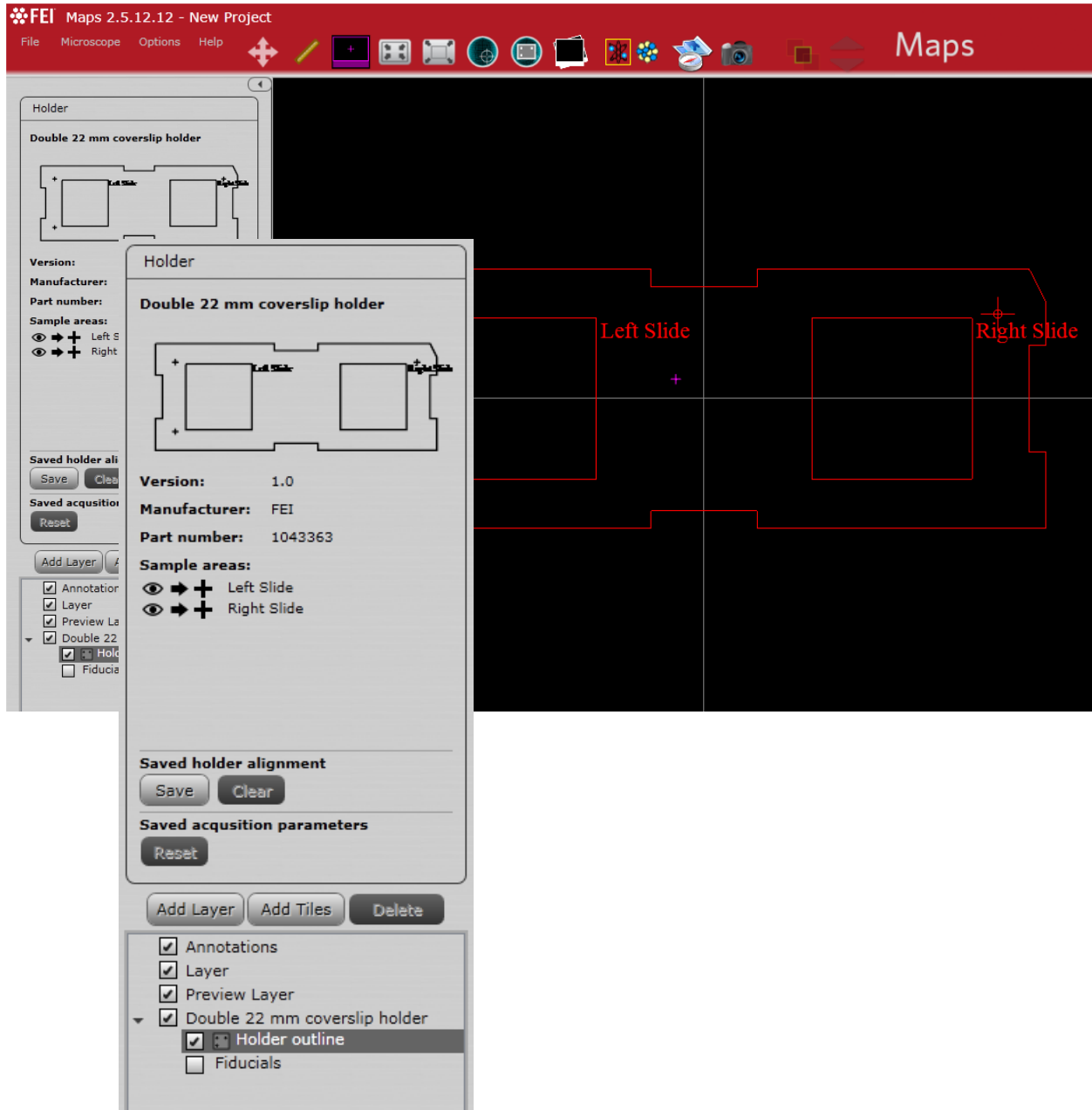
Slide 75mm x 25mm

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



Double 22 mm Coverslip Holder

This carrier holds up to two samples.



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 13 Automated Holder Alignment UI

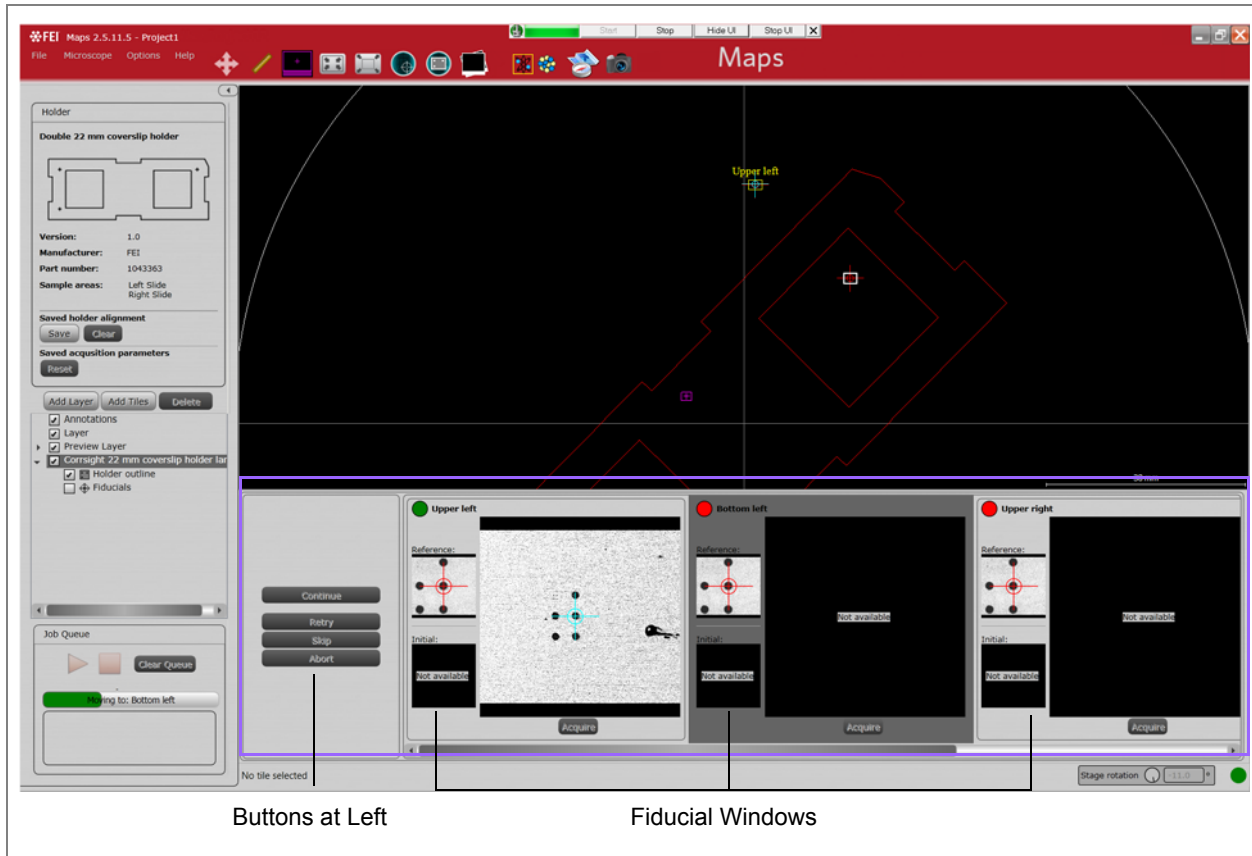



Table 23 Automated Holder Alignment UI Overview (1 of 3)

Interface Item	Description
Buttons at left (Before Starting Alignment):	
Run Alignment	Moves the stage to the position where it expects to find the fiducial, autofocuses, and attempts to match the fiducial.

Table 23 Automated Holder Alignment UI Overview (2 of 3)

Interface Item	Description
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.
Close	Closes the alignment UI and returns to the main viewer before the automated procedure has begun.
Buttons at Left (During Alignment):	
Continue	On EM 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.

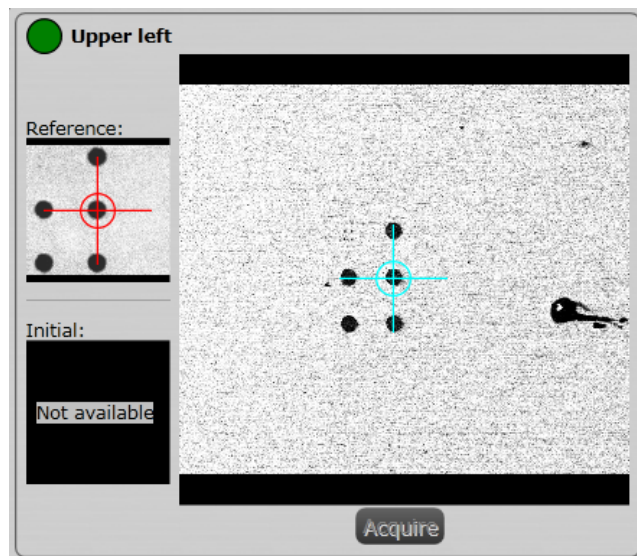
Fiducial Window

Table 23 Automated Holder Alignment UI Overview (3 of 3)

Interface Item	Description
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.
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	<p>The fiducial marker is placed on the fiducial image as a point of reference. Maps will align these reference points during alignment.</p> <ul style="list-style-type: none"> • 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.

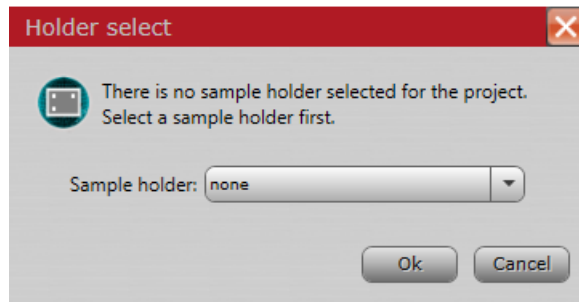
Executing the Holder Alignment

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

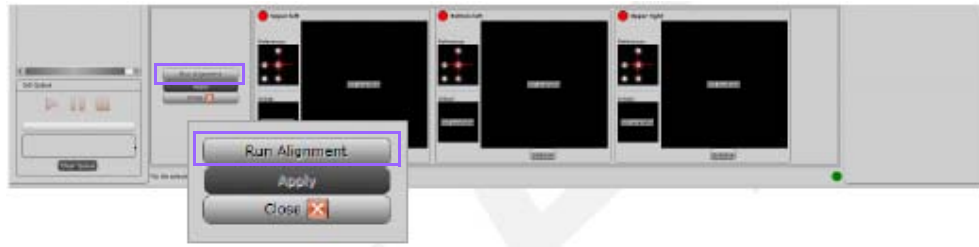
1. Click **Align to Holder**.



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. For the CorrSight see [“LM Holder Calibration” on page 110](#). For the SEM microscope, see [“EM Holder Calibration” on page 117](#).

LM Holder Calibration

NOTE

This procedure is applicable to all holders on LM, only the number of fiducial position is different.

LM Preconditions

- Tool running for > 1 hour
- Transmitted Light channel configured in Maps
- Live imaging functional in Maps
- Camera/Image orientation:
Find a distinguishable feature in live imaging in Maps.
Click in the live imaging window to center the feature in the FOV.
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**.

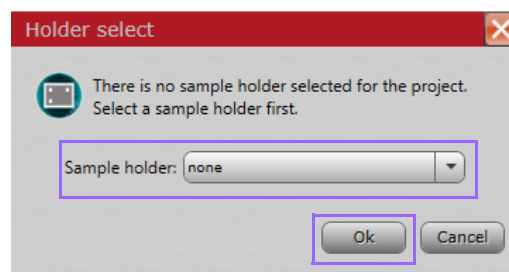
NOTE

Refer to your application specialist for procedural information for each sample type. See ["Sample Holders" on page 98](#) for descriptions of the other types

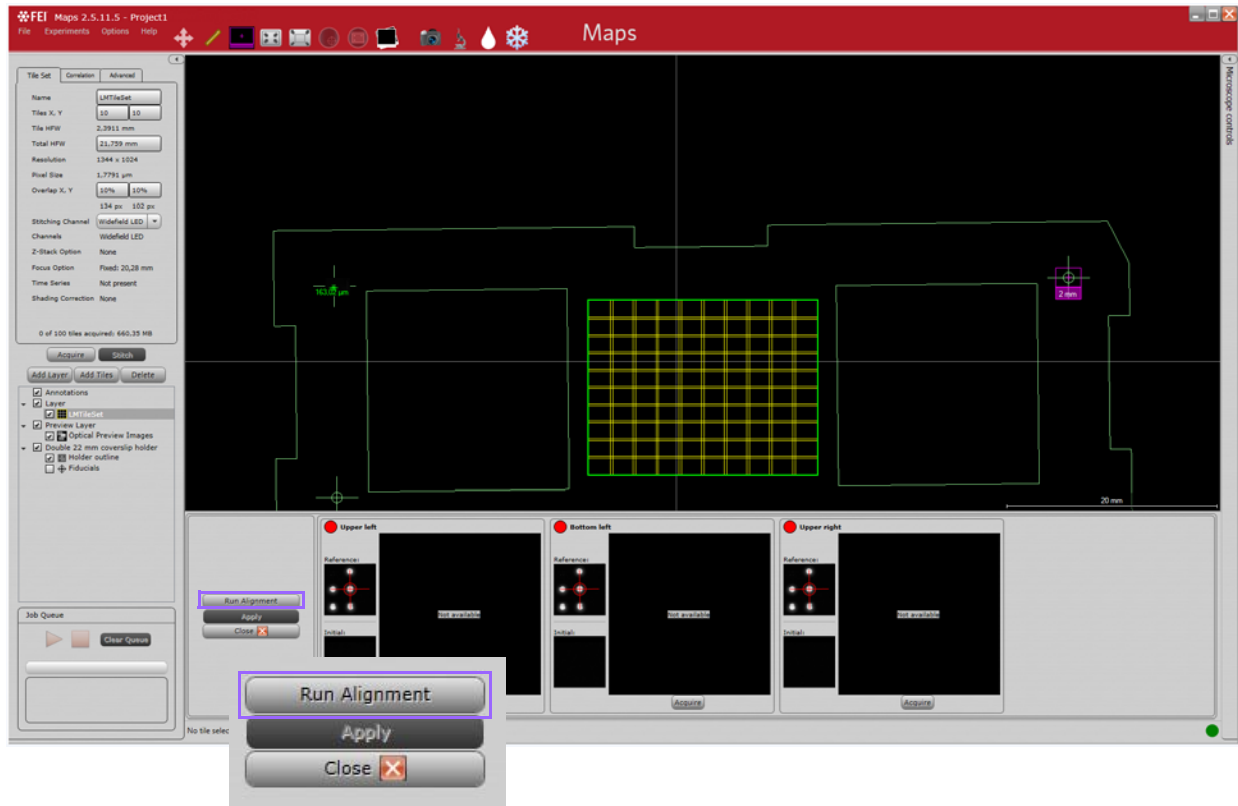
2. Create a New Project or open a project that uses the loaded holder type. You can create a project by selecting **File Menu > New Project...**
3. Click the **Align to Holder** tool box button.



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

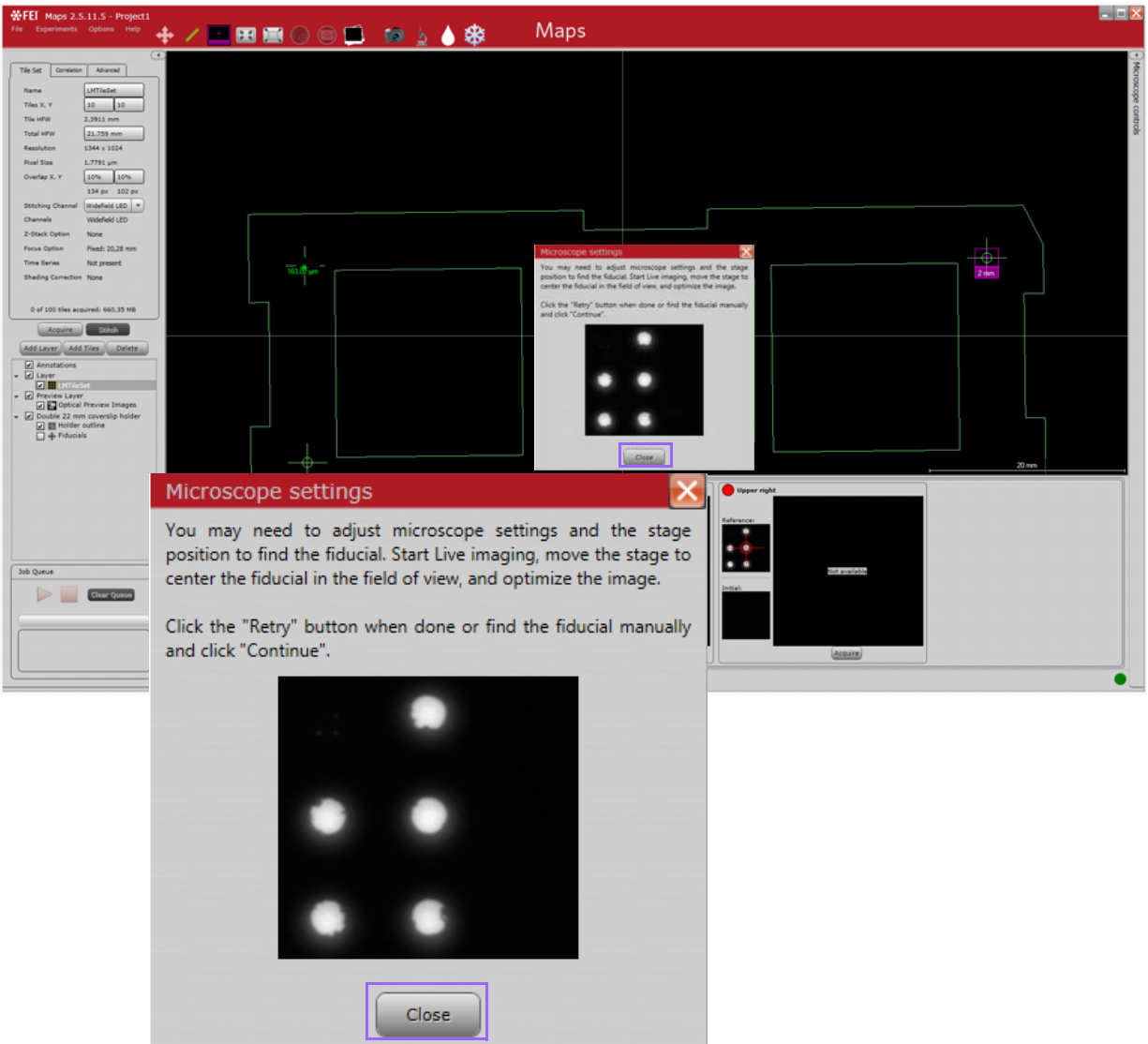


5. In the fiducial alignment controls that appear, click **Run Alignment**.



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.

