MAPS ON THE TALOS L120C
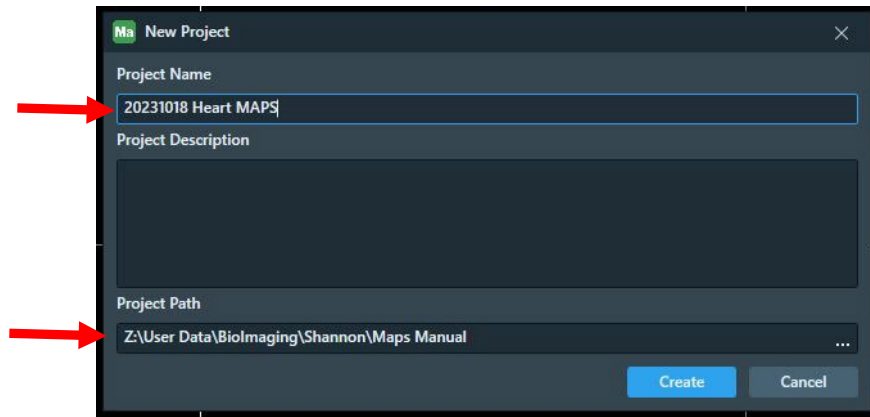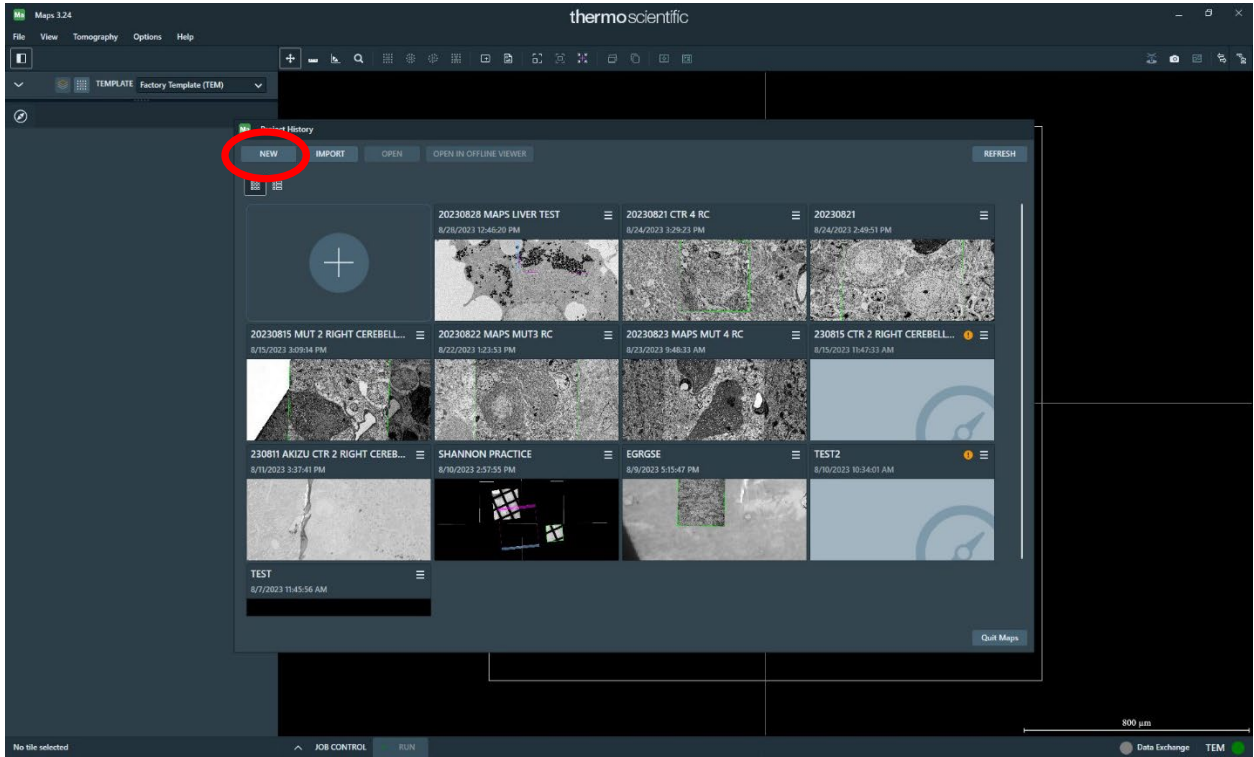
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MAPS is a software that allows you to create large tiles by stitching together multiple images. It is an ideal solution when you need a large field of view with a small pixel size.

