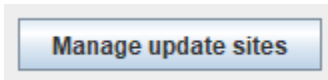
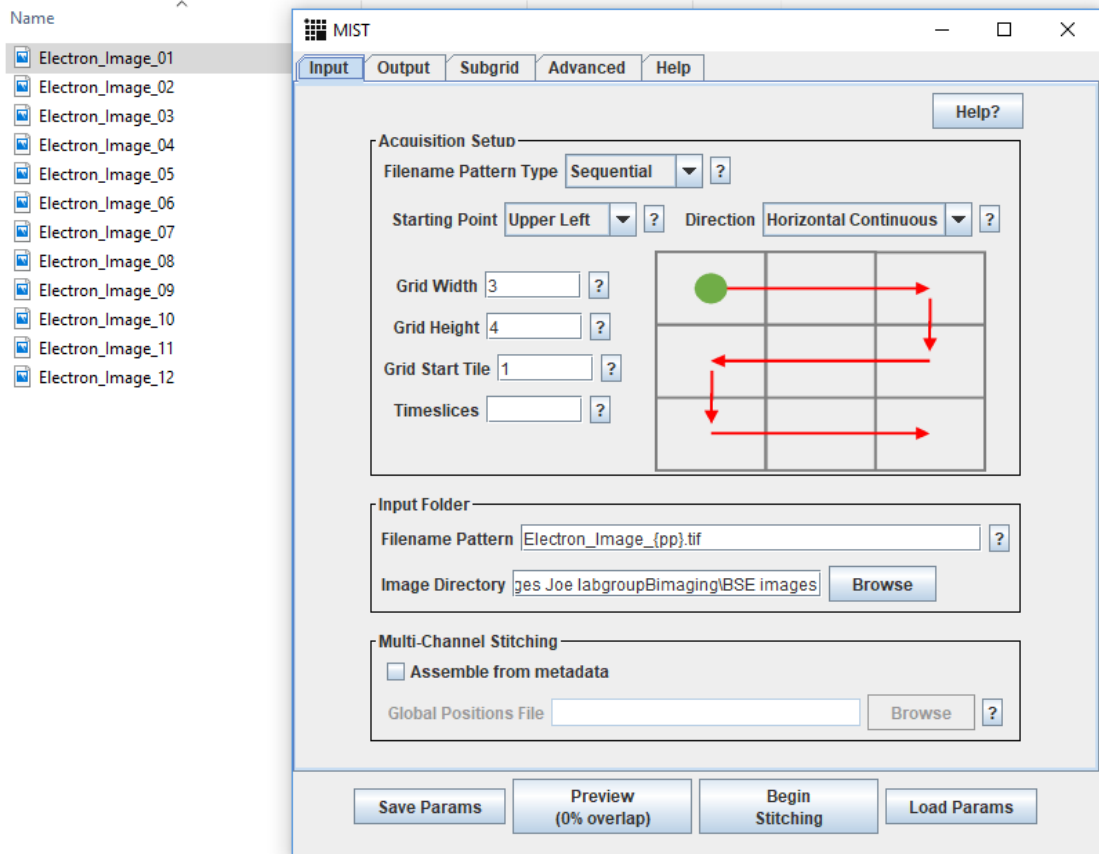