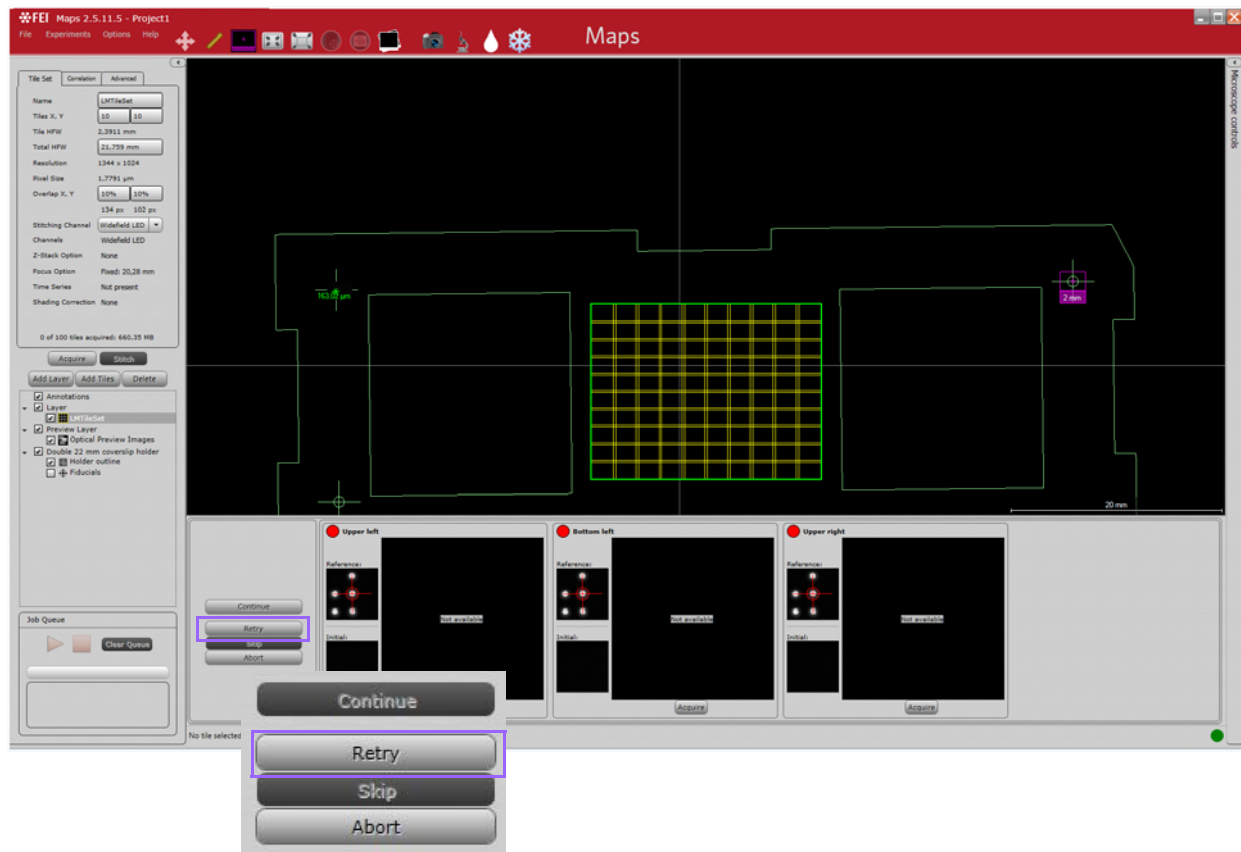


8. Locate the correct fiducial by moving the stage. Center the fiducial in the FOV

NOTE

During calibration, some matches might not select the correct fiducial hole 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 138*.
10. Click **Retry**.

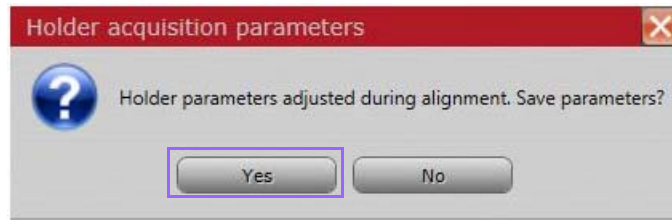


Maps will AutoFocus and attempt to match the fiducial. If the match is successful, the automated alignment procedure will continue to the next fiducial.

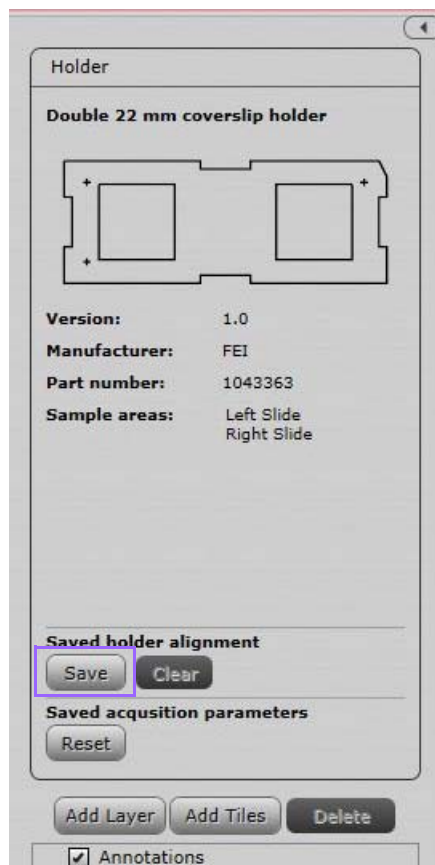
Maps will automatically attempt to match the second fiducial. If the match is not successful, repeat the calibration for the second fiducial (starting from [Step 6](#)).

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 on the Main Controls > Holder > Saved holder alignment > **Save** button in order to commit the calibration to the microscope.



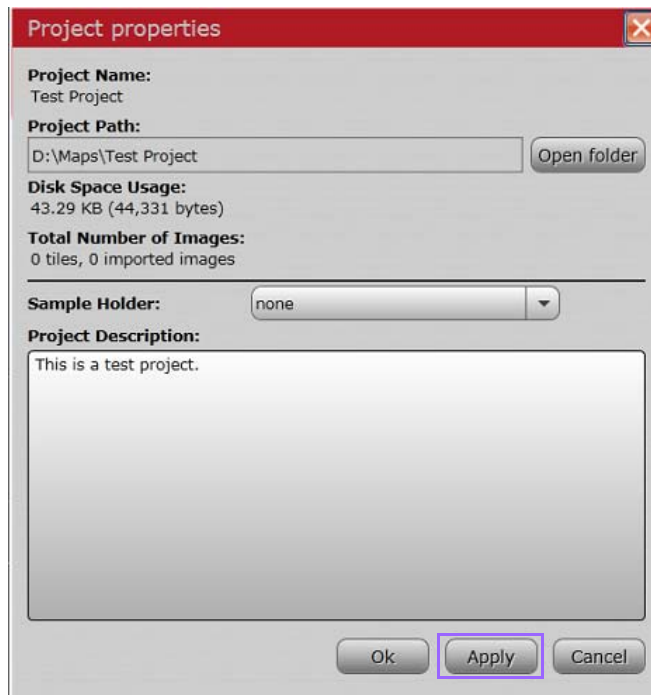
13. Proceed to verify the calibration.

Verifying the Calibration

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



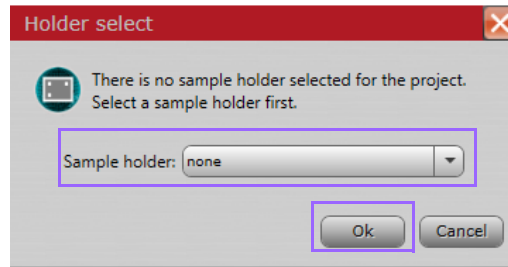
2. Click **OK** to clear the holder from the project. Click **OK** again at the prompt to clear.



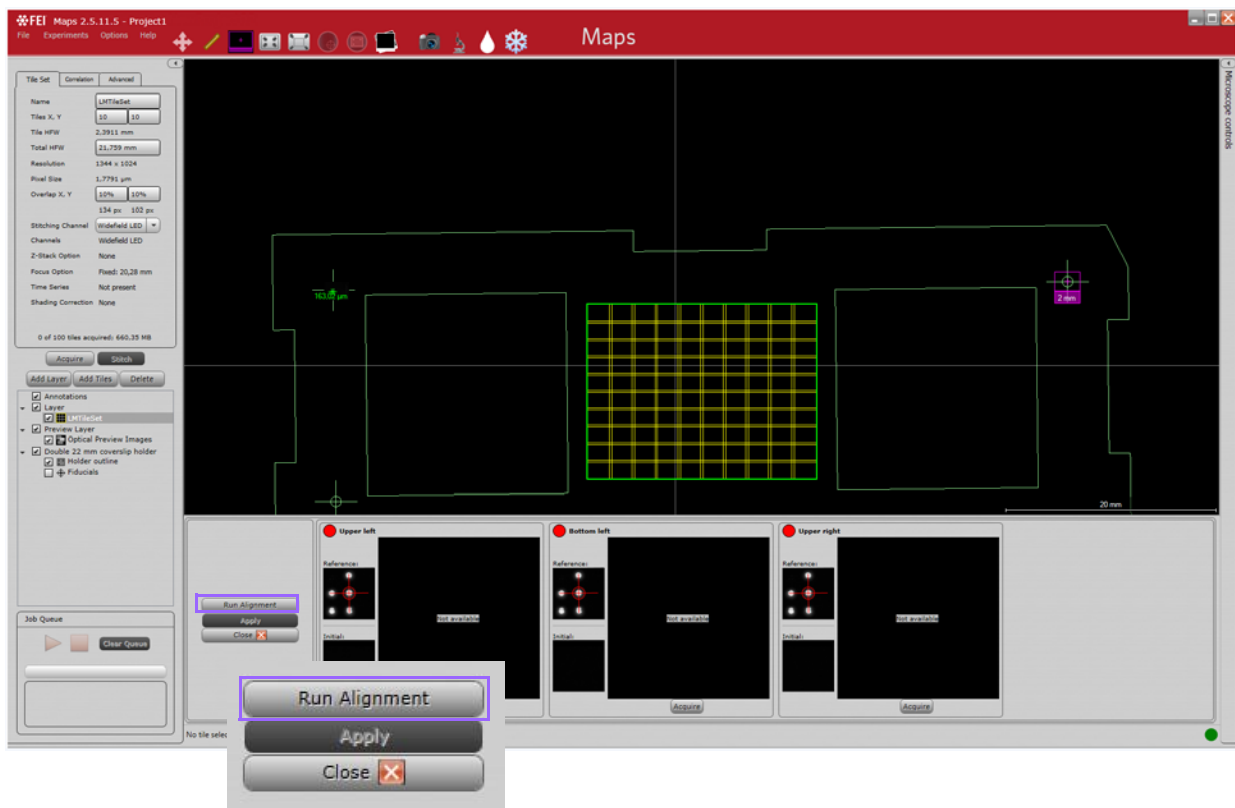
3. Click **Align to Holder**.



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



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



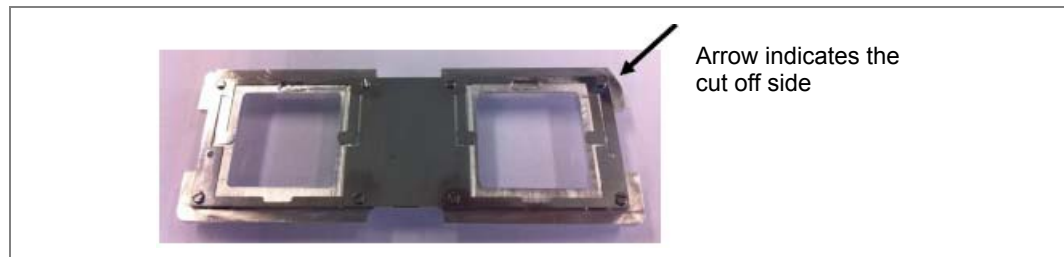
- If the automated alignment is able to match all fiducials successfully, the holder calibration is complete.
- If adjustment is still required, repeat the above procedure.

EM 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 14 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 15](#)) fits the hole (encircled in red in [Figure 16](#)) in the adaptor.

Figure 15 Bottom View of the Holder

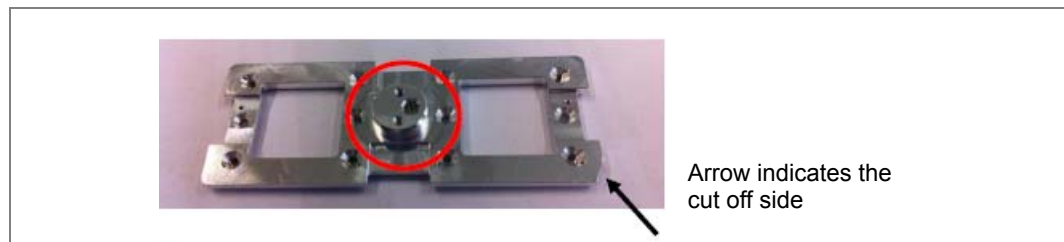
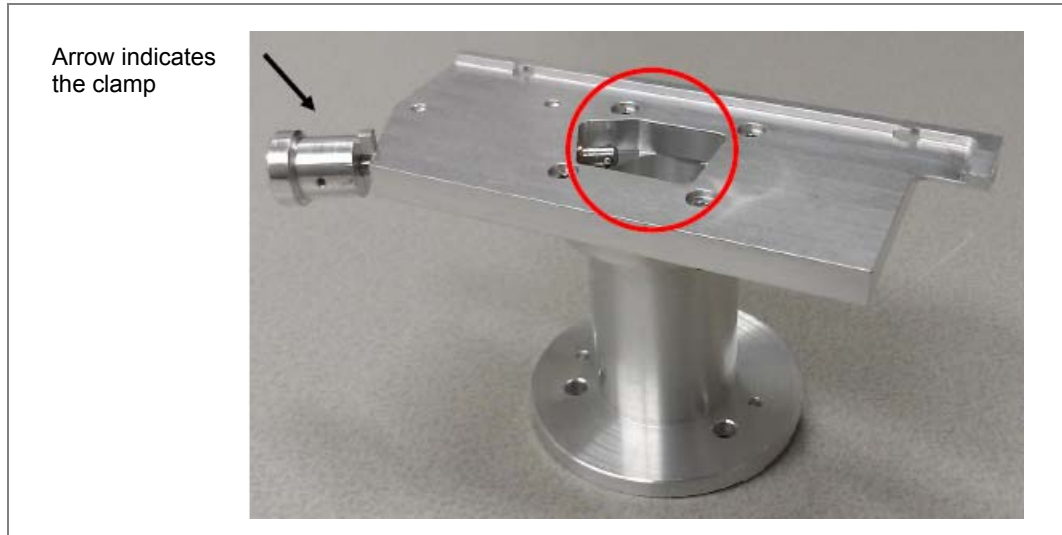
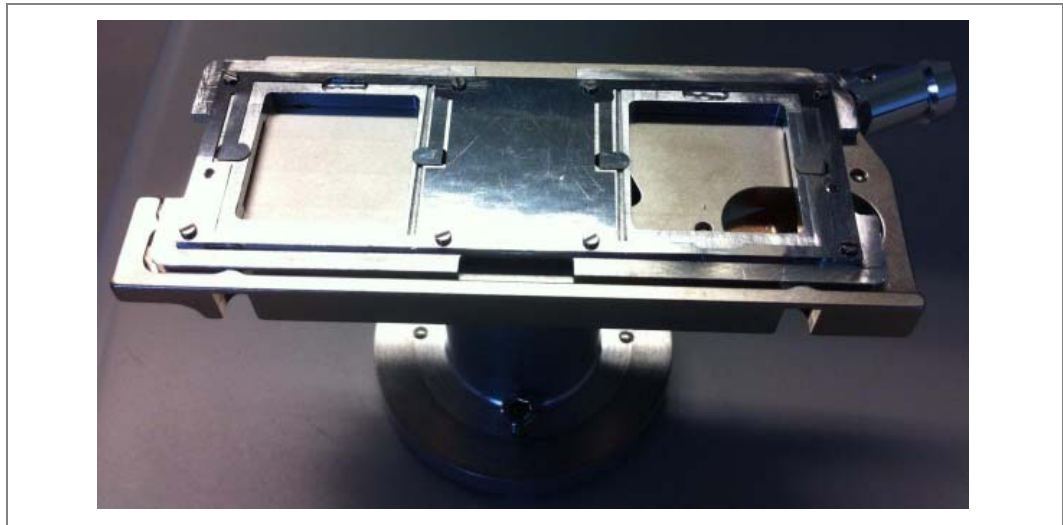


Figure 16 6 inch Stage Adaptor for Double 22 mm Coverslip Holder



2. If the holder is positioned ([Figure 17](#)), release the clamp.

Figure 17 Holder Positioned on Adaptor



- 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 18*) and two pins (encircled in green) that fit nicely into the stage (*Figure 19*).

Figure 18 Adaptor Base with Screw and Pin Positions

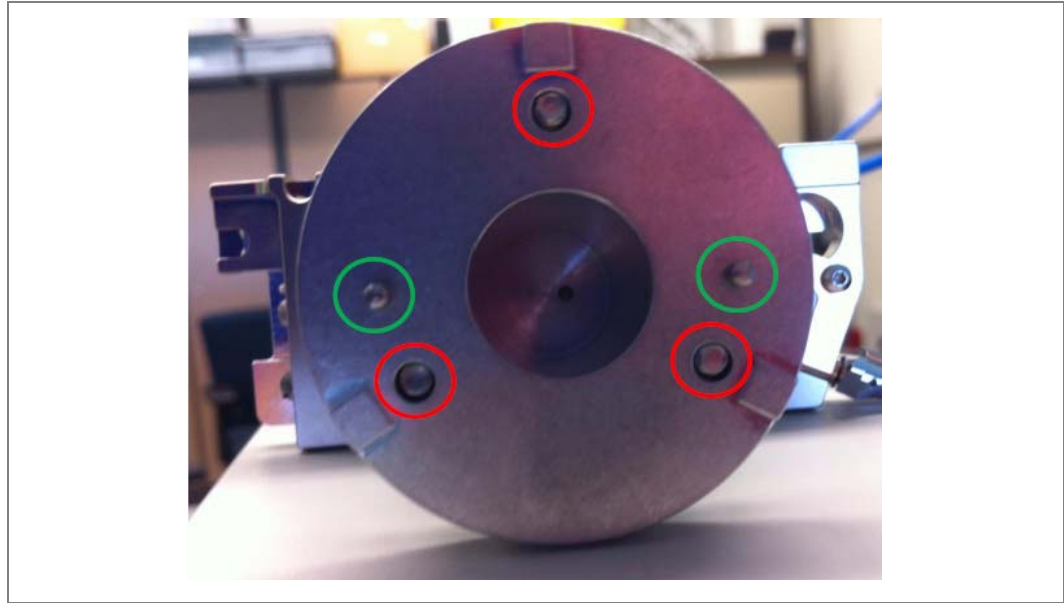
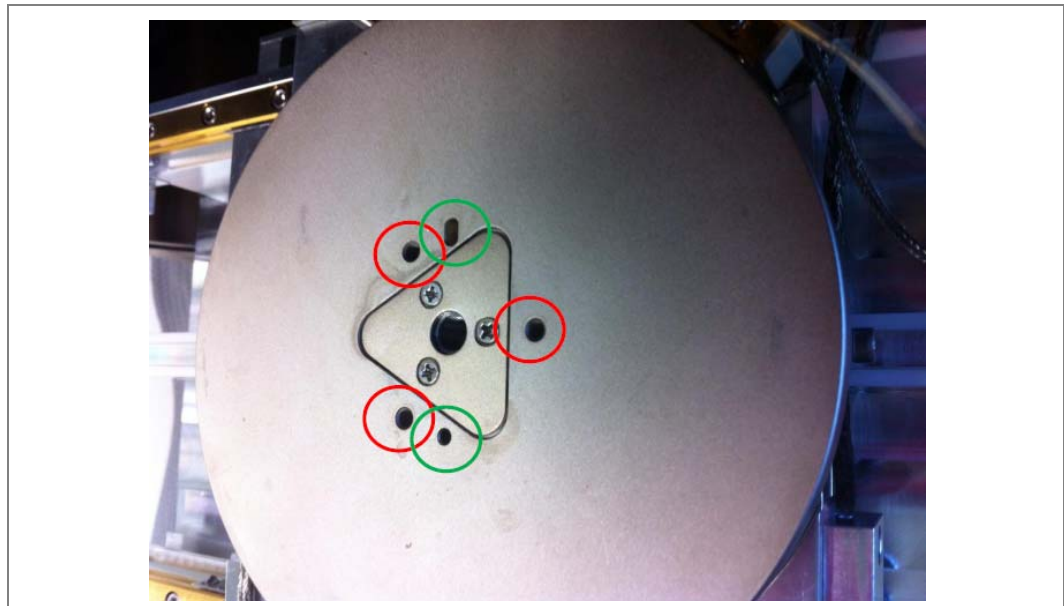


Figure 19 Stage Screw and Pin Placement Indicators



- Tighten the screws so the stage can't move.
- 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 holder type. See [“Sample Holders” on page 98](#) for descriptions of the types of holders.