1. Start the MAPS software and create a New Project.
   a. Define the Project Name and the Project Path.

b. In the MAPS software, the magenta box is where you are currently located on your grid, and the boundaries of the grid are defined by the white box. You can zoom in and out with the middle mouse wheel and pan by left clicking and dragging.
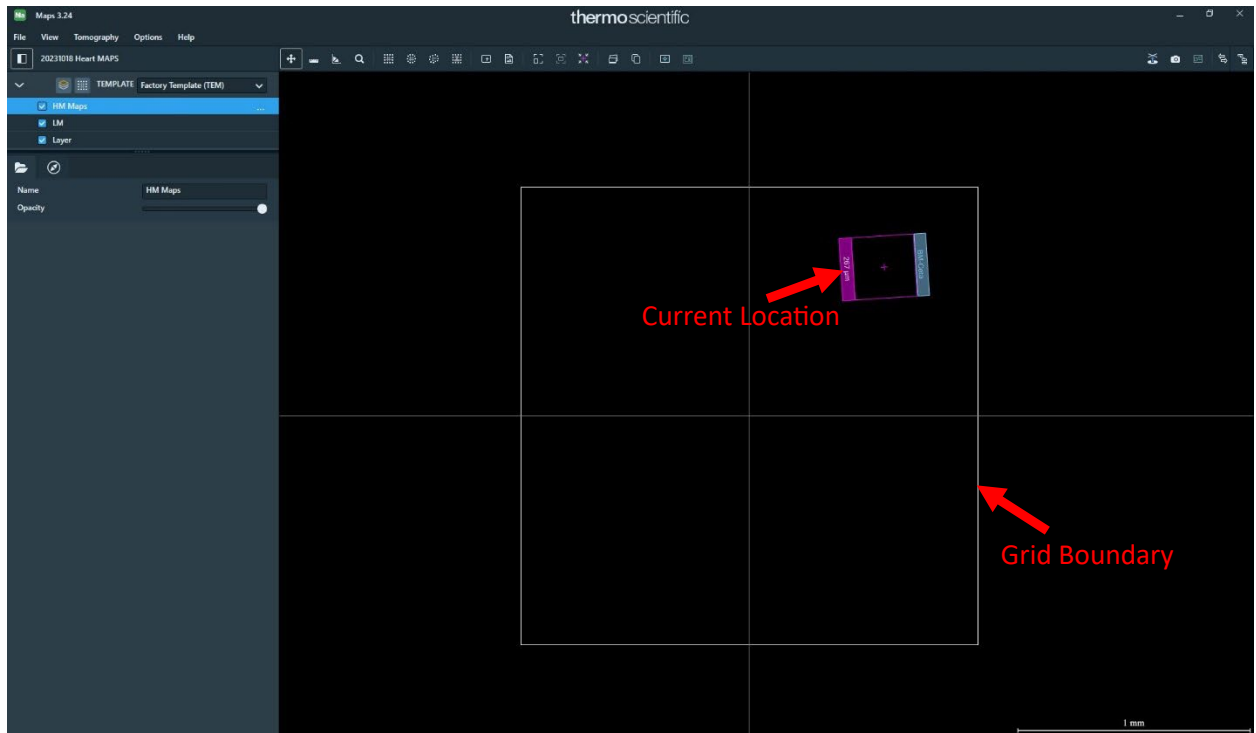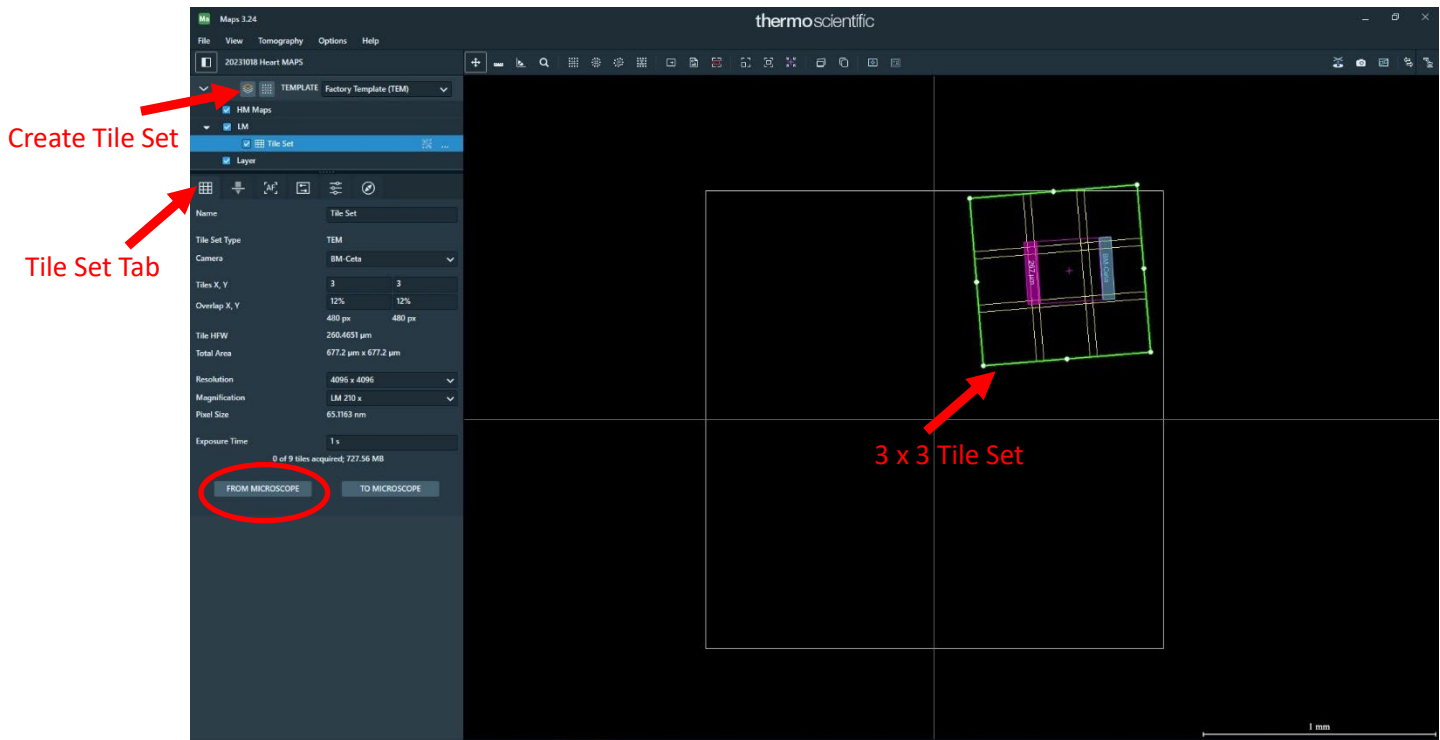

2. Create 2 new layers. Name one LM and one HM Maps.
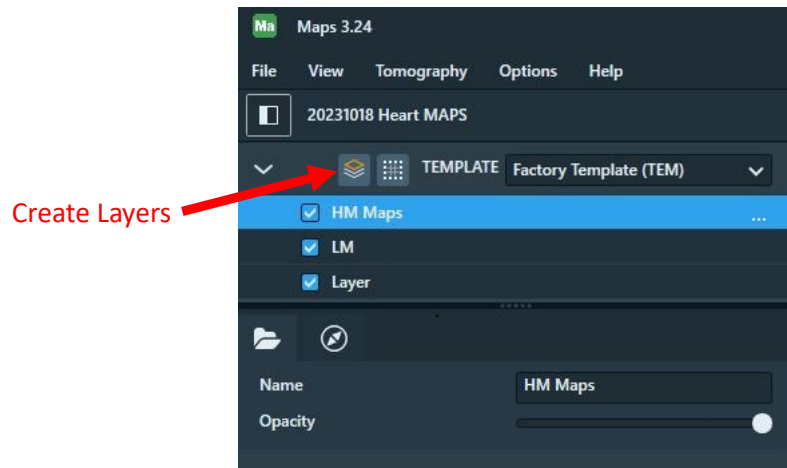
3. In the LM Magnification range, acquire an image in Velox of a region of interest. Ensure the acquired image is in focus and the camera is not oversaturated.

   a. Stop the camera in Velox after the acquisition. If Velox is running, it will not allow MAPS to acquire tiles.