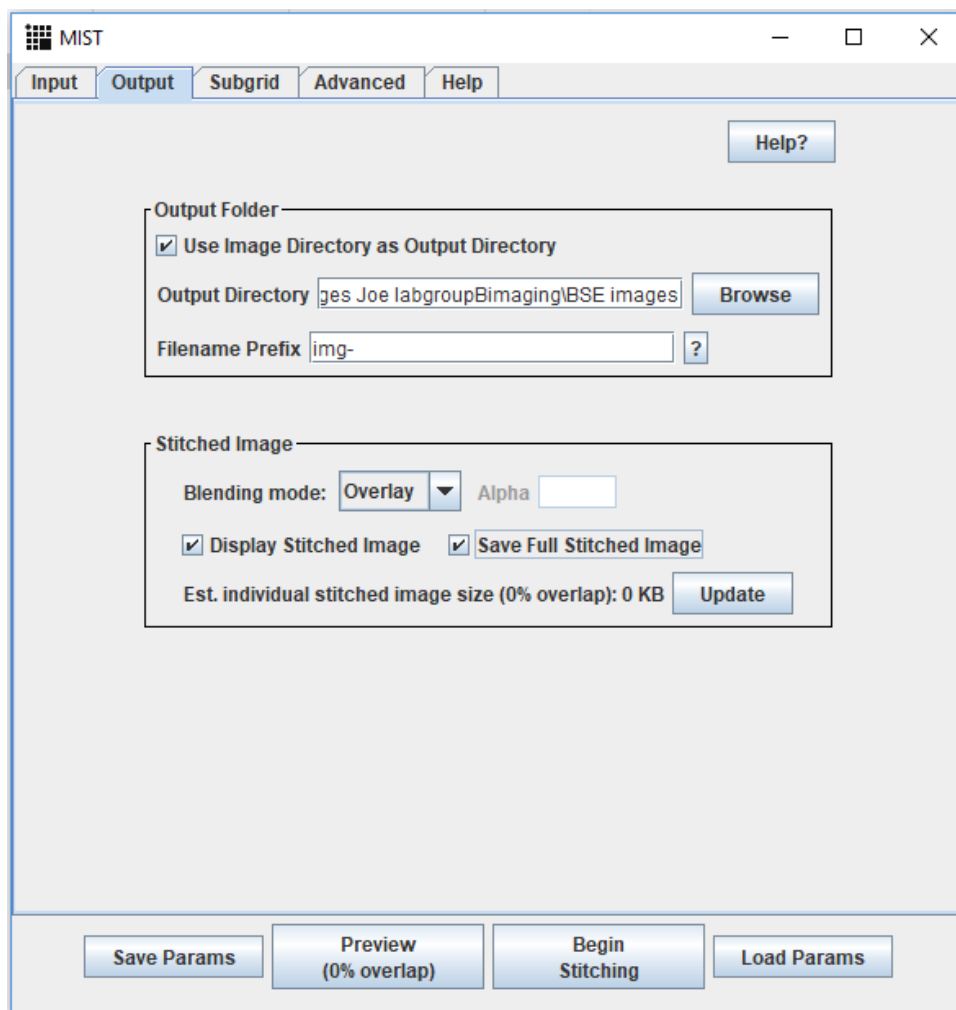
Image stitching using FIJI

1. When capturing images from the SEM, make sure you save without the annotations at the bottom of the screen (e.g. scale bar)
2. Save images as .tiff and make sure the image field of view dimensions or pixel size are included in the meta data
 - a. **NOTE:** You may want to measure the X direction in the SEM software if this information is not provided in the .tiff meta data. You can usually figure this out based on pixel length indicated in meta data.
3. Visit the FIJI website and download FIJI
 - a. Make sure you're running most recent Java
4. Install Plugins
 - a. **Help > Update... > Manage update Sites**
 - b. Check the box next to MIST
 - c. Close the window
 - d. Select "Apply Changes"
5. Select Plugins > Stitching > MIST
6. Select the Filename Pattern Type
 - a. Your images are most likely saved as sequential and not row-column
7. Designate the Starting Point (indicated by the green circle on the schematic)
8. Indicate the direction of the image sequence (shown as red arrows)
9. If your first image is number "1" or "01", indicate the Grid Start Tile is 1.
10. Input Folder > Filename Pattern
 - a. Enter the name of the images followed by {ppp}
 - i. The number of p indicates the digits in your naming scheme
 - ii. **NOTE:** All images must have the same number of digits in the name
 1. E.g. Image_01 to Image_10 not Image_1 to Image_10
11. Browse to the file directory holding the images