EM Preconditions

Before performing calibration on a Maps 2.1 supported holder using an SEM/SDB (small DualBeam) 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

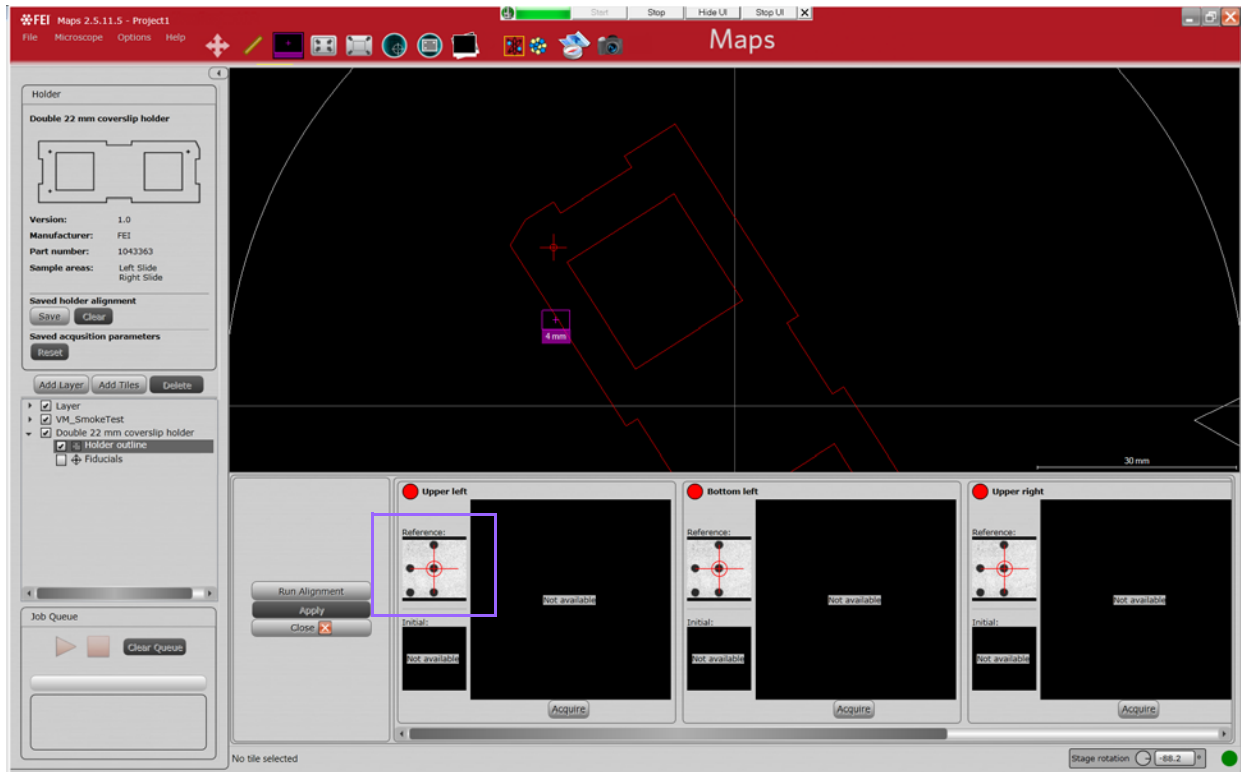
NOTE

This procedure is only necessary when starting Maps for the first time on a 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.

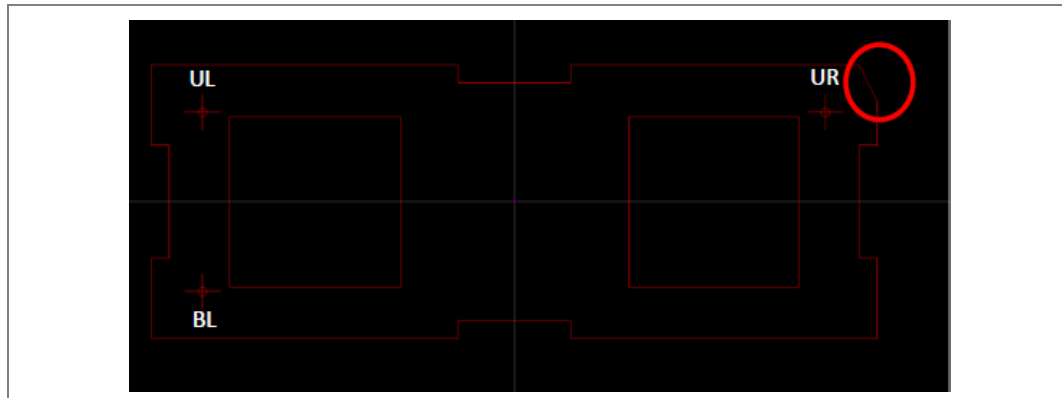
NOTE

The fiducial orientation must match the reference image.



The fiducial next to the chamfer/cut off (encircled in red; [Figure 20](#)) is called upper right.

Figure 20 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 reverse

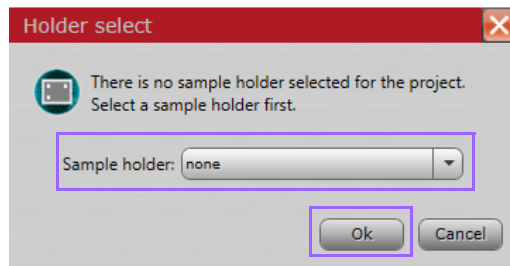
- If you are entirely unable to find the fiducials on the holder, perform overview tile for help in navigation.
- If the system is equipped with a NavCam, use the NavCam navigate assist with finding the fiducials.

Performing the Calibration

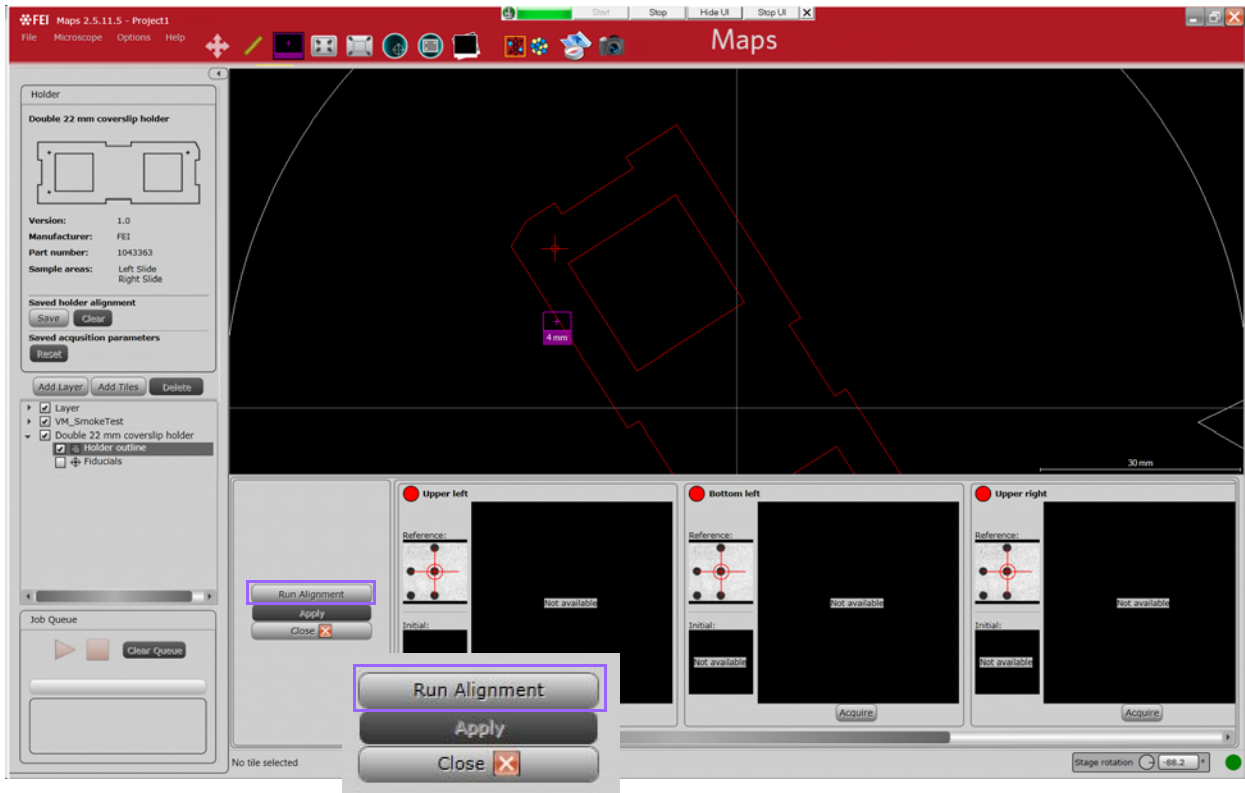
1. Create a new project in Maps.
2. Click the **Align to Holder** icon in the tool box.



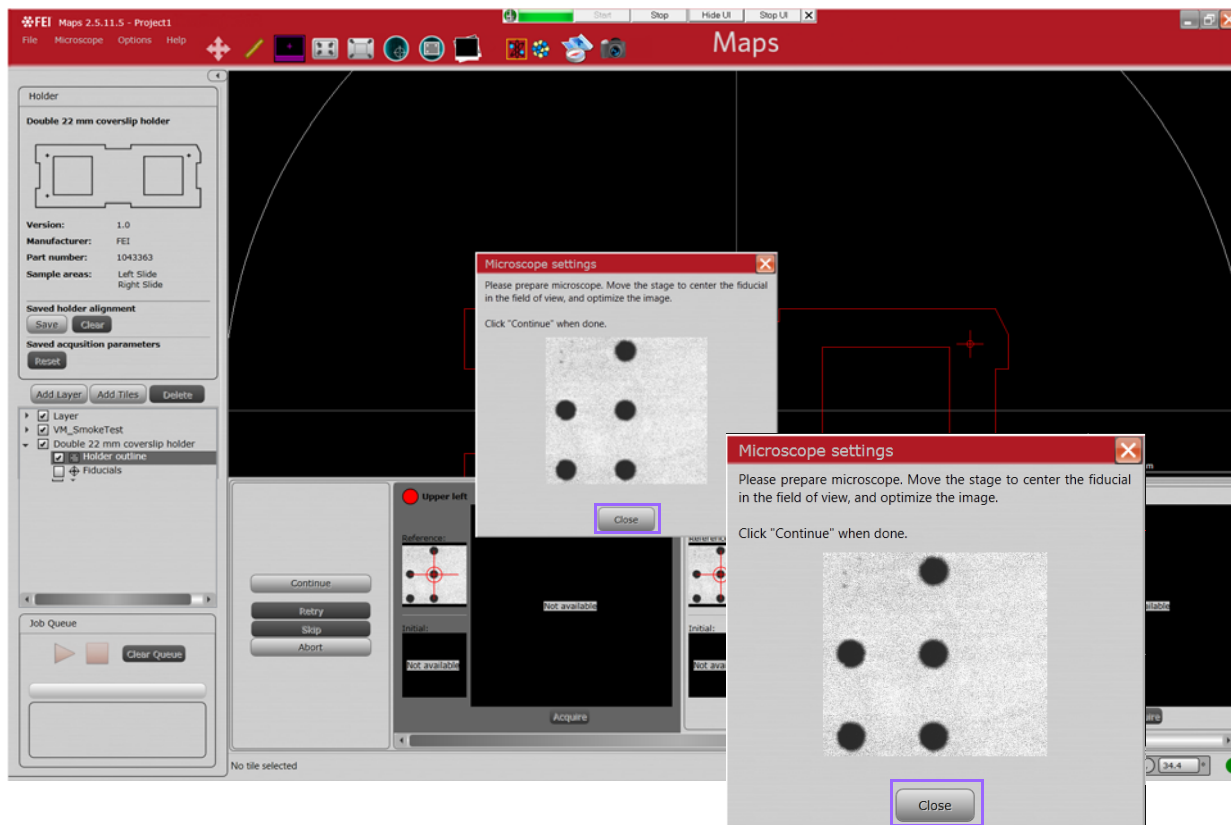
3. Select the appropriate holder from the **Sample Holder** dropdown menu, in this example it is **Double 22 mm coverslip holder**, and click OK.



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

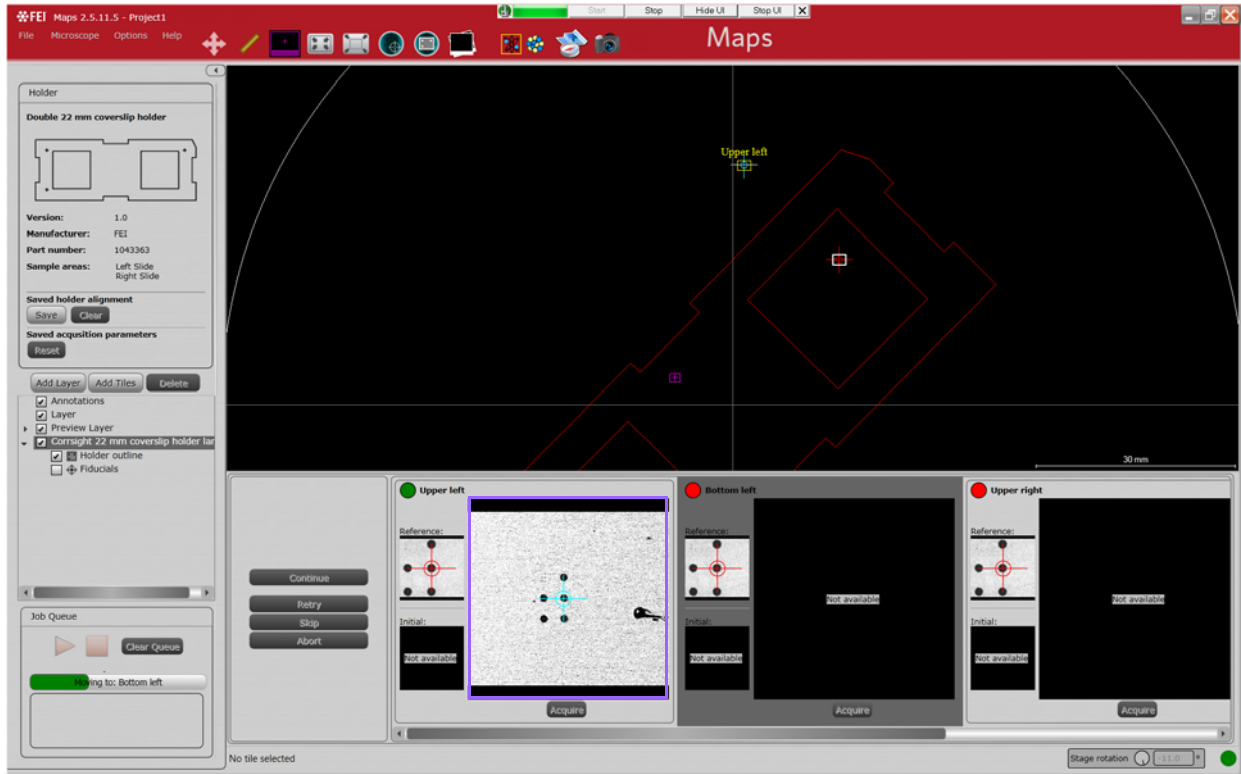


- The stage drives to the position where it expects to find the upper left fiducial. Maps displays a prompt to adjust microscope settings for optimal fiducial image. Click **Close**.



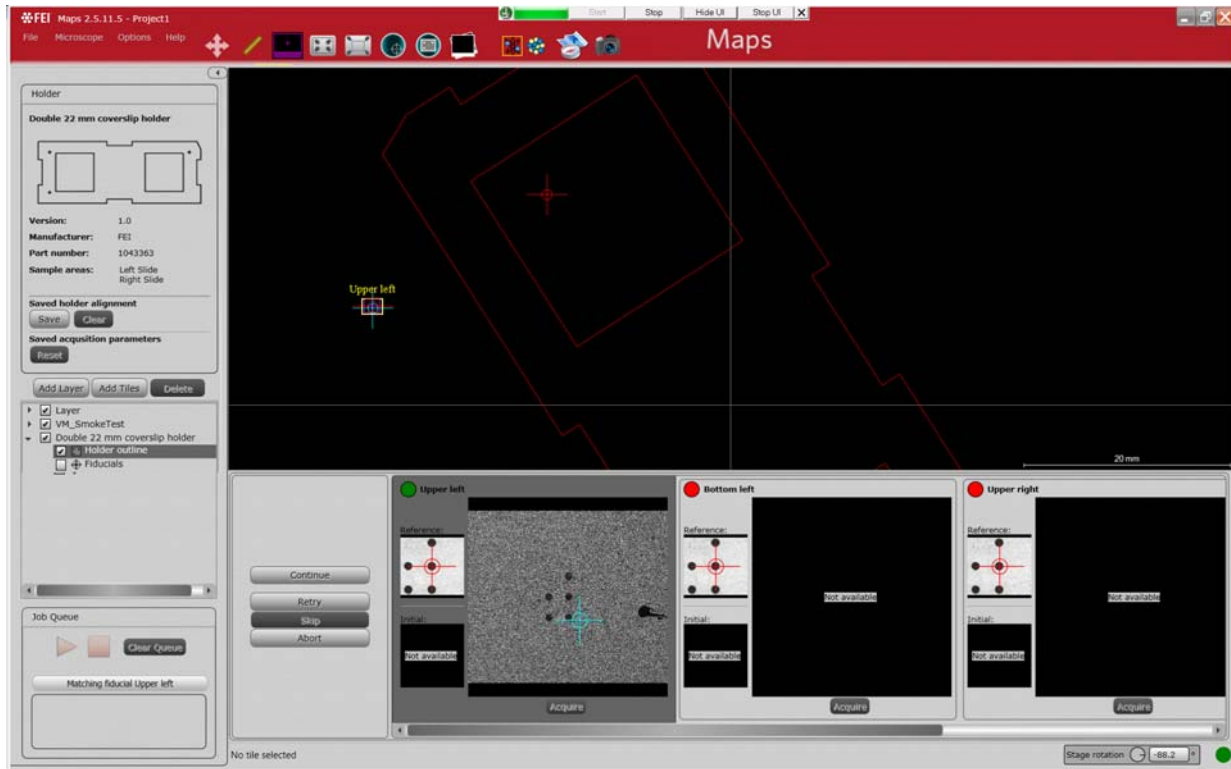
- Go to the xT UI and find the stored position for upper left fiducial. Select the upper left coordinates and click **GoTo**. The stage navigates to the stored coordinates of the upper left fiducial.
- Optimize the image (set correct focus and contrast/ brightness) of the upper left fiducial using the 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	<p>If you are having trouble matching, make sure the:</p> <ul style="list-style-type: none"> ■ Contrast / brightness matches reference ■ Fiducial orientation matches reference ■ HFW is 1.5 mm ■ Sample is clean with clearly distinguishable features and no dust contamination
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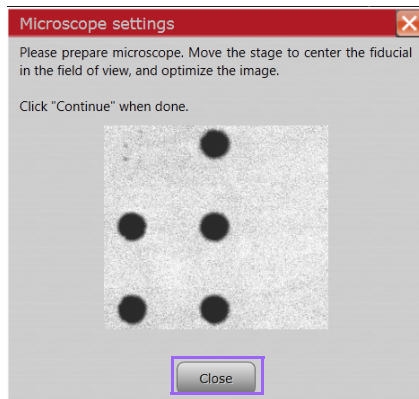