4. In MAPS, under the LM layer, create a new tile set.


a. Under the Tile Set Tab:
   i. Define the size of the tile set in X and Y. In this example, it is a 3 x 3 tile set collected at 210X magnification.
   ii. Define the amount of overlap between adjacent images in the tile set. A 15% x 15% overlap generally works well.
   iii. Define the resolution. If you want the tile set to be acquired at full resolution, select 4096 x 4096.
   iv. To load the same imaging conditions that were used in the LM image you acquired in step 3 above, click "From Microscope". This imports the microscope settings (ie. Magnification, focus, beam intensity, spot size, etc.) from the microscope to MAPS.
   v. Position the tile set over the region of interest (ie. Magenta Box)

b. Under the Advanced Menu:
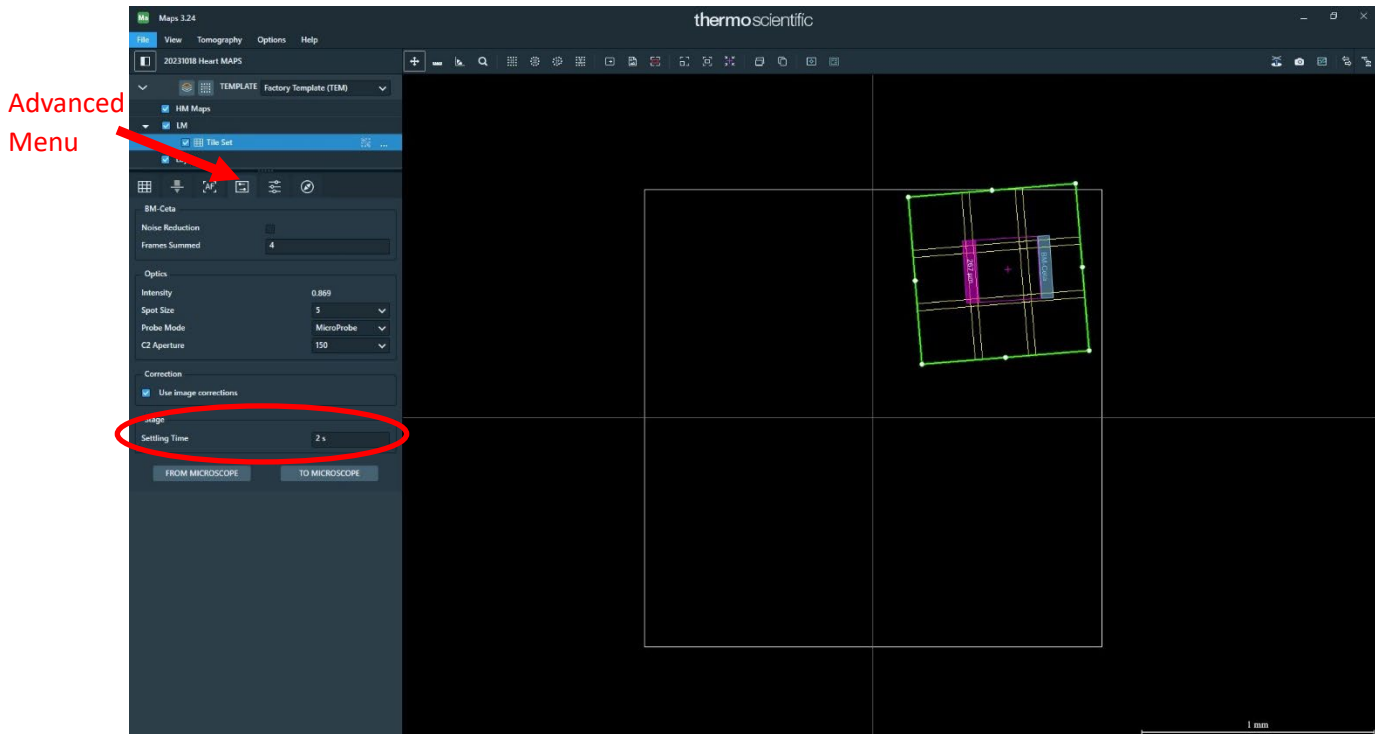
i. You should notice the microscope optics listed here should match that of the microscope if you clicked "From Microscope".

ii. Select a settling time of 2s. This will minimize drift in the tile set. As the stage moves to collect each of the images in the tile set, it will pause for 2s between image acquisitions to allow the stage to settle.

c. Under Job Control (at the bottom of the screen), you will see the jobs listed. If you created more than one tile set, each will be listed as a separate job. If the "Stitch Tile Sets When Done" slider is selected, the tile sets will automatically be stitched by the software.

d. Click the Run button at the bottom of the screen. This will start the acquisition of the LM tile set. You will see a countdown providing the approximate time remaining in the job.

i. When the job is completed, you will see options to view the unstitched tile set and the stitched tile set. The one that is visible has a checkmark next to it.

ii. You can zoom in and out of the stitched tile set using the middle mouse wheel and you can pan by clicking and dragging with the left mouse button.

<div style="text-align: center;">

iii. If you want to rotate the tile set, press SHIFT + Click and drag with the left mouse button.

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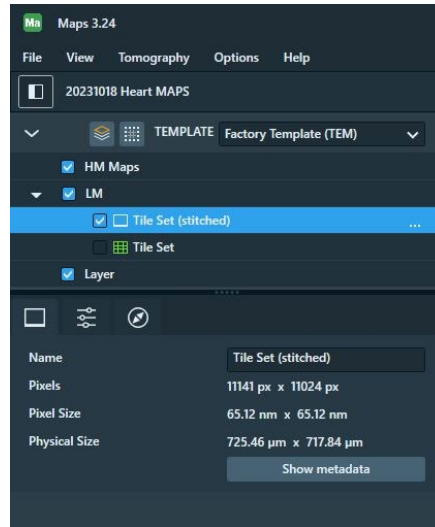
e. Align high mag to low mag
  i. There is some degree of misalignment between low and high magnification settings, so you must perform an alignment step to ensure the high magnification tile sets are correctly positioned relative to the LM tile set.
  ii. Procedure:
      1. Find a landmark in your LM tile set that can be easily identified at both low and high magnifications.
      2. Go to the desired high magnification and adjust the microscope settings (focus, beam intensity, spot size, etc.) to yield a high quality image in Velox. Acquire an image in Velox to ensure the image is in focus and the camera is not oversaturated.