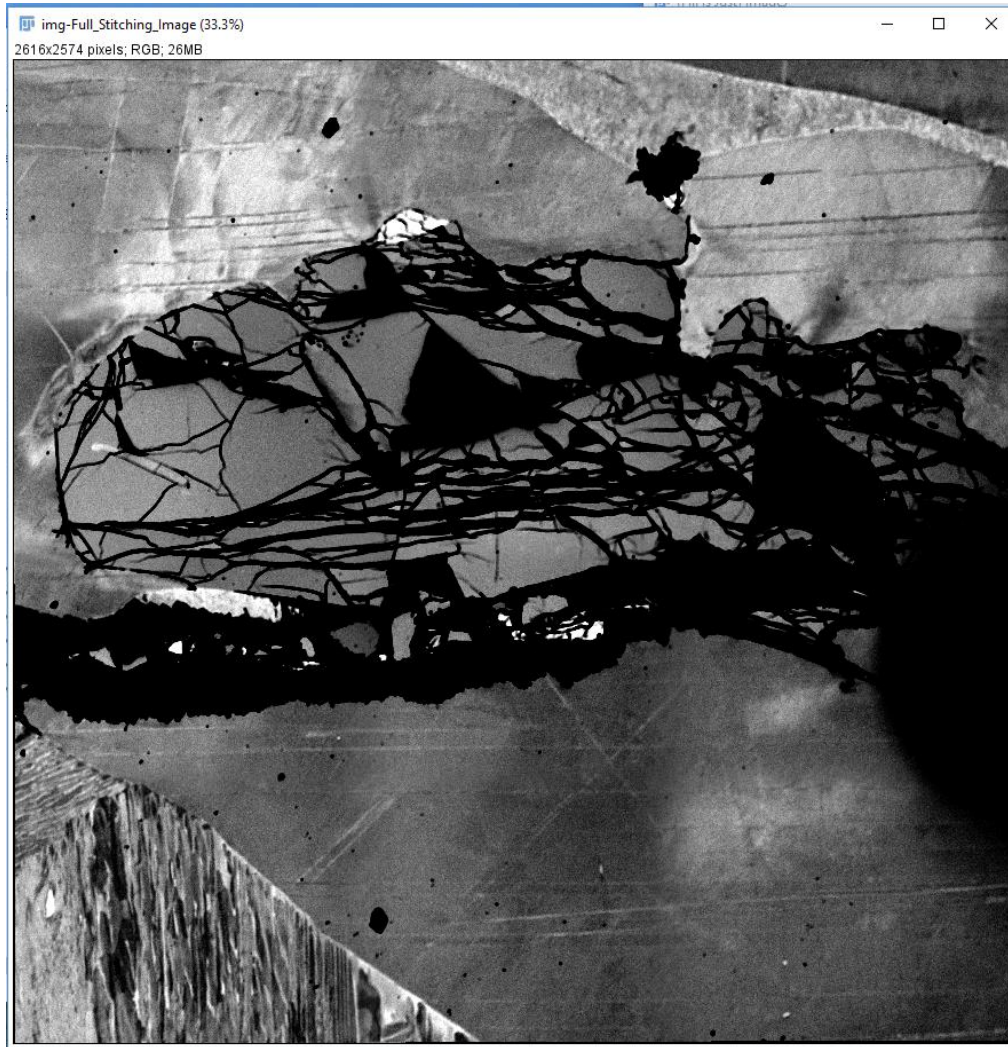




12. Open the Output Tab
13. Indicate the file directory where the image will be saved
14. Name the file to be created
15. Under Stitching Image
 - a. Select Blending mode > Linear
 - i. Overlay – uses one image at image overlap and deletes the information of the previous image
 - ii. Average – averages the pixels over the overlap
 - iii. Linear – Looks at pixels on either side of overlap and blends them together
16. Select Display Stitched image
17. Select Save Full Stitched Image if you want it saved at that directory

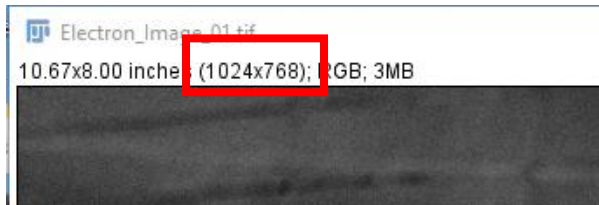


18. Select **Begin Stitching**



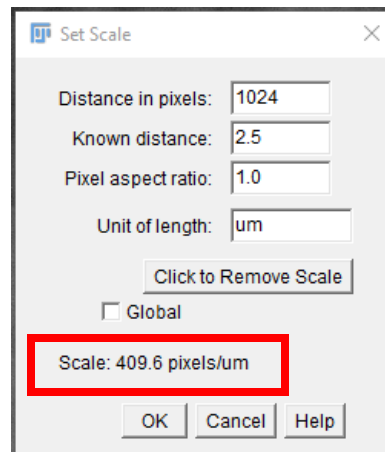
Adding a Scale Bar

19. Open one of the original images (We do not know how many pixels have been overlapped so you cannot use the stitched image)
20. Determine the pixel length in the x-direction at the top of the window



21. Go to Analyze > Set Scale

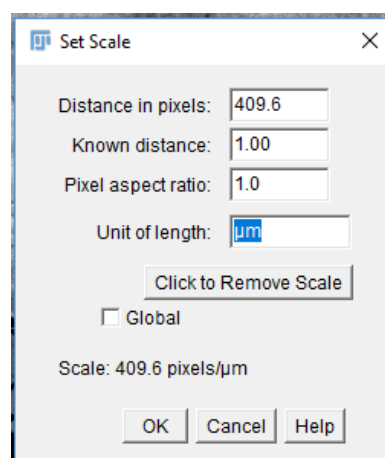
- Enter Pixels in X direction as Distance in pixels
- Enter the field of view distance based on measurement or reading off microscope
- Enter "um" for microns in Unit of Length
- Record the Scale value in pixels/um at bottom of screen
- This indicates how many pixels are in one micron in this image. This will also be true for the stitched image