NOTE

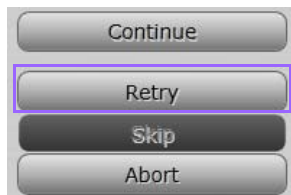
During calibration, Maps does not know the orientation of the holder. This results in false positive matches, as shown in the diagram below. This is normal as false positives will not occur after the holder has been successfully calibrated



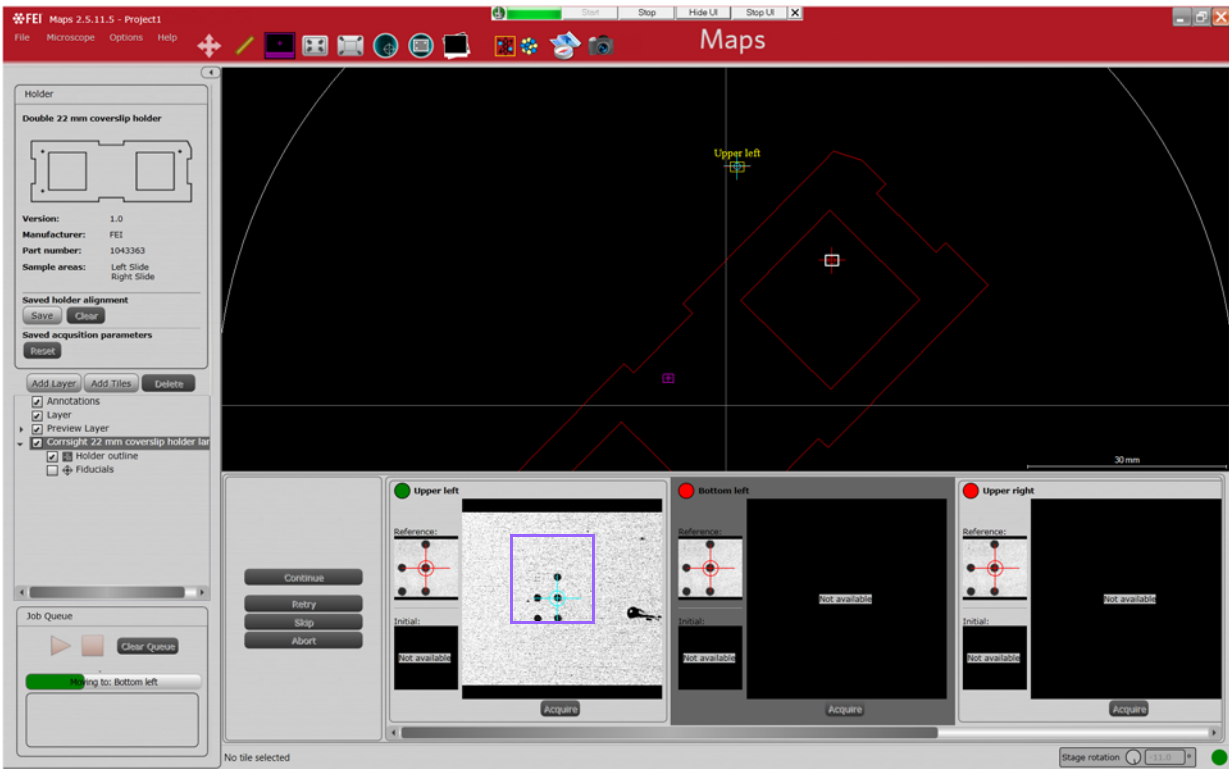
- The Microscope Settings box displays again if Maps still does not recognize the acquired fiducial. Click **Close**.



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



- 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.

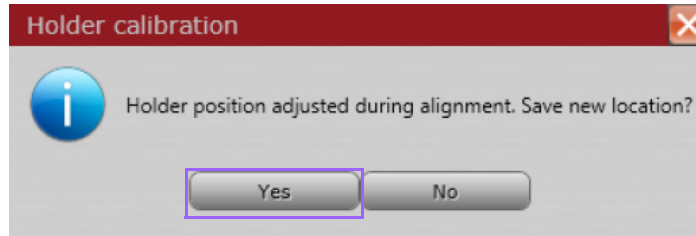


The red button (encircled in red) turns green if Maps recognizes the fiducial.



- If Maps does not automatically navigate to the second fiducial, click **Continue**.
- 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 calibrated fiducial positions and will not allow you to save the potent calibration values. This can occur if there is a large unexpected rota fiducials.

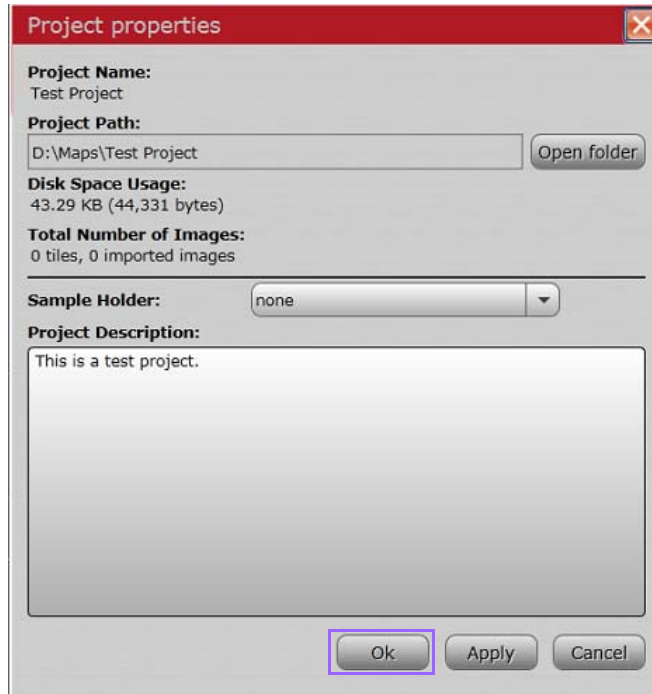
Perform the calibration a second time, and make sure fiducials are \ and are oriented properly to match the reference.

Verifying the Alignment

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



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



- Proceed to the procedure, *"Executing the Holder Alignment"* on page 109.

Correlating Projects

Pre-requisites 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

5 Light Microscopy

Overview

This chapter describes how to use the Maps software on a light microscope.

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

Topics include:

- *“Microscope Controls” on page 133*
- *“Live Imaging” on page 139*
- *“Live Imaging Controls” on page 141*
- *“Z-Stack Browser” on page 144*

Microscope Controls

Figure 21 Microscope Controls

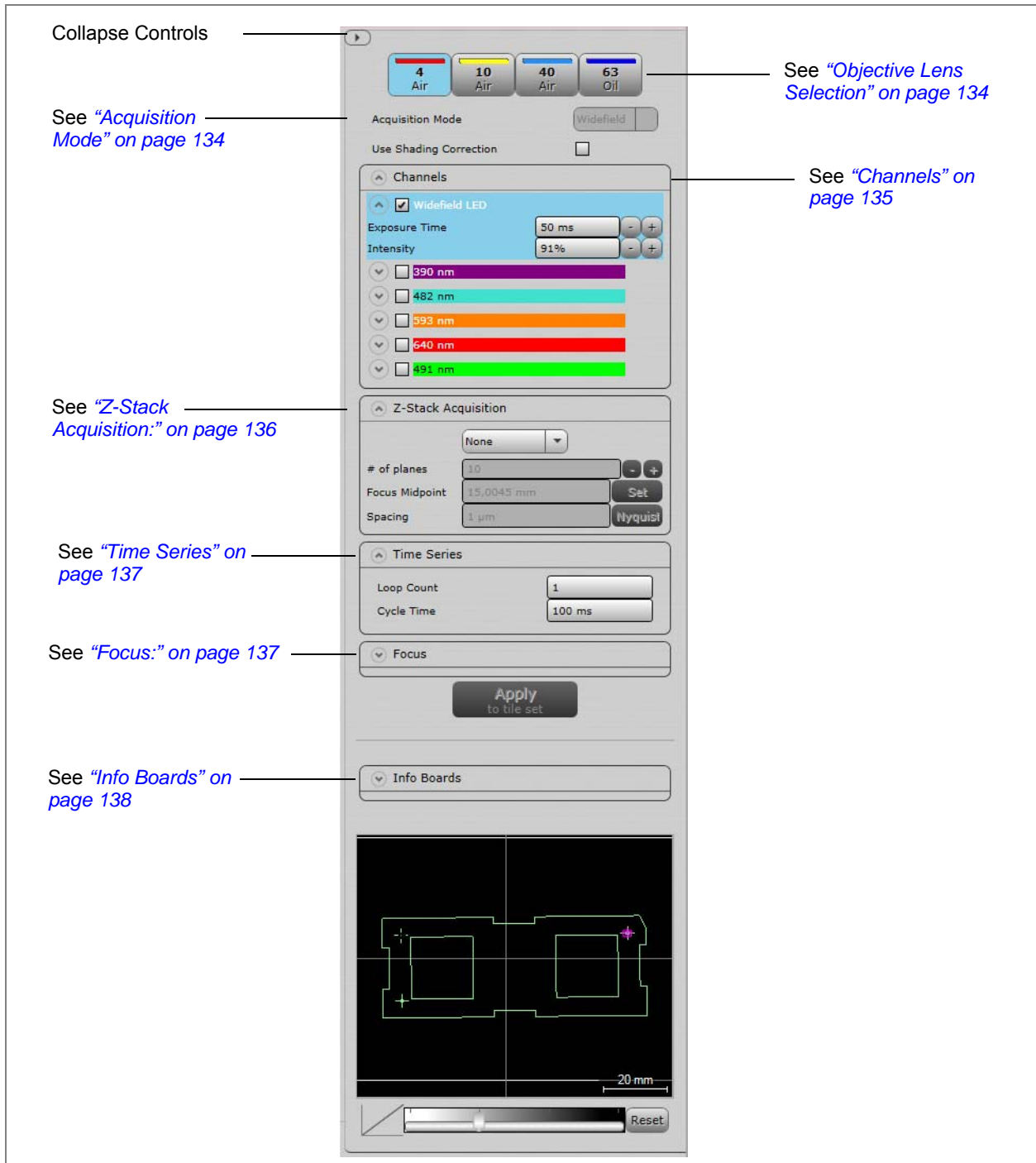


Table 24 Microscope Controls Overview (1 of 5)




Interface Item	Description
Objective Lens Selection	 <p>Displays the objective lens selections: 4X, 10X, and 40X, and the immersion type: Air, Oil, Glycerol, Water, or Special installed on your microscope. <i>The display is configured in the FEI Live Acquisition software.</i></p> <p>The depressed button (blue) is the selected objective lens.</p> <p>Note: Take care when changing between objective lenses with different immersion types.</p>
Acquisition Mode	 <p>Specifies the Acquisition mode (hardware specific):</p> <ul style="list-style-type: none"> • Widefield • Structured Illumination • Spinning Disk <p>Note: Some acquisition modes are not available on all systems.</p>
Use Shading Correction	 <p>Uses a blank reference image to correct (remove) shading effects in all images acquired inside the tile set.</p> <p>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.</p> <p>Note: Before this feature can be used in the Maps application, the reference must be acquired and stored using the Live Acquisition application.</p>

Table 24 Microscope Controls Overview (2 of 5)

Interface Item	Description
<p>Channels</p>	<div data-bbox="522 359 1005 709" data-label="Image"> </div> <p>The channel names and colors are configured in the FEI Live Acquisition software. The selected channel is highlighted in blue. At least one channel must be selected.</p> <p>As soon as you create a tile set, all enabled channels are applied when the tile set is acquired.</p>
<p>Expand/Collapse (down/up arrow)</p>	<p>Clicking the arrow expands the selected channel to show the Exposure Time and Intensity for that channel.</p> <div data-bbox="526 982 943 1136" data-label="Image"> </div> <p>Enter a value or click - or + to change a value.</p>
<p>TL Contrast</p>	<p>Transmitted light contrast is a software supported method to improve the contrast of transmission images for CorrSight instruments. The TL Contrast control will be selectable from the Channels section. For more details on this, see the Live Acquisition user doc at http://www.live-acquisition.com/37LA_/Online_Manual/index.html#!Documents/transmittedlightcontrast.htm</p>
<p>Exposure Time</p>	<p>Specifies the time (in milliseconds) that the sample is illuminated for imaging.</p>
<p>Intensity</p>	<p>Specifies the percentage of maximum (full) illumination.</p>

Table 24 Microscope Controls Overview (3 of 5)

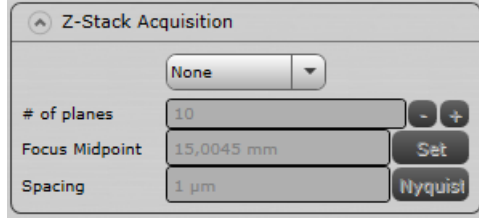
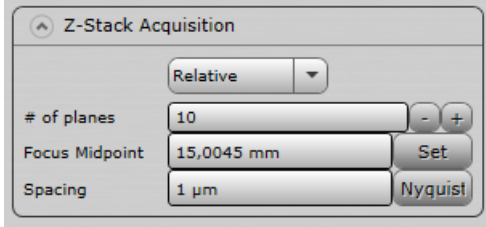
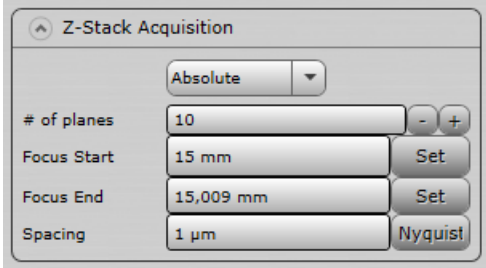
Interface Item	Description
Z-Stack Acquisition:	
	<p>A Z-stack is a series of multiple images taken with varying focus. Each tile in a grid is a separate stack for a Z-stack acquisition.</p>
Relative or Absolute	<p>Defines the Z-stack as:</p> <ul style="list-style-type: none"> • None: No Z-stack defined • Relative: To the defined midpoint of the stack
	
	<ul style="list-style-type: none"> • Absolute: To the top and bottom of the stack
	
Focus Midpoint	<p>When Relative is selected, specifies the center or midpoint of the stack. Type in a value and click Enter.</p>
Set	<p>This uses the current focus position for the focus midpoint. Dial in a focus midpoint with the mouse scroll wheel and click Set.</p>
# of Planes	<p>When Relative is selected, specifies the number of planes (images) in the stack. Use the - and + buttons to change the number.</p>
Focus Start	<p>When Absolute is selected, specifies the top of the stack. Note: <i>Z must be smaller than the Focus End value.</i></p>
Focus End	<p>When Absolute is selected, specifies the bottom of the stack.</p>

Table 24 Microscope Controls Overview (4 of 5)


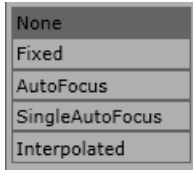
Interface Item	Description
Set	Pulls the current microscope position and displays it in the Focus Start and Focus End fields.
Spacing	Specifies the distance the objective moves between images in the stack. Choices are: <ul style="list-style-type: none"> • Nyquist: A calculated value based on the selected objective that produces the optimal sample rate. • Custom: Spacing is defined by the user in this field.
Time Series	Allows a tile set acquisition to be acquired multiple times at a specified time interval to collect images of a sample that is changing with time.
Focus:	
Focus Strategy	Specifies the focus strategy.  <ul style="list-style-type: none"> • None: Maps will not change the objective position during acquisition of the tile set. • Fixed: Uses a fixed objective position for the tile set. This is useful for queuing tile sets with different objective positions. • AutoFocus: Performs the AutoFocus routine at each tile before acquisition. • SingleAutoFocus: Performs a single AutoFocus routine at the center of the tile set and uses the resulting objective position for the entire tile set. • Interpolated: User will select three focus points on the sample and a focus plane will be defined by these points. Each tile's objective position will be set based on where it is located in this plane. See "Focus Strategy: Interpolated Procedure" on page 138 for the procedure.
Auto Focus Channel	Specifies the channel upon which the focus strategy for each tile in the set will be applied.

Table 24 Microscope Controls Overview (5 of 5)

Interface Item	Description
Step Size	Specifies the step size of the auto focus.
Focus	Specifies the current focus position.
Auto Focus	Uses the step size for live imaging Auto Focus.
Apply to tile set	Modifies the existing selected tile set.
Info Boards	For microscopes with the a Cryogenic cooling system.

The screenshot shows a window titled 'Info Boards' with a collapse icon. It contains four rows of controls:

- Sample 1: Not Connected
- Sample 2: Not Connected
- Chamber 1: Not Connected
- Chamber 2: Not Connected

Focus Strategy: Interpolated Procedure

1. Navigate to the first location.
2. Manually focus the image. When the image is in focus, click set **WD1** button. The current objective position will be remembered for that point and the button will now be checked.
3. Repeat for the other two Set WD 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 tile objective position as defined by this plane. Choosing points that are ends of the sample is important to get the best results.

4. Click **Reset** to clear all three WD settings.

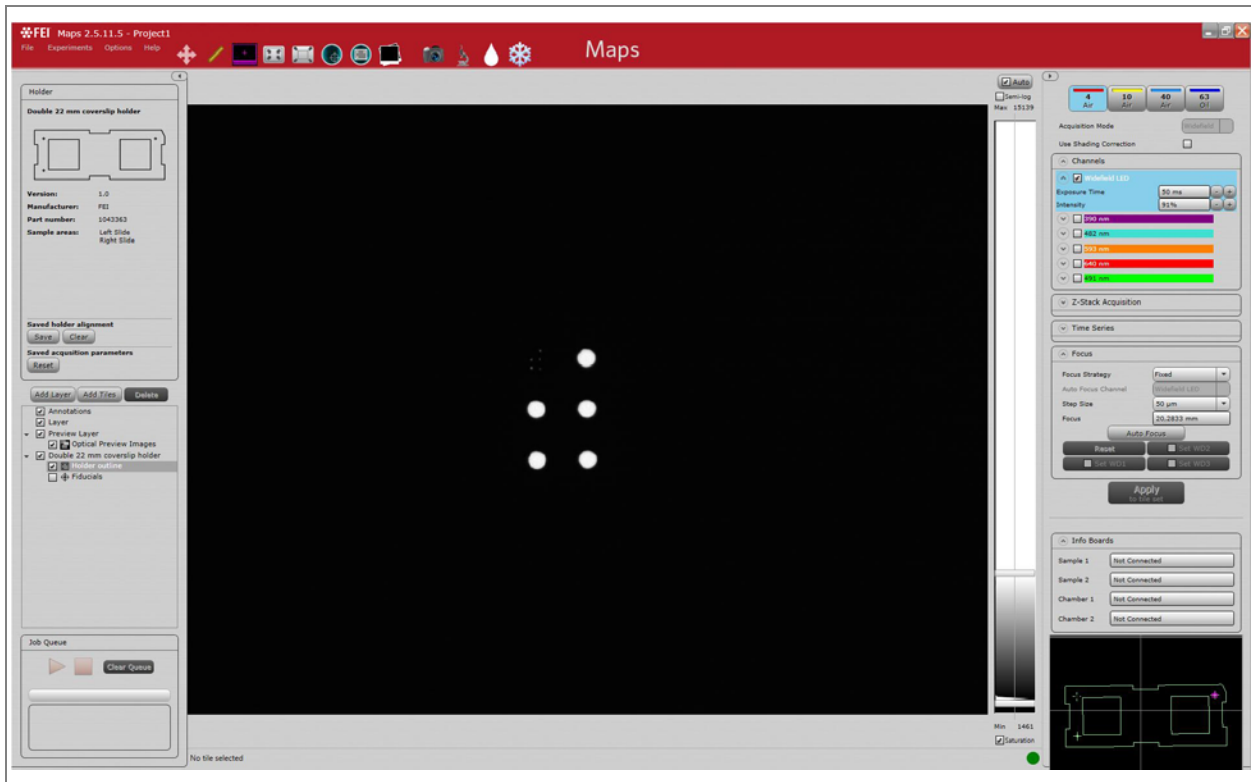
Live Imaging

Click the **Live Imaging** icon in the Tool Box to start live imaging.