3. In MAPS, click on the LM Layer and click on the snapshot icon (looks like a camera). This will take a snapshot at the high magnification settings.
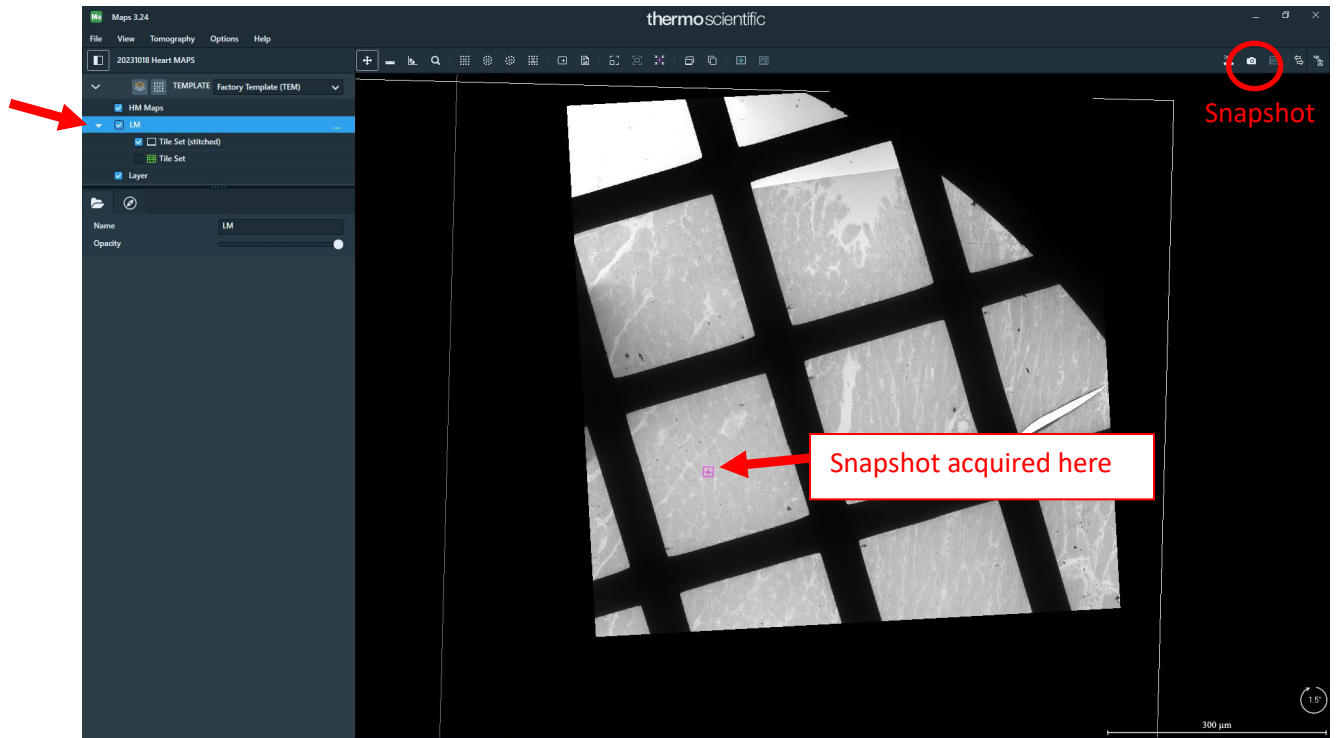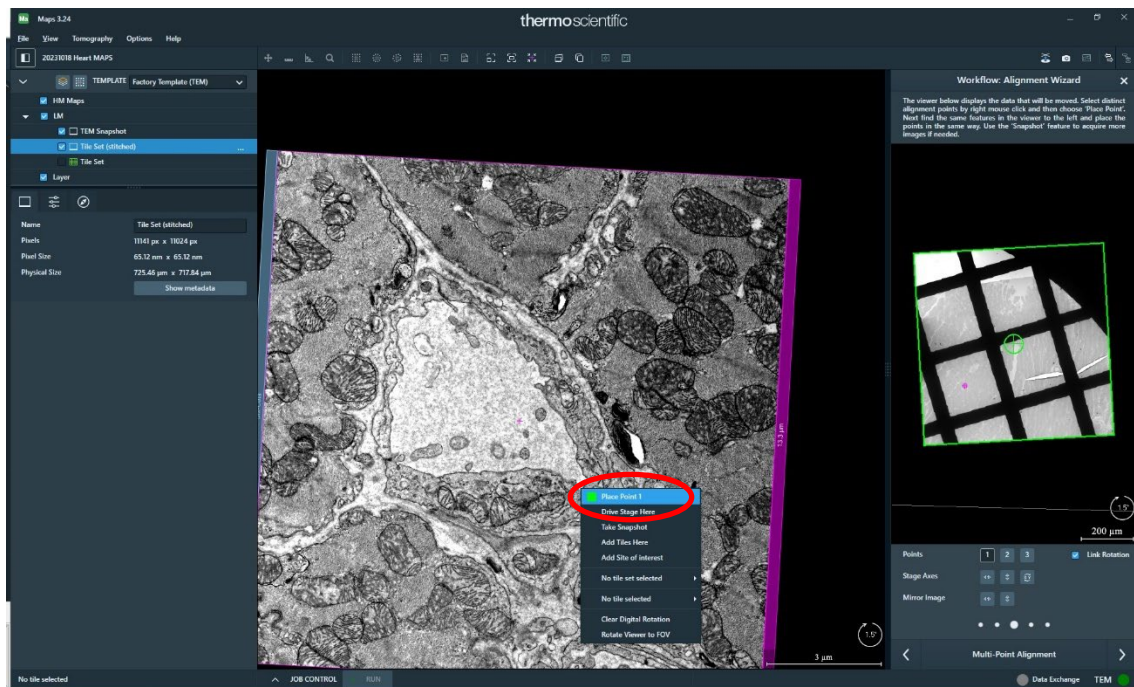