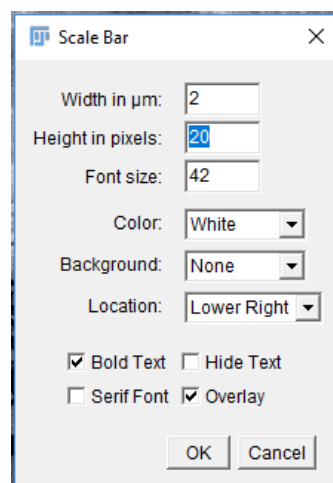
22. Go back to the stitched image window

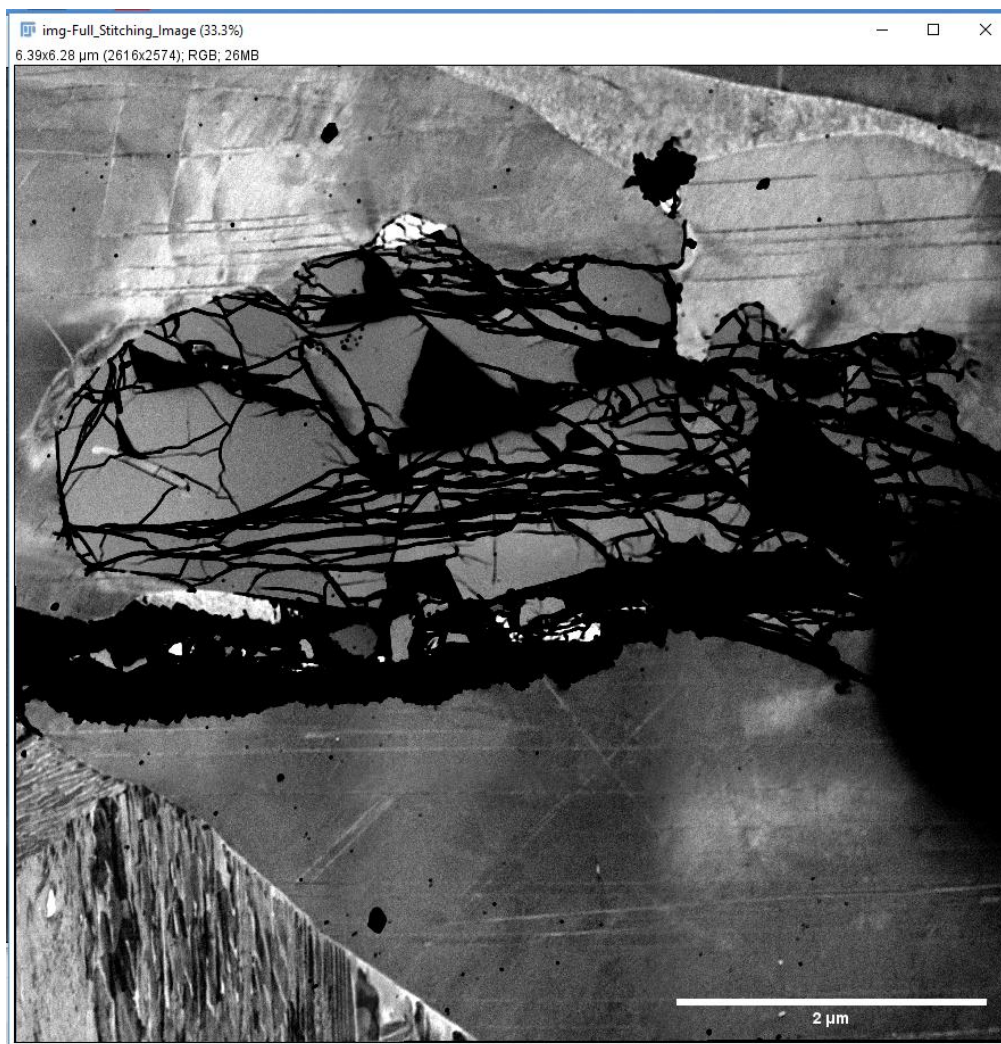
23. Go to Analyze > Set Scale

- Enter the scale from the previous measurement in Distance in Pixels
- Enter 1 in known distance
- Enter "um" for Unit of Length
- Click OK



24. Go to Analyze > Tools > Scale Bar





25. Save image