Live Image Window

Figure 22 Live Image Window, Microscope Controls Expanded



Click the arrow to expand/collapse the Microscope controls.

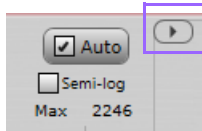
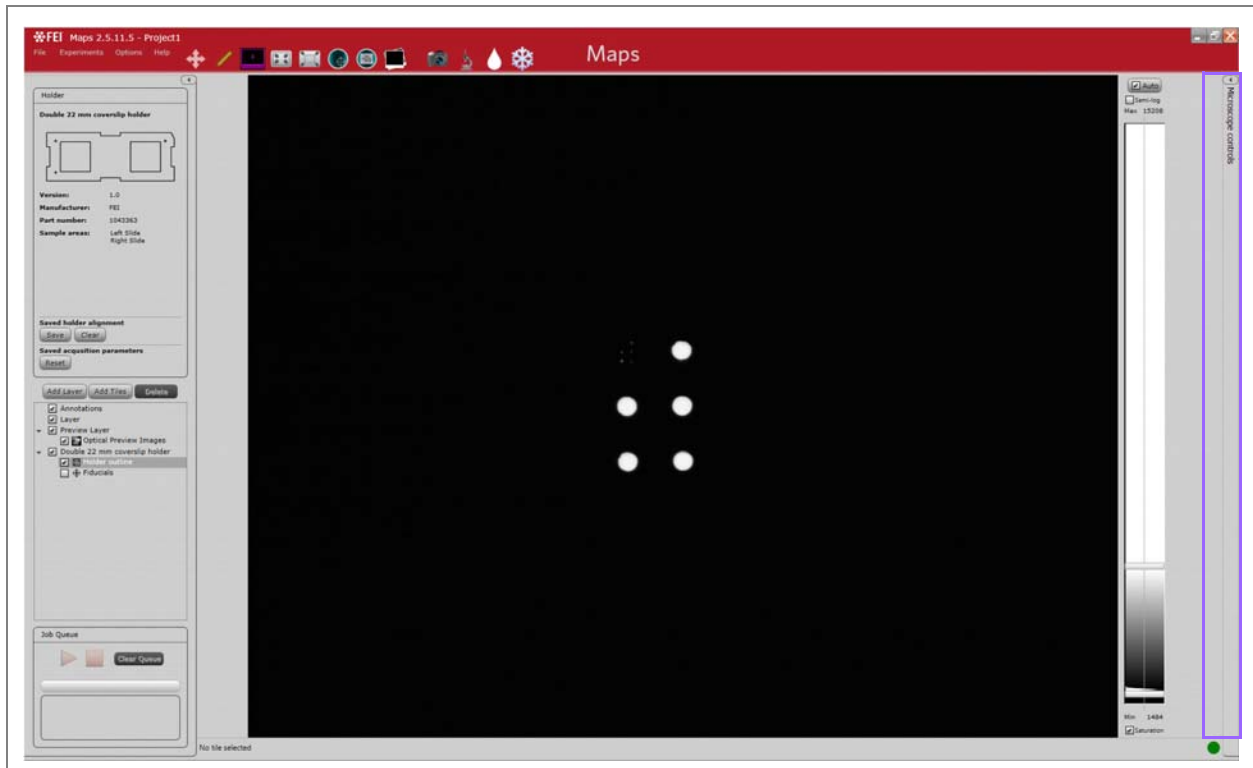


Figure 23 Live Image Window, Microscope Controls Collapsed



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

Use the mouse scroll wheel inside the Live Image window to change focus at the selected step size.

Live Imaging Controls

Figure 24 Live Imaging Controls

The screenshot shows the Live Imaging Controls software interface. On the left, a vertical histogram displays the signal intensity distribution, with a 'Max' value of 15305 at the top and a 'Min' value of 1485 at the bottom. A 'Saturation' checkbox is checked at the bottom of the histogram.

The main control panel includes the following sections:

- Top Controls:** An 'Auto' checkbox is checked. Below it are four buttons labeled '4 Air', '10 Air', '40 Air', and '63 Off'.
- Acquisition Mode:** A 'Widefield' button is selected.
- Use Shading Correction:** An unchecked checkbox.
- Channels:** A 'Widefield LED' checkbox is checked. Below it are 'Exposure Time' (50 ms) and 'Intensity' (91%) controls. A list of channels is shown with checkboxes: 390 nm (purple), 482 nm (cyan), 593 nm (orange), 640 nm (red), and 491 nm (green).
- Z-Stack Acquisition:** A dropdown menu.
- Time Series:** A dropdown menu.
- Focus:** 'Focus Strategy' is set to 'Fixed'. 'Auto Focus Channel' is 'Widefield LED'. 'Step Size' is '50 μm'. 'Focus' is '20,2833 mm'. There is an 'Auto Focus' button and four 'Set WD' buttons (WD1, WD2, WD3, and an unlabeled one).
- Info Boards:** A table showing the status of Sample 1, Sample 2, Chamber 1, and Chamber 2, all of which are 'Not Connected'.
- Mini Viewer:** A bottom window showing a tiled image of a sample with a green outline and a pink crosshair.

Callout boxes on the left side of the interface point to the following features:

- 'Auto' checkbox: See "Auto" on page 142
- 'Semi-log' checkbox: See "Semi-log" on page 142
- Histogram: See "Histogram" on page 142
- 'Saturation' checkbox: See "Saturation" on page 142

A callout box on the right side points to the 'Widefield LED' and 'Channels' section: See "Microscope Controls" on page 133

Another callout box on the right side points to the 'Mini Viewer' window: See "Mini Viewer" on page 143

Table 25 Live Imaging Controls Overview (1 of 2)

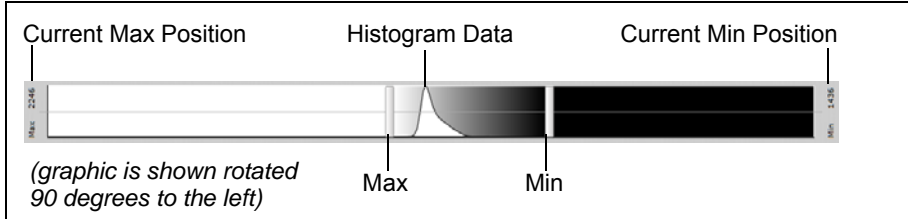
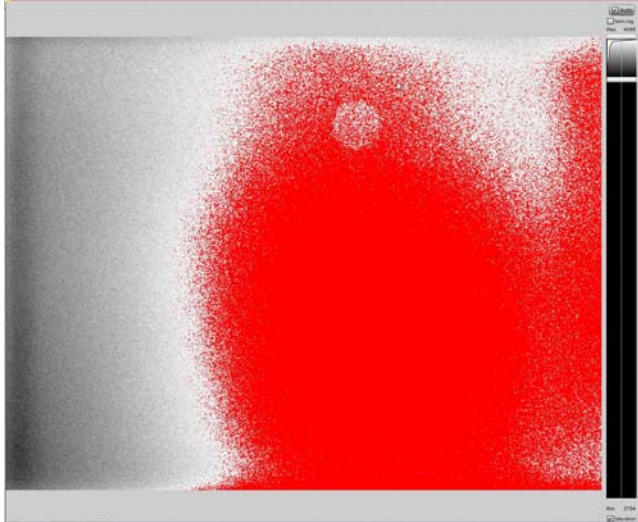
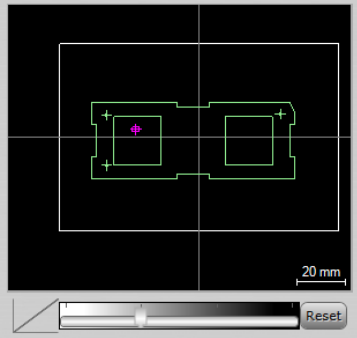

Interface Item	Description
Auto	When selected, continuously adjusts the min and max for the histogram display.
Semi-log	When selected, changes the histogram intensity display to logarithmic.
Histogram	<div data-bbox="521 541 1422 758" style="border: 1px solid black; padding: 5px;">  <p>(graphic is shown rotated 90 degrees to the left)</p> </div> <ul style="list-style-type: none"> • 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	<p>When selected, the saturated pixels are colored red.</p> 

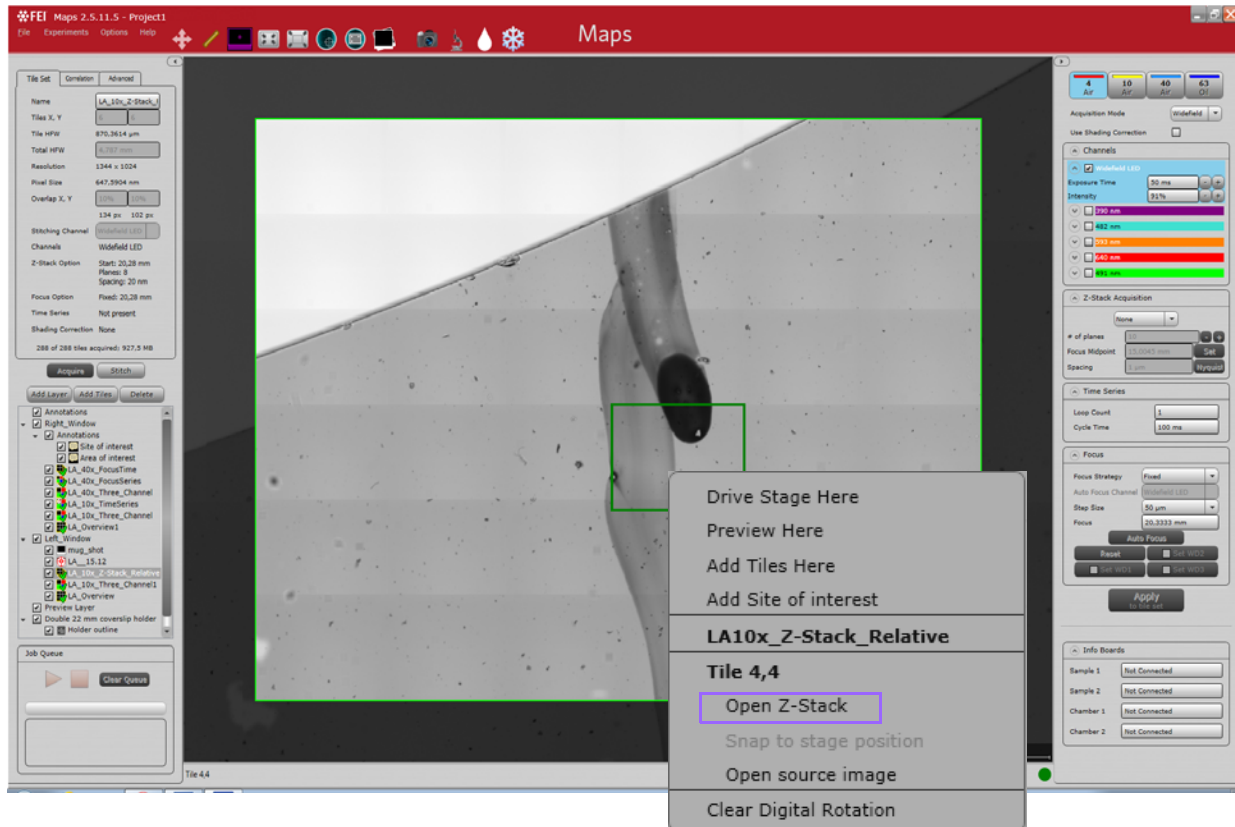
Table 25 Live Imaging Controls Overview (2 of 2)

Interface Item	Description
<p>Mini Viewer</p>  <p>The Mini Viewer has all of the functionality of the main viewer.</p>	<p>The Mini Viewer has all of the functionality of the main viewer.</p> <p>Gamma control slider</p> 
<p>Gamma Control Slider</p>	<p>Adjusts the gamma for the live image.</p>
<p>Reset</p>	<p>Returns to the default gamma setting.</p>

Z-Stack Browser

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

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



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

Z-Stack Browser Controls

Figure 25 Z-Stack Browser Controls

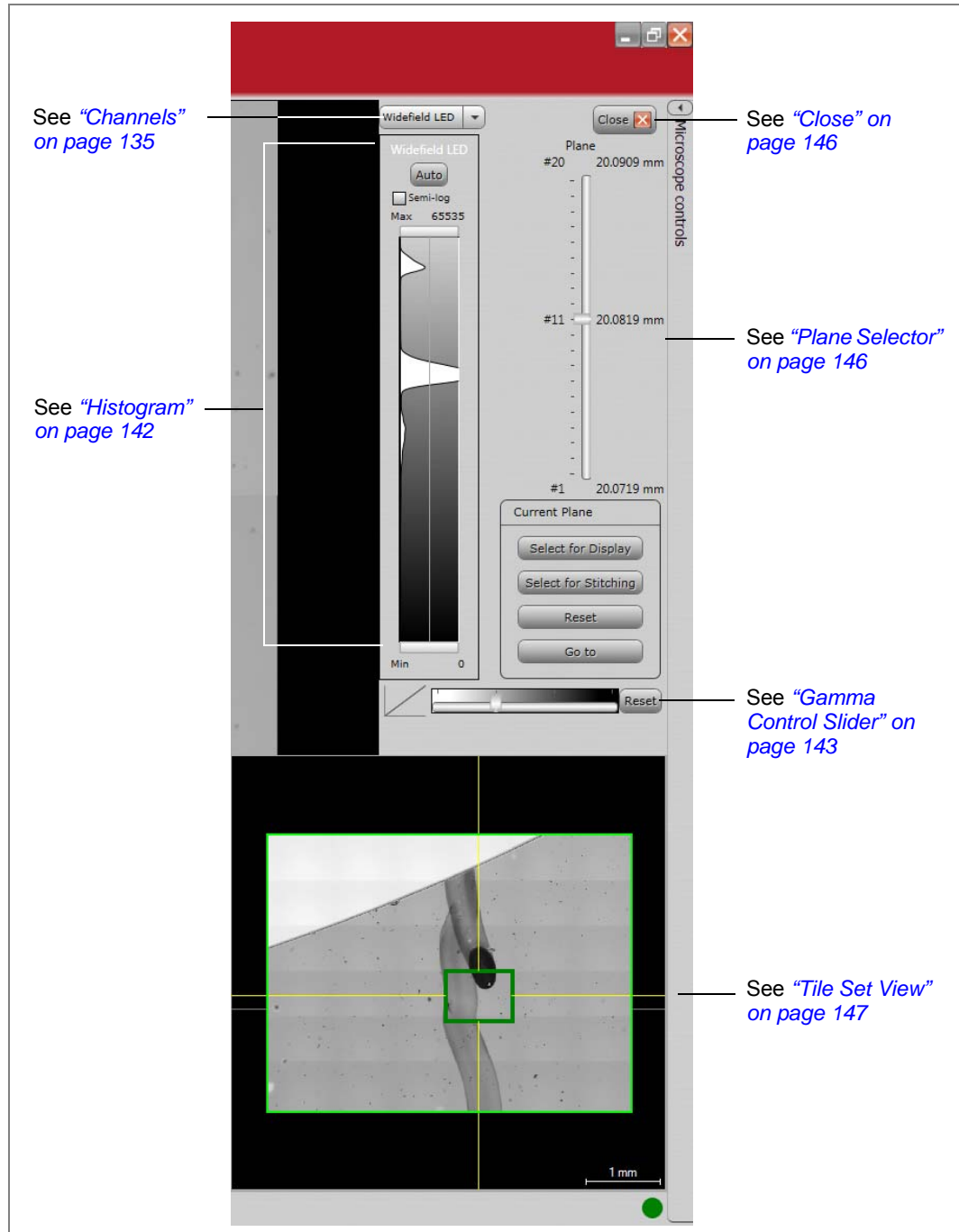


Table 26 Z-Stack Browser Controls Overview (1 of 2)

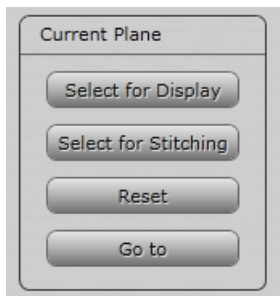
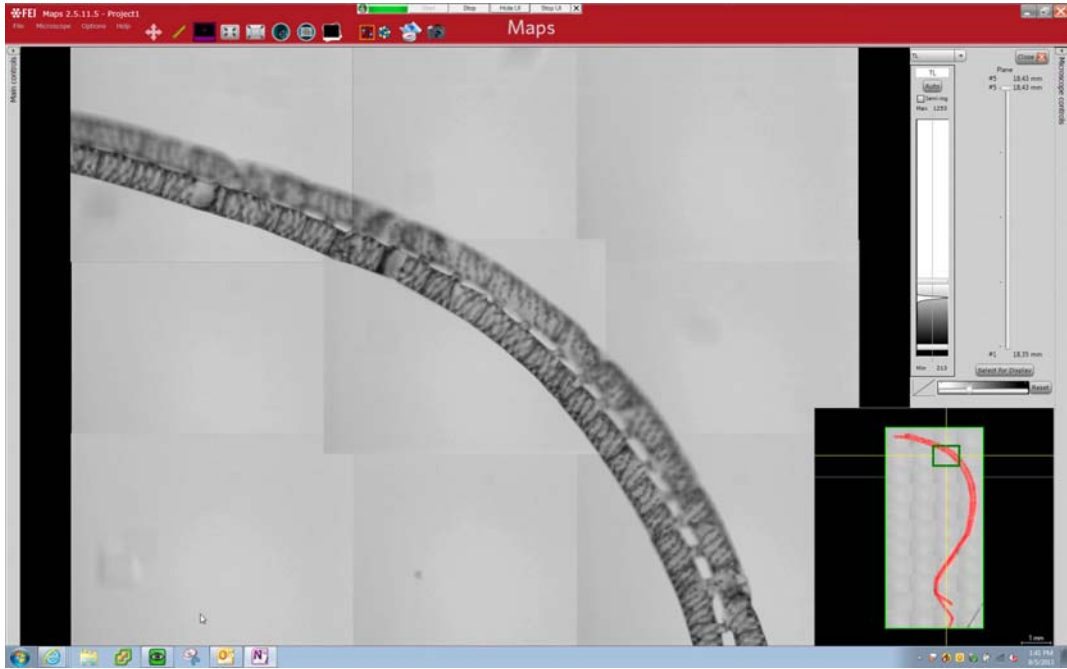
Interface Item	Description
Channel Selector	Selects the channel to be displayed in the multi-channel Z-Stack. The pulldown list contains the same choices as in the Microscope controls. See <i>"Channels"</i> on page 135.
Histogram	Displays the same histogram as in the Microscope controls. See <i>"Histogram"</i> on page 142.
Close	Closes the Z-Stack Browser.
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. <i>Note: Only one plane can be displayed in the main viewer.</i>
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 26 Z-Stack Browser Controls Overview (2 of 2)

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



Nav-Cam Support

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

6 Correlation and Alignment

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.