4. Right click on the stitched LM tile set and select Align. This will open the alignment page that will allow you to align the LM tile relative to the high mag snapshot.
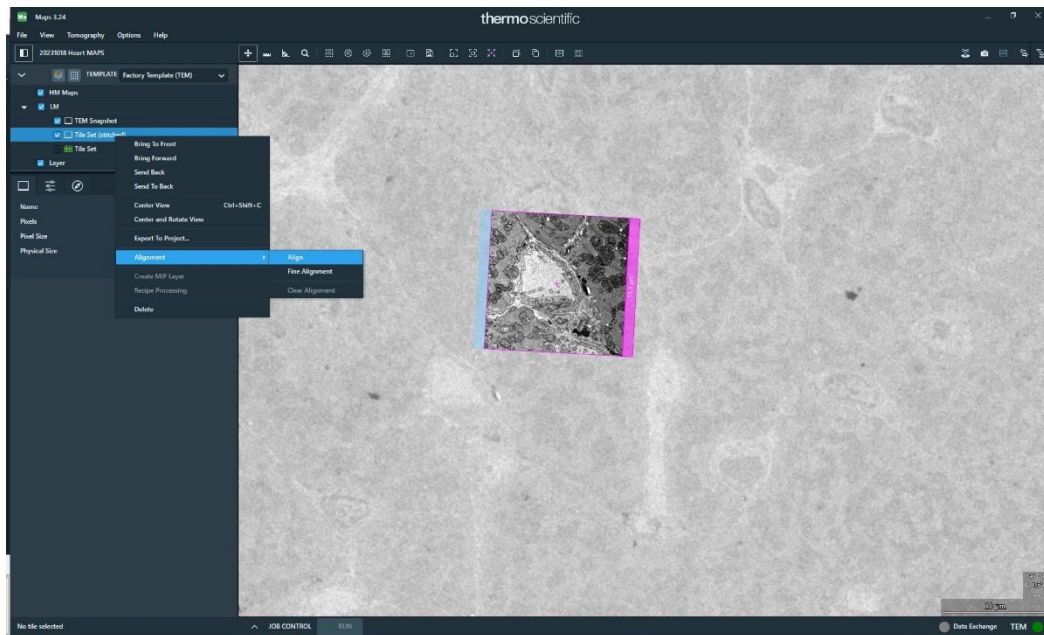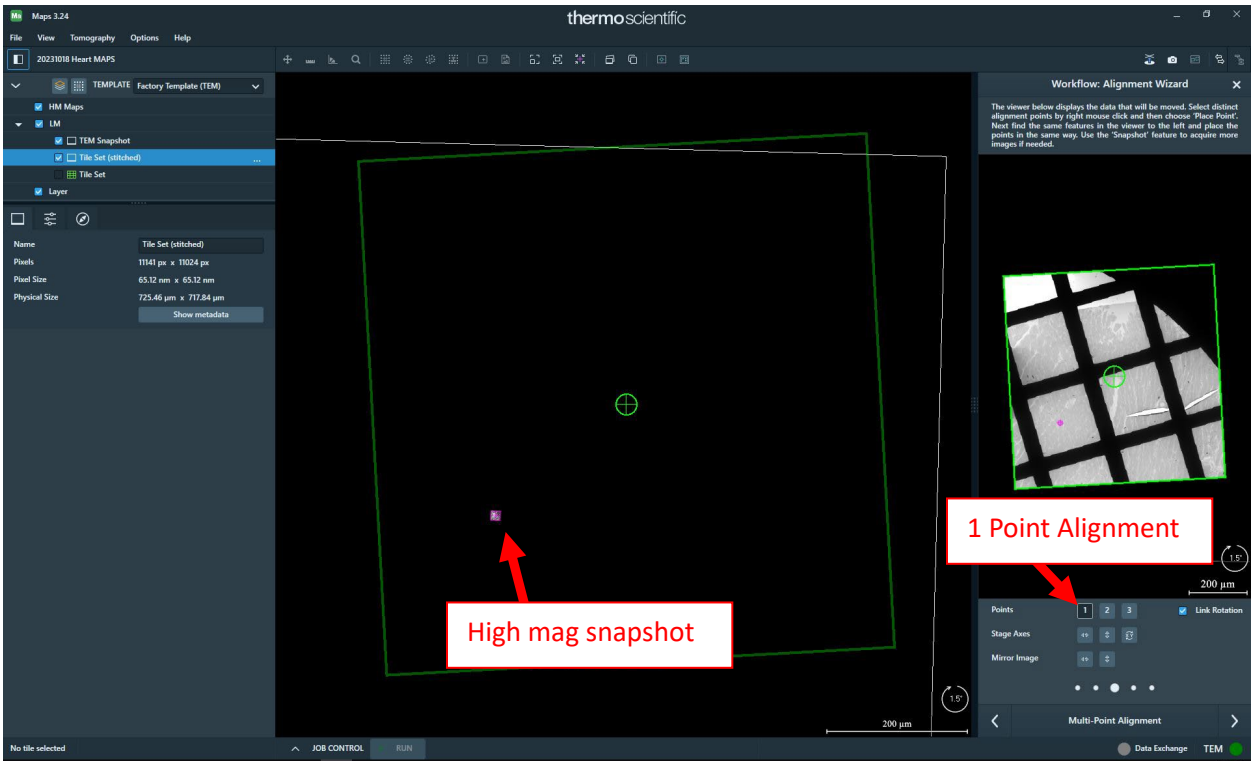
a. A one-point alignment is sufficient. Click 1 Points.
b. Zoom in on the snapshot in the left box. Find a recognizable feature in the snapshot, right click on it, and select Place Point 1. In this example, I'm using the bottom right edge of a capillary as my reference point.