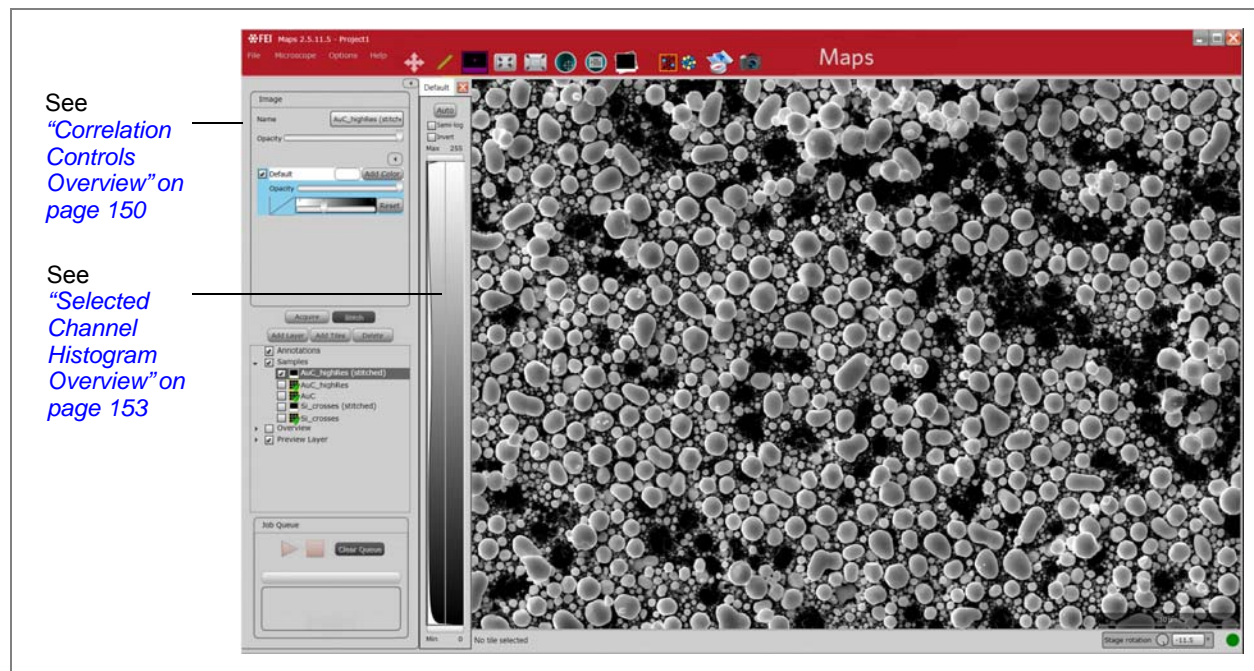
It also describes Alignment procedures.

Topics include:

- [“Correlation Controls” on page 148](#)
- [“Segmentation” on page 154](#)
- [“Alignment” on page 159](#)

Correlation Controls

Figure 26 Correlation Controls



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

Figure 27 Correlation Controls

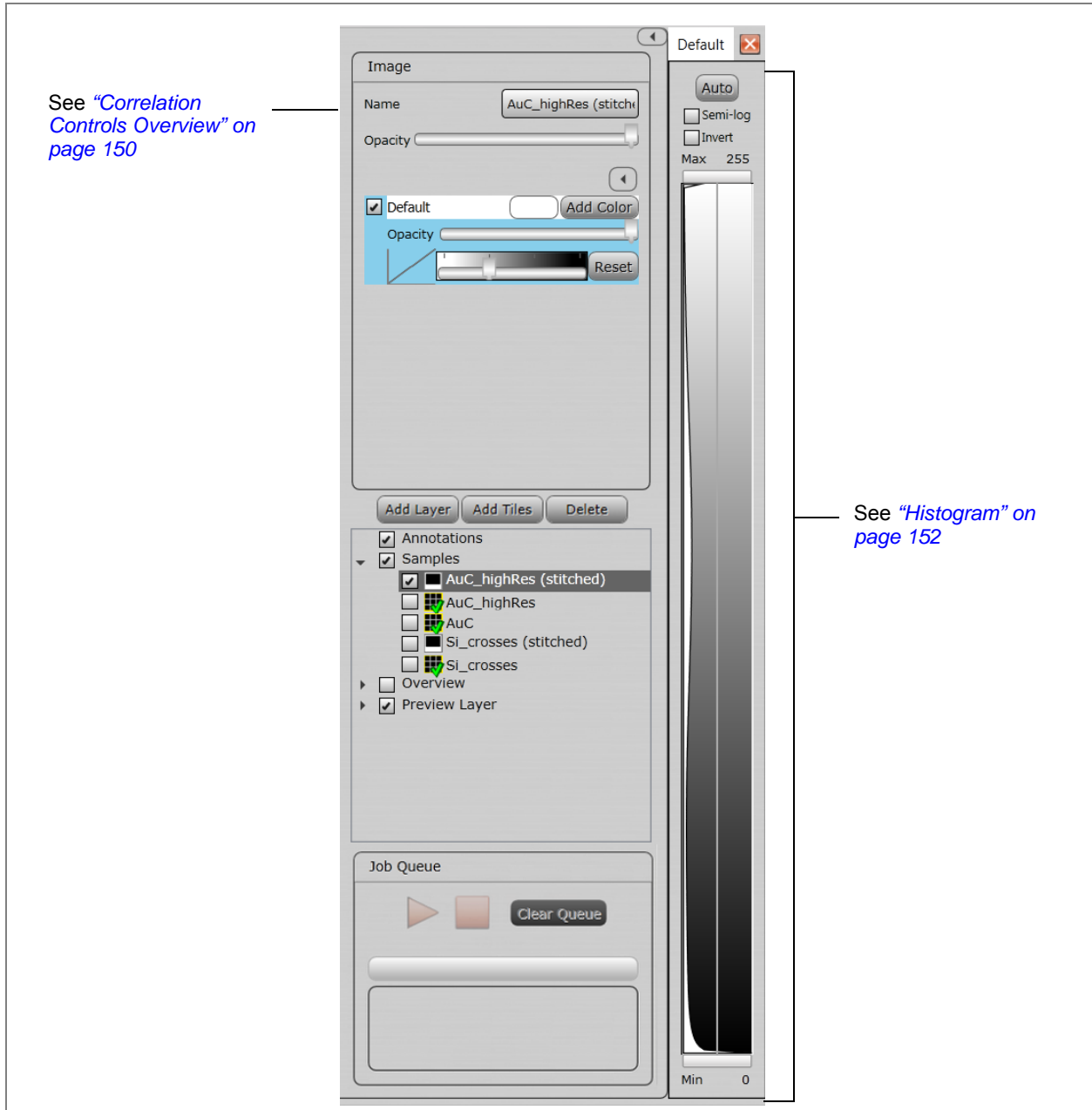


Table 27 Correlation Controls Overview (1 of 2)

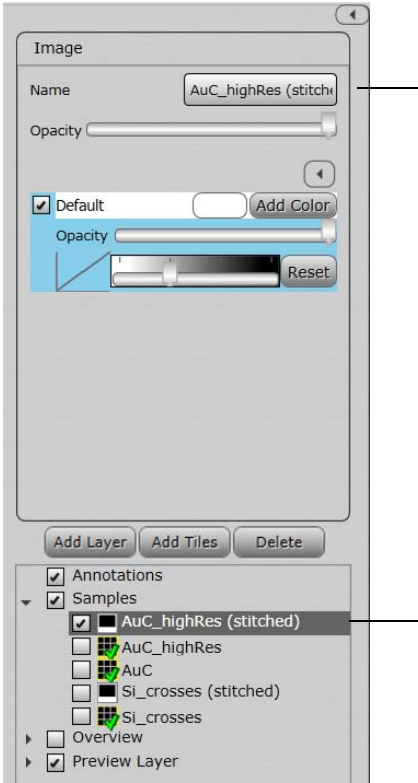
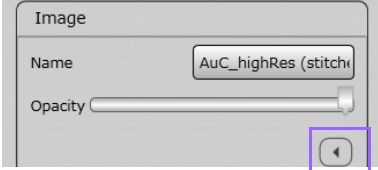
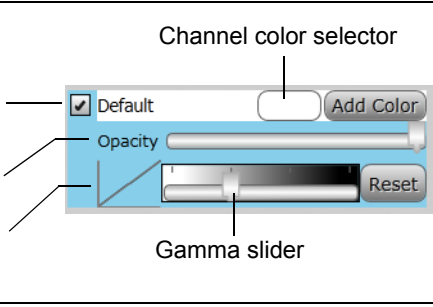
Interface Item	Description
<p>Name</p>	<p>Displays the selected layer item (a tile set or image) from the Layer control.</p> 
<p>Opacity</p>	<p>Sets the measure of opaqueness for the entire image. The less opaque, the more transparent it becomes. Transparent images are useful when overlaying on image over another.</p>
<p>Expand/Collapse Histogram View</p>	<p>Shows/hides the histogram. See <i>"Histogram" on page 152.</i></p> 

Table 27 Correlation Controls Overview (2 of 2)

Interface Item	Description
Channels with Opacity slider and Gamma adjustor	<div style="display: flex; align-items: flex-start;"> <div style="flex: 1;"> <p>Enables/disables display of that channel</p> <p>Channel opacity slider, adjusts opacity for this channel only</p> <p>Graphical display of gamma curve adjustment</p> </div> <div style="flex: 2; border: 1px solid black; padding: 5px;">  <p style="text-align: right; margin-right: 50px;">Channel color selector</p> </div> </div>
Add Color	<p>Launches Segmentation. Displays an additional histogram every time you click the button. See “Segmentation” on page 154.</p>
Channel color selector	<p>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.</p>

Histogram

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

Figure 28 Selected Channel Histogram

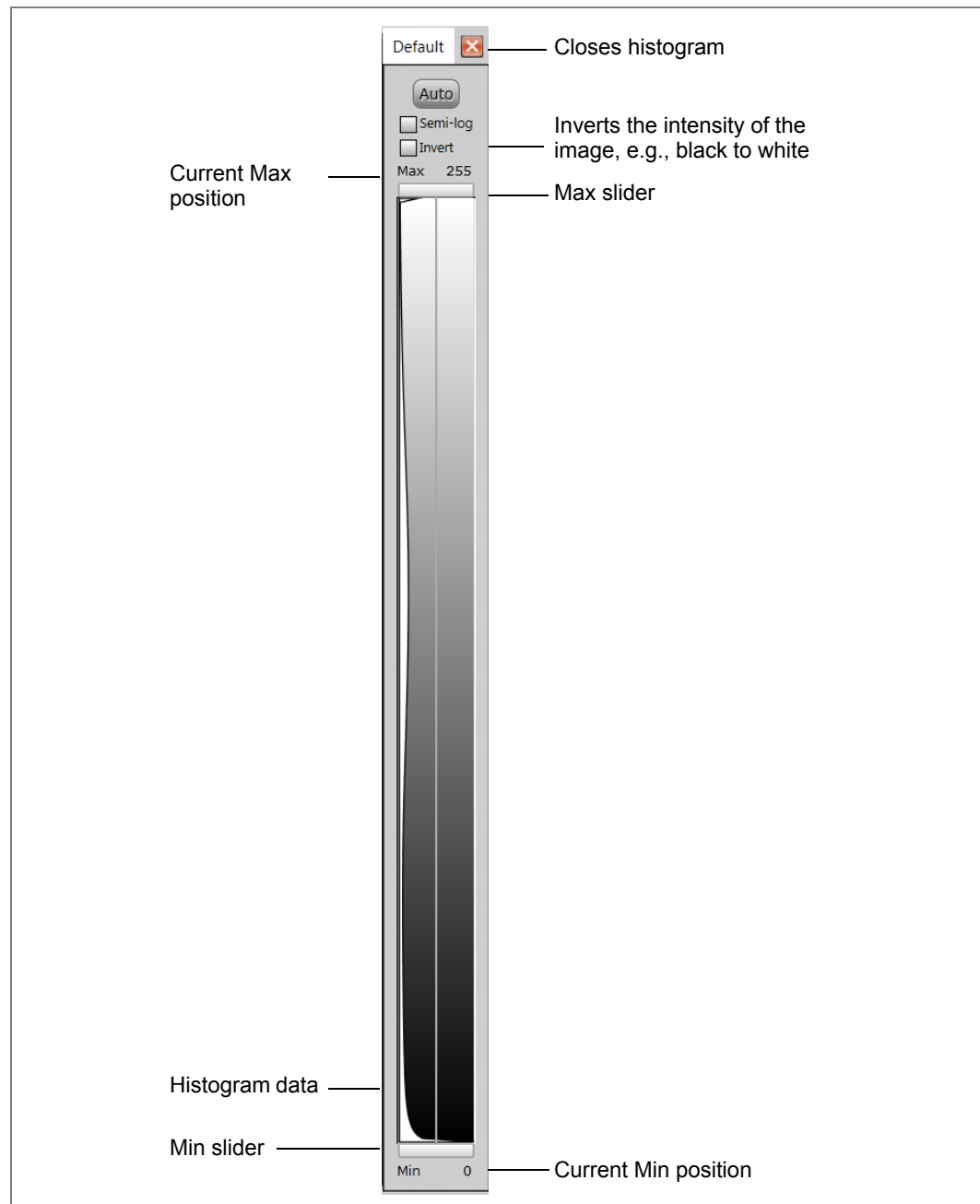
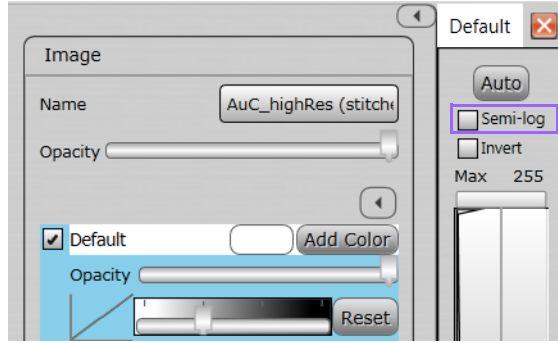
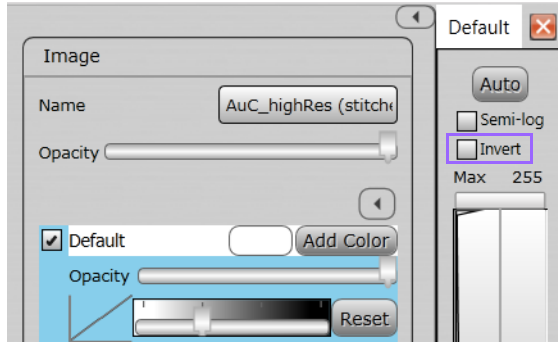


Table 28 Selected Channel Histogram Overview

Interface Item	Description
Selected channel name	Displays the name of the selected channel.
Close	Closes 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.
	
Invert	Inverts the intensity of the image, e.g., black to white.
	
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

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 157](#)

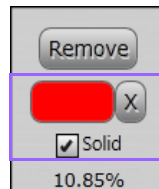
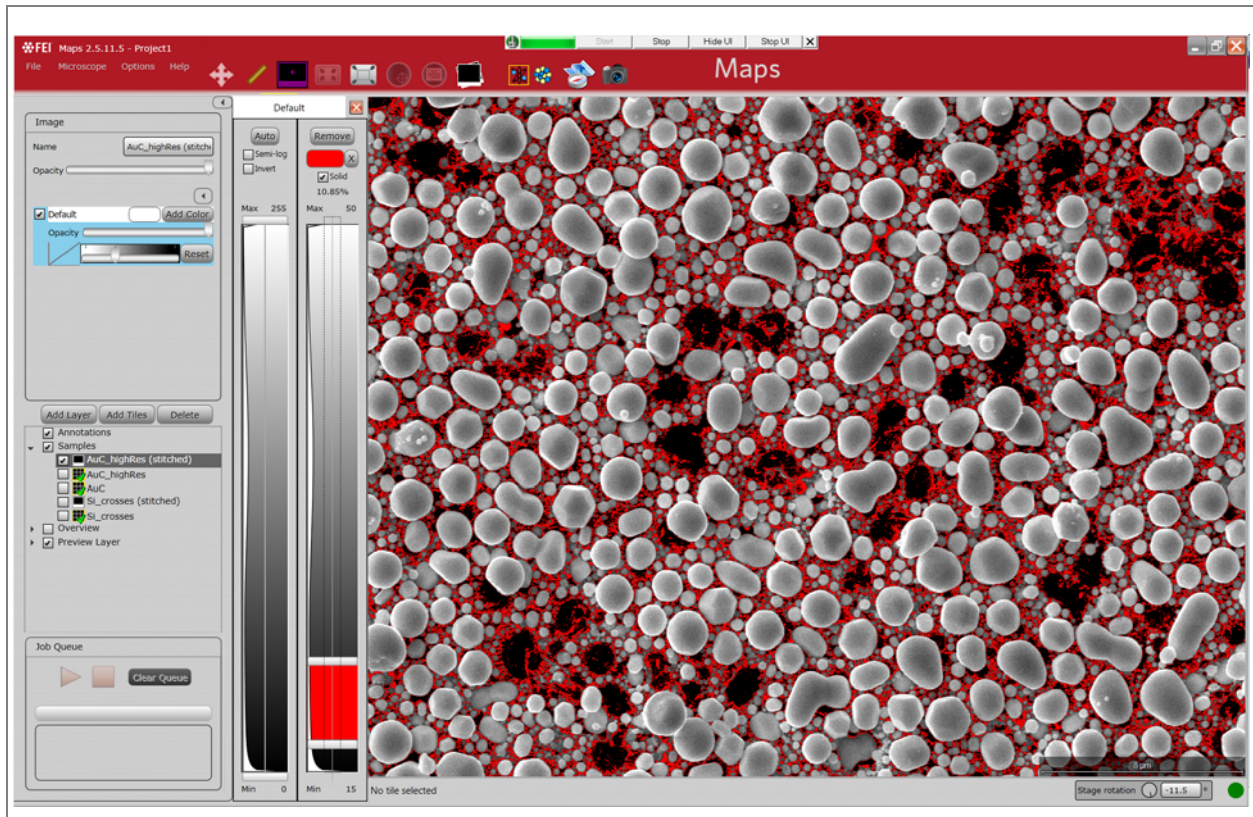


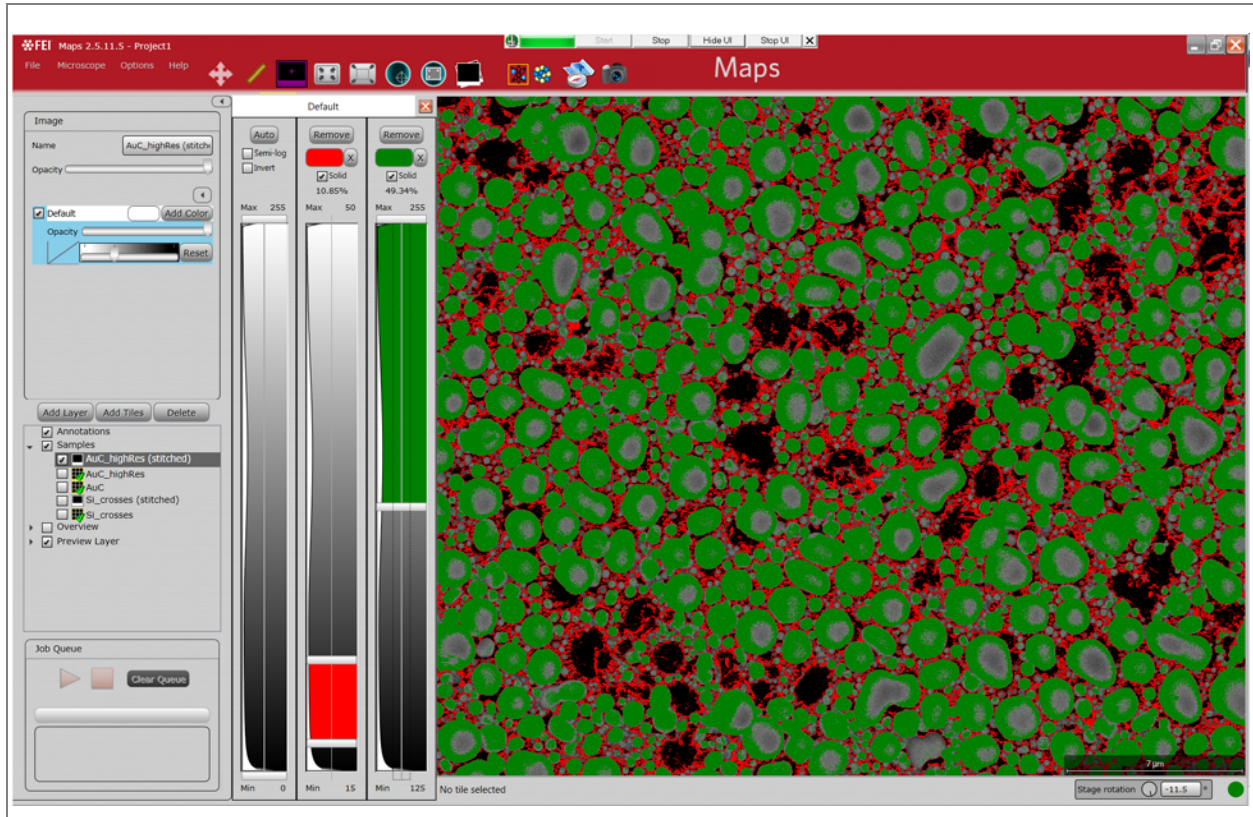
Figure 29 Segmentation, One Color



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

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

Figure 30 Segmentation, Two Colors

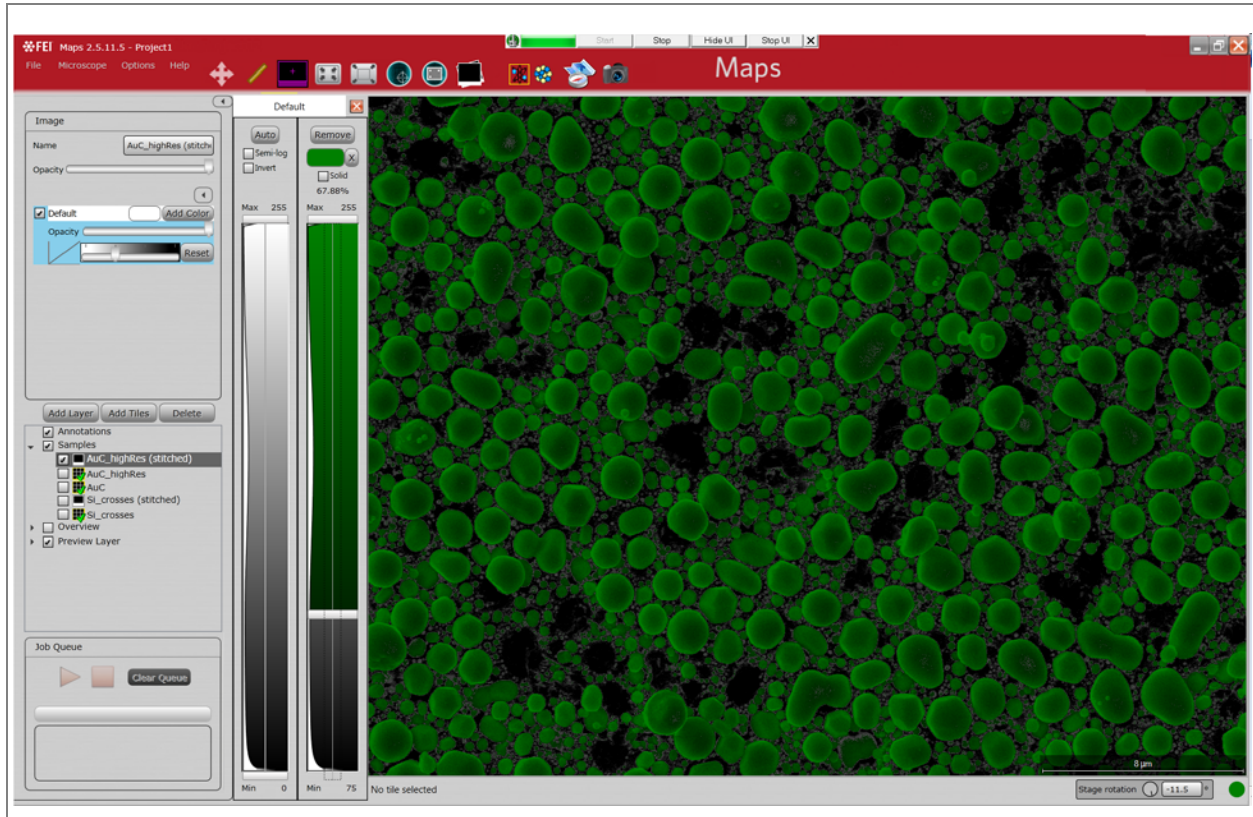


- Adjust the **Max** and **Min** sliders to select a different range of the histogram data to be assigned to the second image segment.

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 31 Segmentation, One Color with Gradient



Segmentation Controls Overview

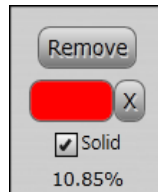


Table 29 Segmentation Controls Overview

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.
X	Clears the color and makes the segment transparent.
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.

Alignment

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 three levels of alignment:

- **Align:** Aligns a single tile set or image. Selected from the *"Tile Set Right-Click Menu"* on page 47.
- **Align Layer:** Aligns an entire layer, including all tile sets and images at once. Selected from the *"Layer Right-Click Menu"* on page 45.
- **Global Alignment:** Performs an alignment to align all previously acquired data to the stage. Selected from the *"Tool Box"* on page 29.

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

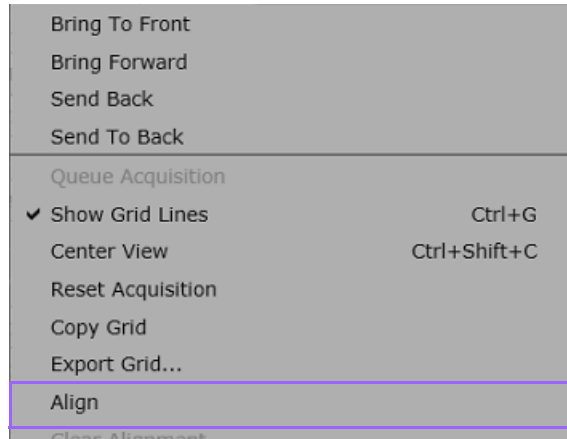
Use 1-, 2-, or 3-point alignment to match up a previously acquired image with the current stage position.

- **1 point:** Adjusts the data using translation only.
- **2 point:** Adjusts the data using translation, rotation, and resizing along one direction.
- **3 point:** Adjusts the data using translation, rotation, and resizing along two directions.

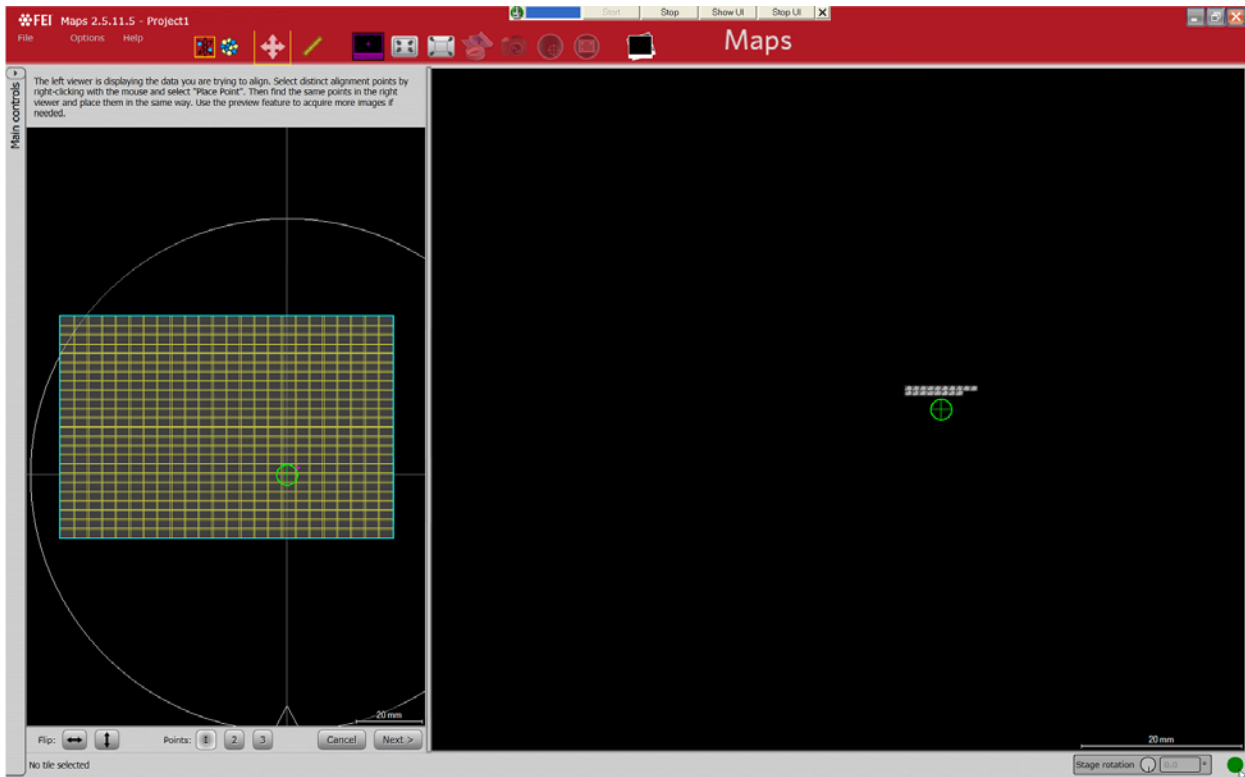
Procedure

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

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

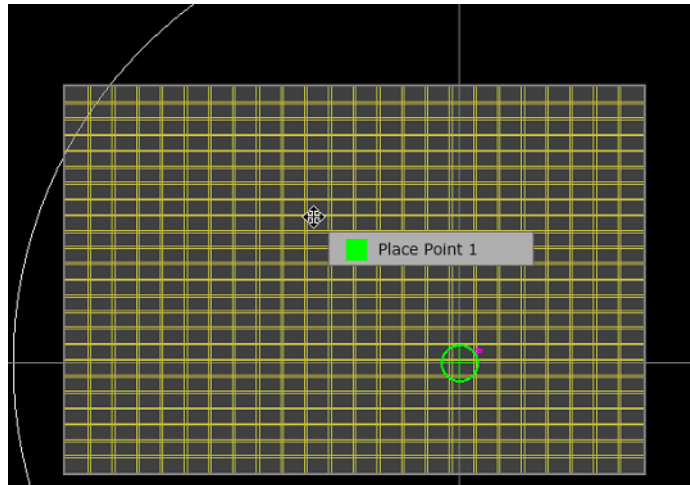


A split-window appears with guided instructions.



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

The left viewer is displaying the data you are trying to align. Select distinct alignment points by right-clicking with the mouse and select "Place Point". Then find the same points in the right viewer and place them in the same way. Use the preview feature to acquire more images if needed.



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



- Select 1, 2, or 3 point alignment.
- Click **Next** to enter Fine Alignment.

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.

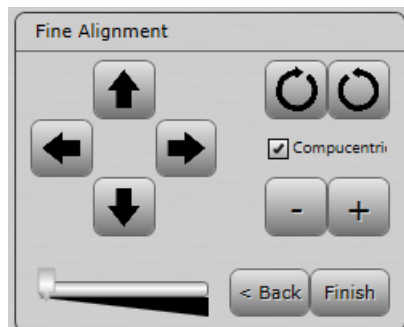


Table 30 Fine Alignment Controls Overview

Interface Item	Description
Up, Down, Left, Right Arrows	Moves the image in the direction of the arrow.
Rotate Right, Rotate Left	Rotates the image in the direction of the circular arrows.
Compucentric check box	<ul style="list-style-type: none"> • 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.
-/+	Zooms in and out on the image.
Sensitivity Slider	Sets the sensitivity for all of the fine alignment controls: Left = fine; Right = coarse.
Back	Returns to the selection of alignment points in the coarse alignment procedure. From there, click Cancel if you want to undo the coarse alignment.
Finish	Closes the Fine Alignment control area.

7 Array Tomography

Overview

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

Topics include:

- [“About Array Tomography” on page 163](#)
- [“Terminology” on page 165](#)
- [“Creating and Defining Frames” on page 166](#)
- [“Adding a Tile Set” on page 170](#)
- [“Editing the Tile Set” on page 171](#)
- [“Acquiring a Tile Set” on page 172](#)
- [“Navigating Through the Tile Sets” on page 173](#)

About Array Tomography

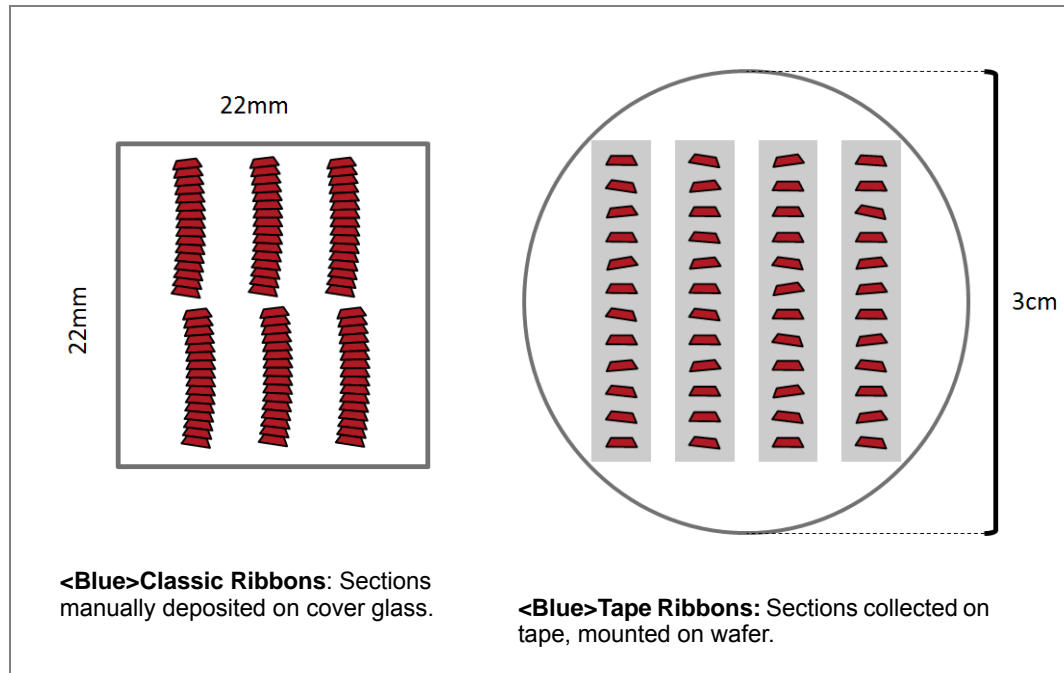
Array Tomography is an imaging technique in which 3D image data is reconstructed from serial recordings on serial sections that are spread out on a large sample carrier.

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 (i.e., 22 x 22mm cover glasses) or on wafers.

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

Figure 32 Classic and Tape Ribbons



Frames and Tile Set Arrays

The main purpose of the AT feature in Maps is to make it as easy as possible to align high resolution imaging on areas of interest on all sample sections.

The AT feature set consists of a new concept for setting up local coordinate systems around the individual sample sections, called **<Blue>Frame**, a quick and easy way to create a collection of tile sets across all sample sections called a **<Blue>Tile Set Array**, and smart extrapolation of frame position based on the relative position of the first two frames that the user positions manually.

The option to **<Blue>Use Rotational Extrapolation** when adding new frames speeds up positioning of frames on classic ribbons.

As the Maps AT extension also runs on FEI's light microscopes, frames can also be created on light microscope overview scans with or without specific fluorescent labels.

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.

- **Frame**

An area of interest used to outline a sample slice and provide a relative coordinate system for tile sets within it.

- **Sample Array Group**

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

- **Tape Ribbons**

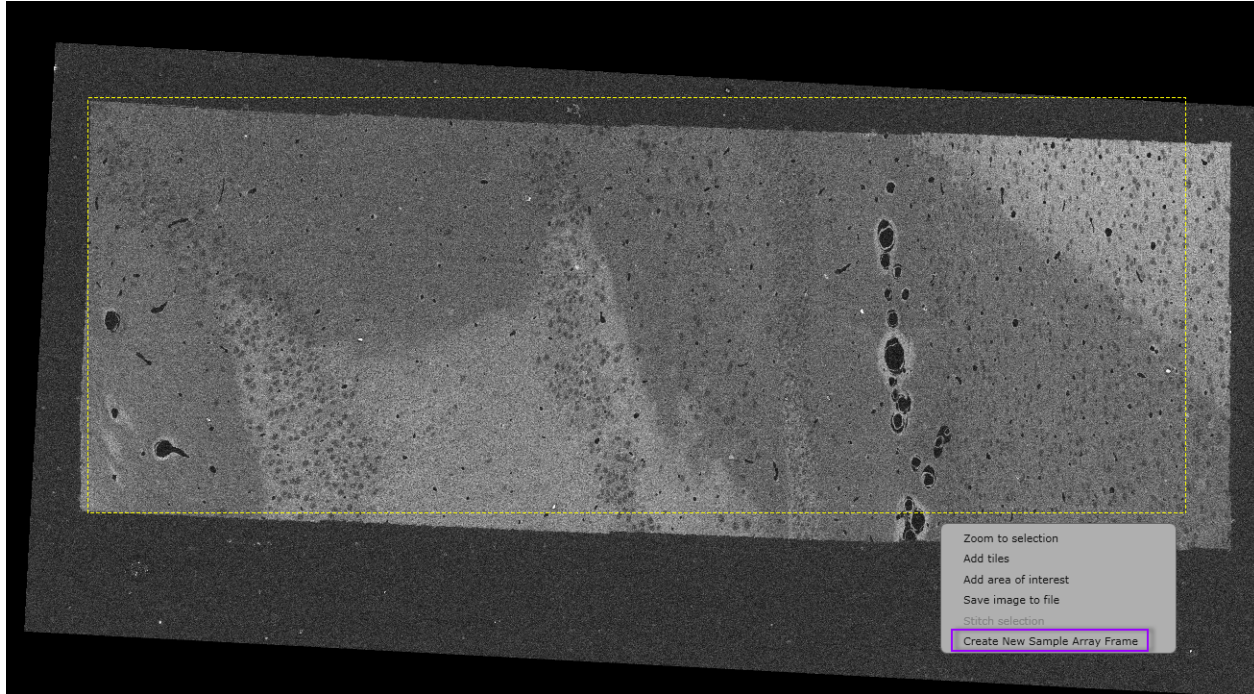
Sample sections are regularly spaced and the angle between each slice varies slightly.