c. Now zoom in on the LM tile set on the right. Find the same recognizable feature, right click on it, and select Place Point 1.
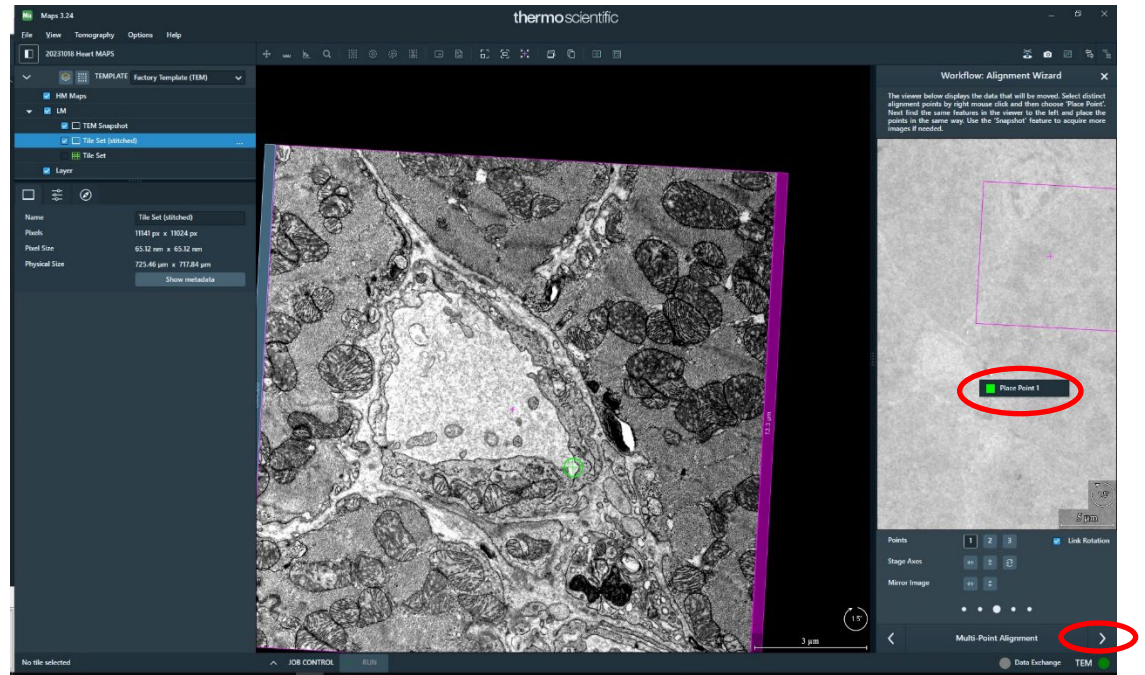
d. Click the right arrow after Multipoint Alignment. Now you can check the alignment by clicking on the high mag snapshot, and under the Visualization tab, use the slider to adjust the opacity so you can see how well the LM tile and high mag snapshot align. If you are satisfied, click the right arrow after Interactive Alignment then click the checkmark after Fine Alignment.
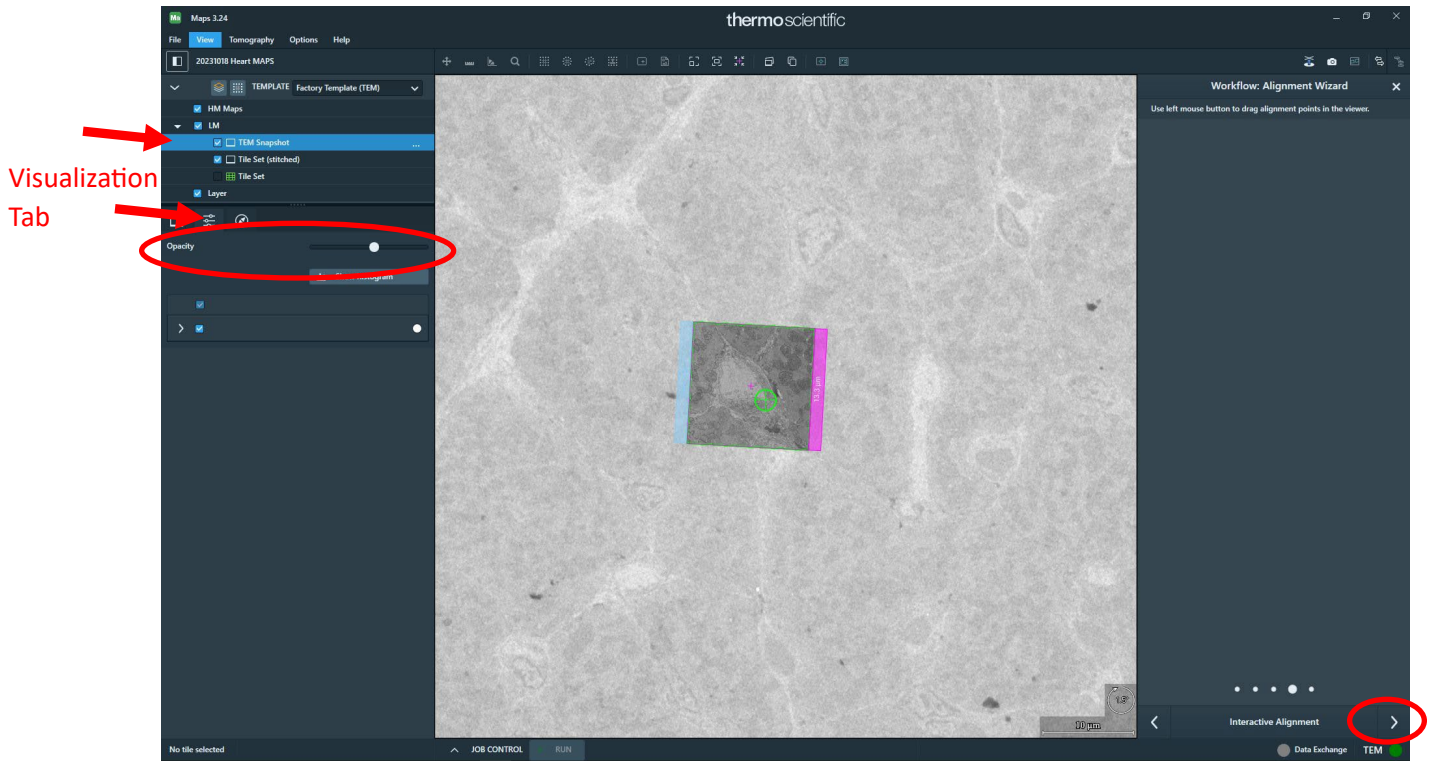

e. Now your LM tile is aligned to the high mag snapshot. If you double click somewhere on the LM tile, it will drive the stage to that location, and it should correspond to the same area at high magnification.

f. Note. The alignment is only valid to a localized area around where the alignment was performed. If you plan on acquiring tiles from a completely different area of the grid (ie. several grid squares away), you will have to redo this alignment step for each localized area.

f. Set up and acquire high magnification tiles
    i. Under the HM Maps Layer, click add tile set.
    ii. If you want the high magnification tile set to be acquired using the current microscope conditions (magnification, beam intensity, spot size, etc), click "From Microscope." This imports the current microscope settings to MAPS.
    iii. Define the Tile size (X, Y) and Overlap. For high magnification tile sets, full resolution (4096 x 4096) is typically desired.

High Mag Tile Set
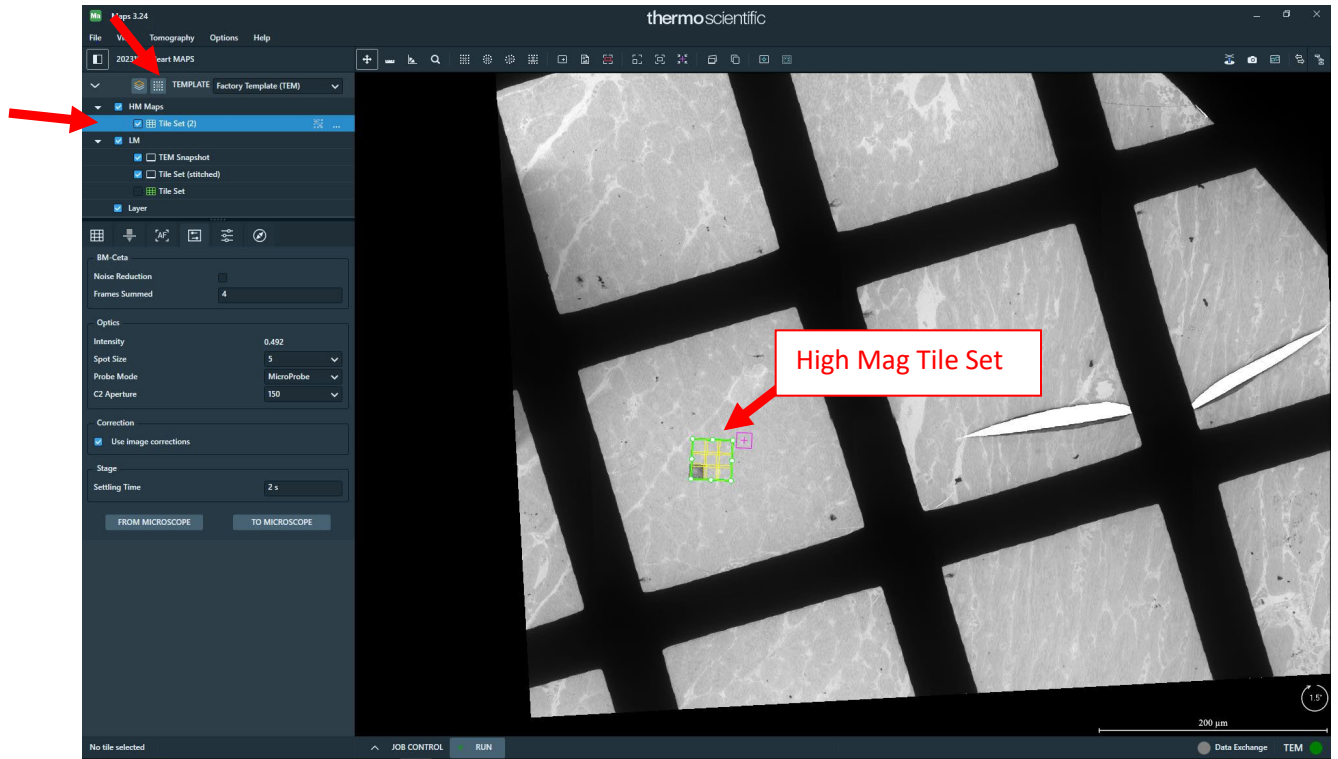
iv. Under Advanced, select a settling time of 2s (as you did above for the LM tile set).
v. Position the tile set where you want within the LM tile set.
vi. If you want to duplicate this tile set, right click on it and select Copy Grid. This will copy all the parameters from the first high magnification tile set. Simply click on the copied tile set and then position it where you desire on the LM tile.
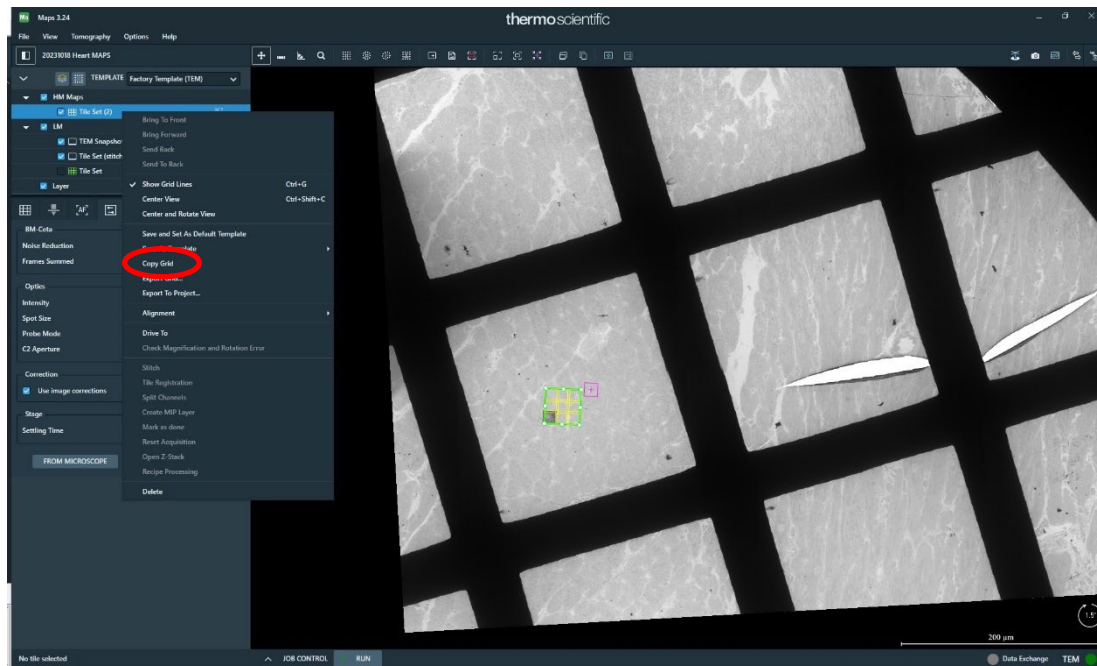
If the copied tile set is positioned far from the original tile set, you will want to go to Velox and redo focus at the copied tile set's location. Once you are satisfied with the focus, click "From Microscope" to send the new focus values to MAPS for that specific tile set.
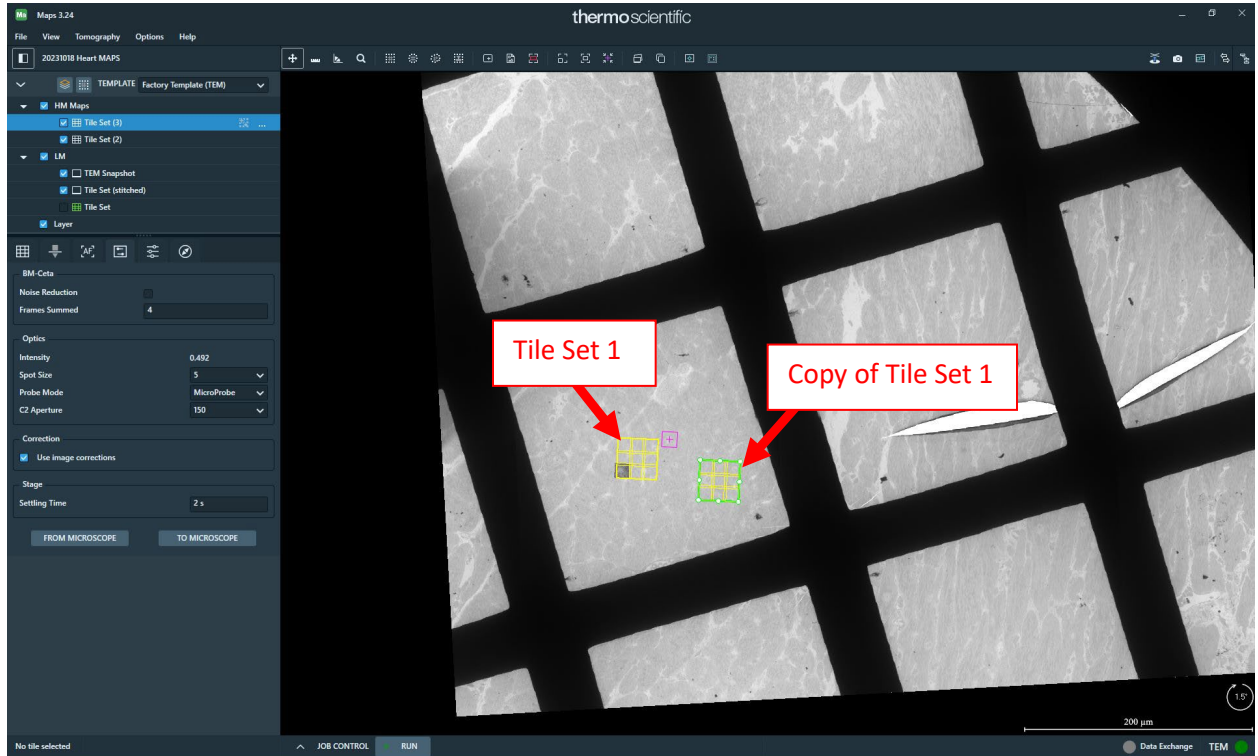
vii. Under job control, you can see the tile sets in the queue. If you do not want the system to collect a particular tile set for any reason, simply unclick the checkmark. If the "Stitch Tile Sets When Done" slider is selected, the tile sets will automatically be stitched by the software.
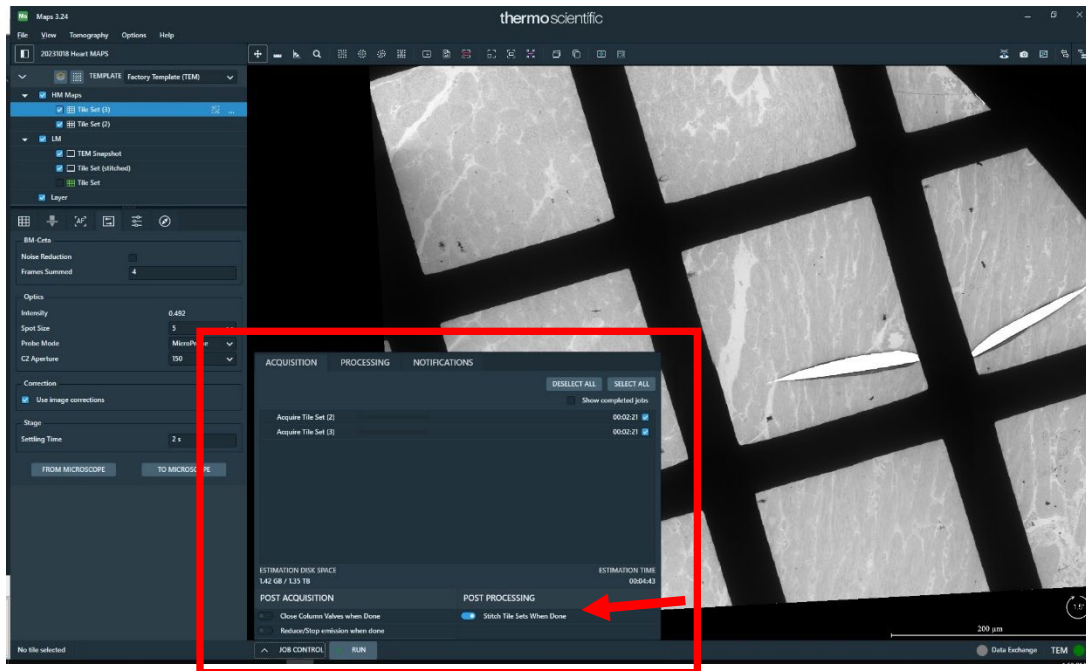
viii. Once you've created all the high magnification tile sets, click Run. The system will automatically collect all the tiles defined under Job Control and stitch them.

g. Exporting Tiles
   i. The tiles can be viewed in either the BioImaging Center's offline copy of MAPS (full functionality) or they can be viewed with the free offline MAPS viewer (not full functionality).
   ii. If you want to export tiles as tifs, you will need to use the BioImaging Center's offline copy of MAPS
      1. Zoom in on a stitched tile set
      2. Click the "Save image to file" icon. The mouse courser now changes to crosshairs. Click and drag to create a box around the high magnification tile set.
         a. Define a Destination File Name and File Location
         b. This will save a full resolution tif of the high magnification tile set

Export

Save Selection Options

Image Size: 10699 x 10634 pixels

Physical Size: 34.79 µm x 34.58 µm

Pixel Size: 3.25 nm Use full resolution

☑ Add scale bar

Destination file name:

Z:\User Data\BioImaging\Shannon\Maps Manual\20231018 Heart M ...

...t:Tiff

OK Cancel

Choose file name and destination for export as a tif