- **Tile Set Array**

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

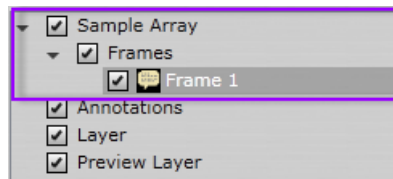
Creating and Defining Frames

To start an Array Tomography job, right-click in the viewer and select **Create New Sample Array Frame** to create and define the first frame.



This will create a frame from the dragged area in the viewer.

New items for **Sample Array**, **Frames**, and **Frame 1** are automatically populated in the Layer tree view.



The first frame you just created will be selected and some new controls display in the Annotation panel for the layer. A frame is just an extended annotation layer.

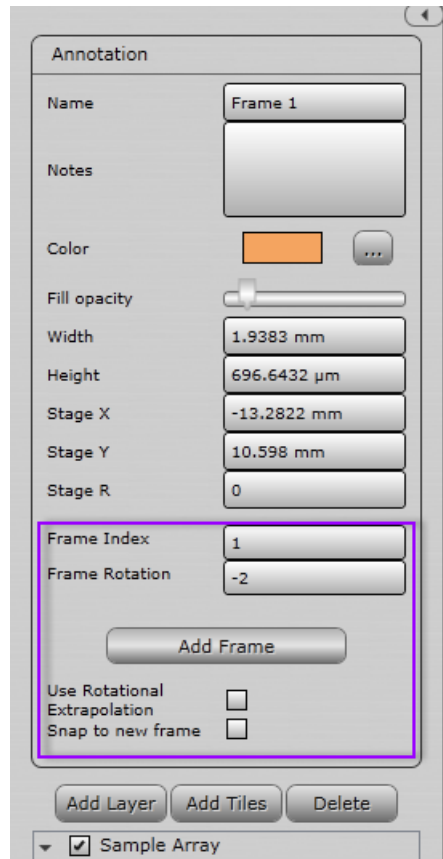
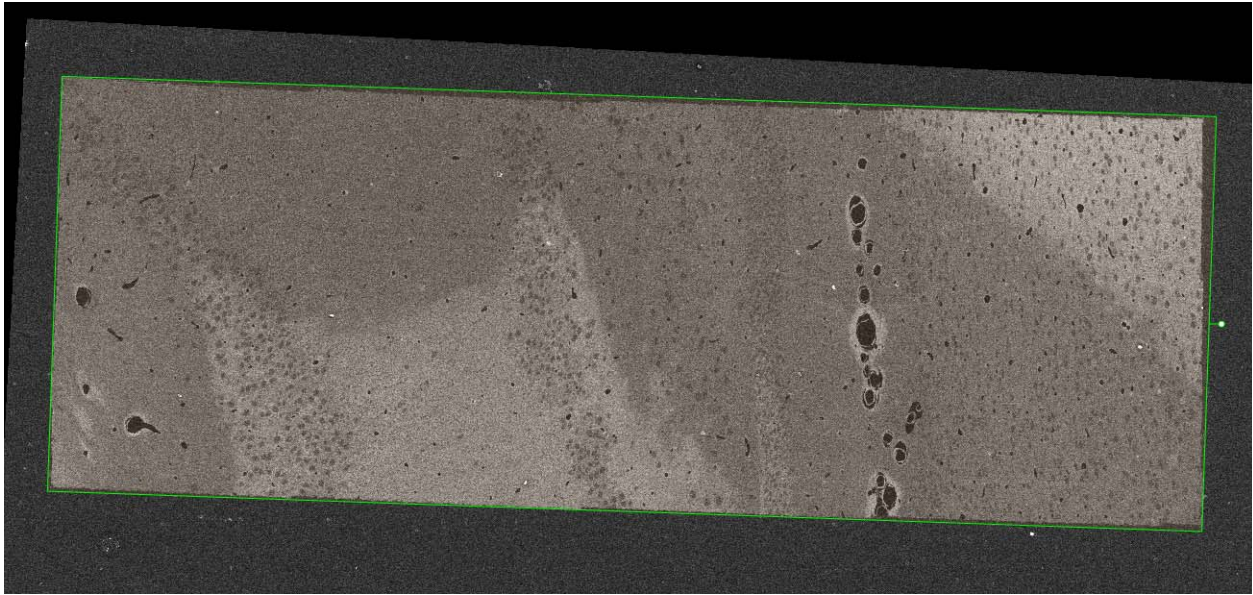


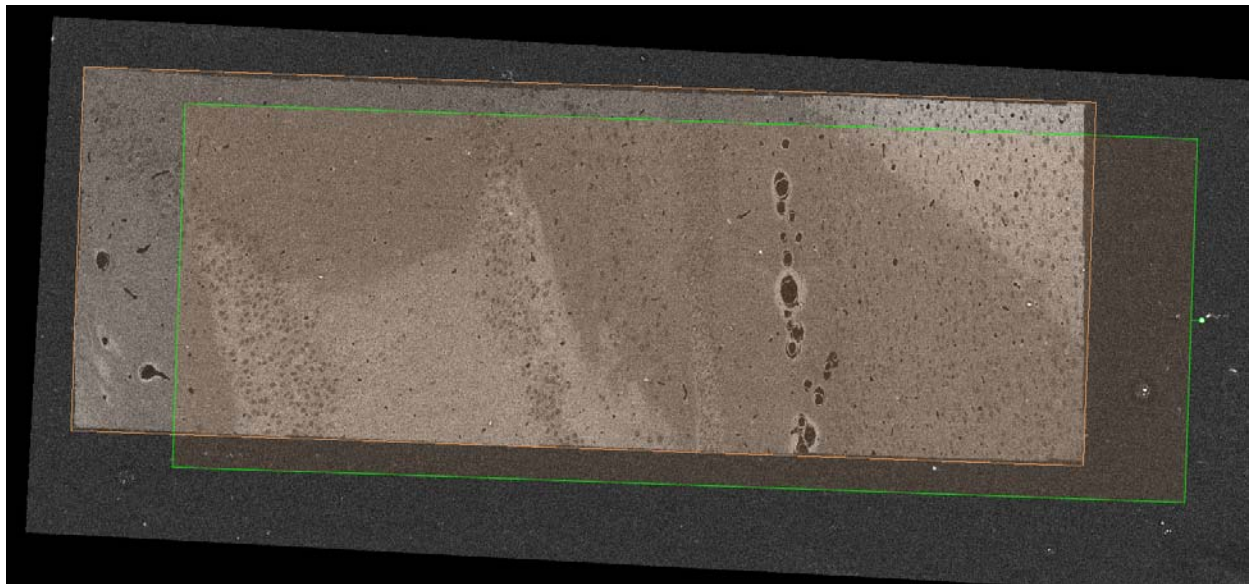
Table 31 Annotation Panel Layer Controls for Frames

Interface Item	Description
Frame Index	The index of the current frame in the frames collection.
Frame Rotation	The rotation of the frame around the origin of the frame.
Add Frame	Adds a new frame to the frame collection.
Use Rotational Extrapolation	When a new frame is added, the location of the new frame will be adjusted to follow a path created by the rotational offset of the previous two frames. This option is typically only used on classic samples.
Snap to new frame	When a new frame is created, the view will retain its zoom and rotation while moving by the offset between the previous two frames.

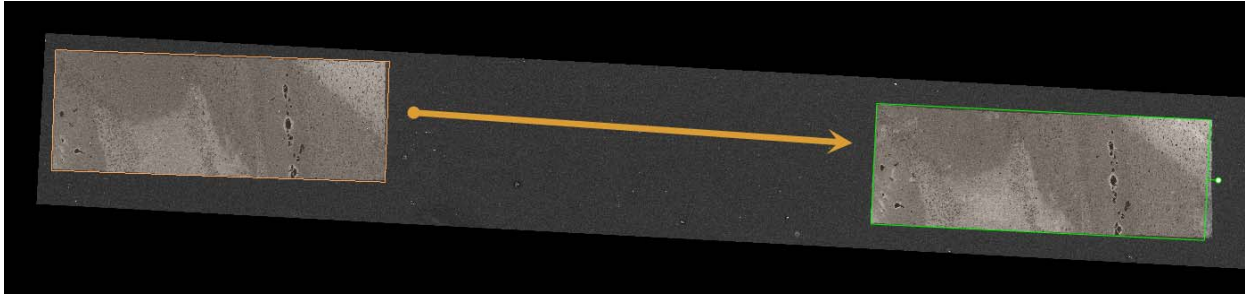
Before adding a second frame, adjust the first frame to fit the sample slice. Change the width, height, and rotation of the frame easily with the mouse.



When you click the **Add Frame** button for the first time, the second frame is created with a slight offset from the original frame. (The green outline is the new frame.)

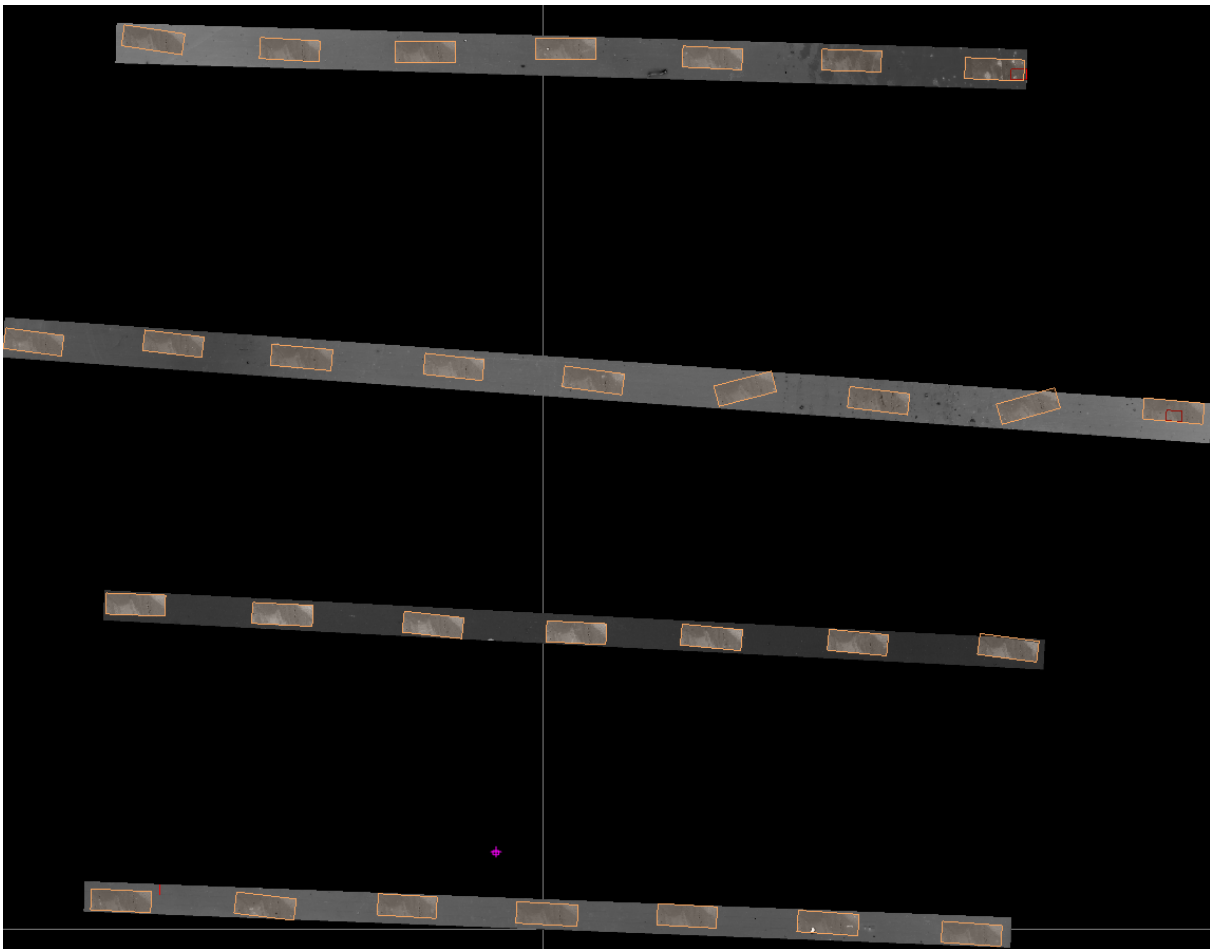


Drag this second frame over the next sample slice and rotate it to fit the second slice.



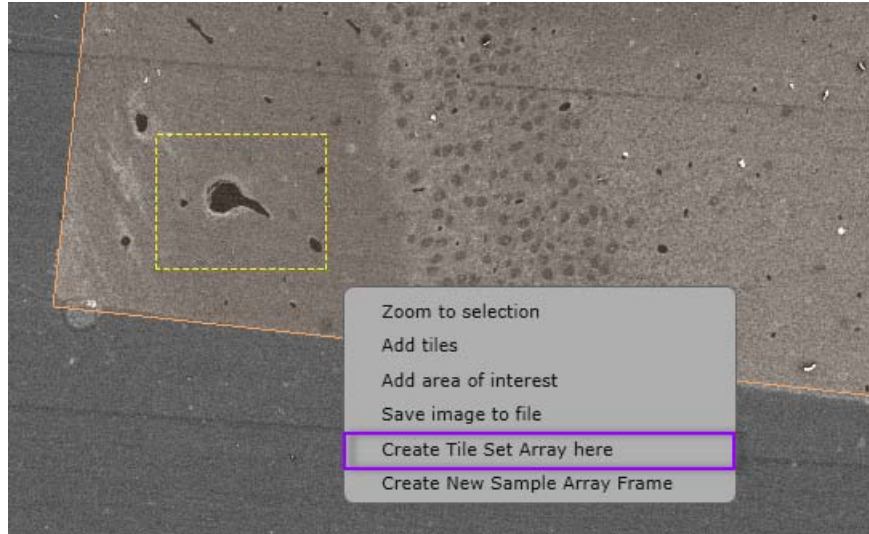
Now when you click **Add Frame**, the third frame will be placed at an offset from the second frame that matches the offset between the first and second frame. This should place the new frame close to the next sample slice. It may not be perfect, so you will need to adjust each frame when it is added.

Once all of the slices have been covered by a frame, the viewer may look like this (tape sample).

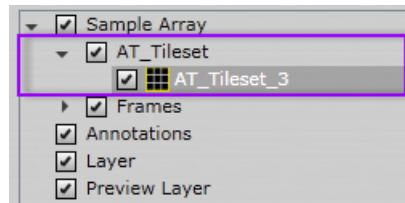


Adding a Tile Set

Adding a tile set is an easy and quick process once all of the frames are set up. First, go to a frame with a visible feature of interest. Drag a selected area in the UI around the feature of interest. If the selected area is completely contained within a frame, a new option for **Create Tile Set Array Here** appears in the context menu.



This option will prompt for a tile set type, template, and name to use for the new tile set array just like adding a normal tile set. This will create a new layer group with the tile set name under the sample array group in the layer tree. This new layer group will contain the array of tile sets that have the name of the tile set array followed by an underscore and the index of the frame of which they are in.



The new tile set will automatically be selected when it is created. If the tile set or tile set array group are selected, then a new slider bar control is enabled on the bottom of the UI as seen below.



The name of the tile set array is displayed on the left hand side and indicates which frames contain a tile set from this array. Simply move the slider control bars around to add or remove tile sets from frames or use the single incrementing buttons on either side of the slider for more precise adding and removing of tile sets.

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

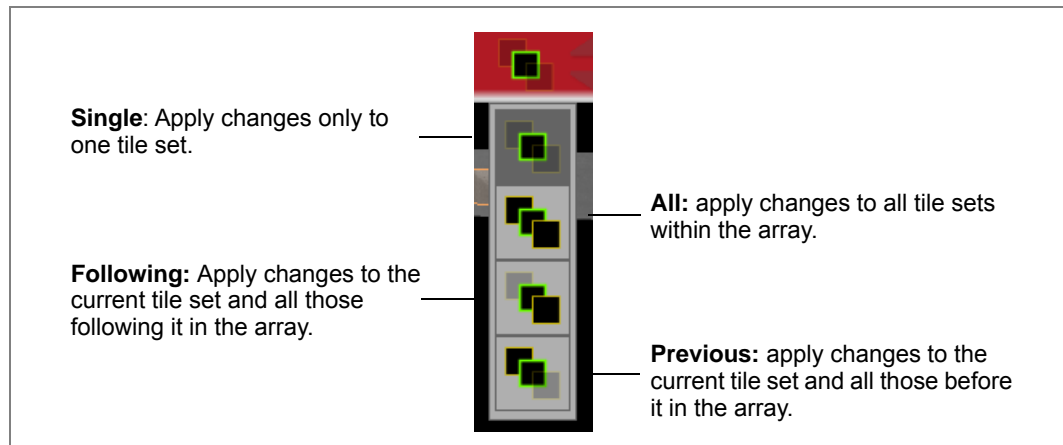
Editing the Tile Set

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** button in the tool bar.



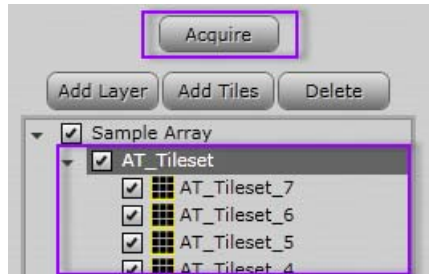
The **Edit Mode** button displays the current edit mode for tile set arrays. To change the edit mode, click the button to access a dropdown menu of options.

Figure 33 Tile Set Edit Mode Options



Acquiring a Tile Set

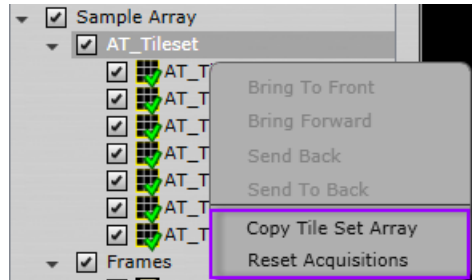
Once all changes are made, select the tile set array group.



The **Acquire** button will queue (line up in order) all tile sets from the array that have not been acquired and then proceed to acquire them.

The right-click context menu for the tile set array group has two new context menu options in the Layer tree:

- **Copy Tile Set Array:** Creates a copy of the existing tile set array group and places it within the sample array group with a name chosen by you from a popup window
- **Reset Acquisitions:** Resets all tile sets acquisition within the tile set array group.



Navigating Through the Tile Sets

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

Select a tile set from an array or a frame to enable the **Toggle View** buttons (stacked vertically) in the toolbar.



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

The viewers rotation and offset from the selected frame will be maintained when moved to the next/previous frame. This allows for quick viewing of a collection of tile sets or frames at a custom zoom and rotation.