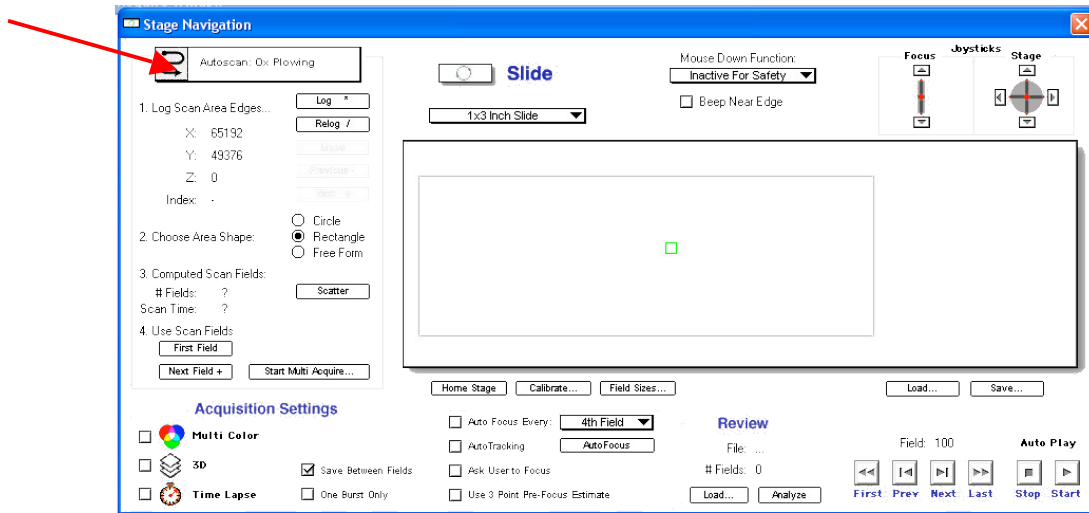
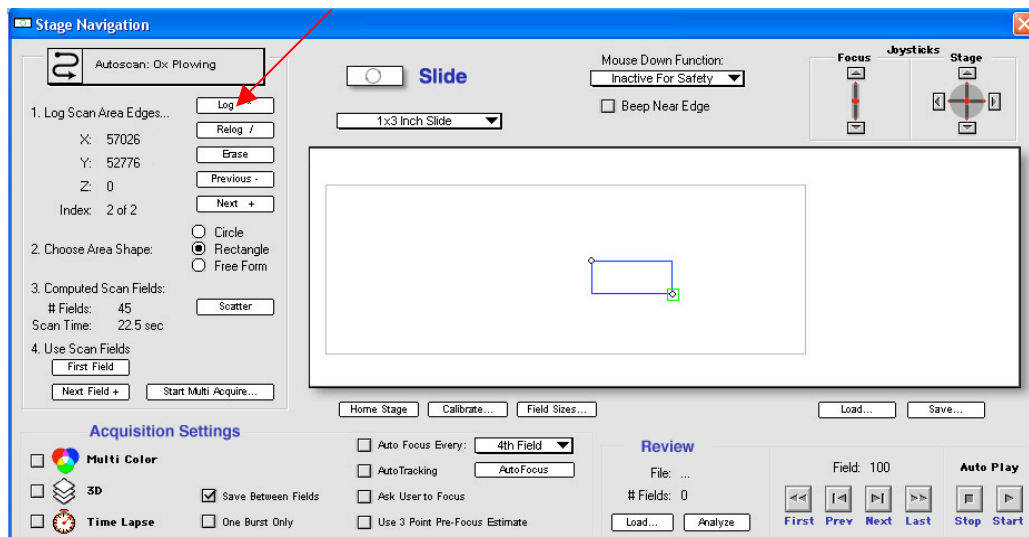


Protocol for Tiling Fluorescence Image

1. Focus your sample and locate the field of interest.
2. Set the gain and exposure time for each channel under the Imaging Modes window.
3. Open the stage navigation window.
 - a. Select “autoscan: ox plowing” in the top-left corner of the window.

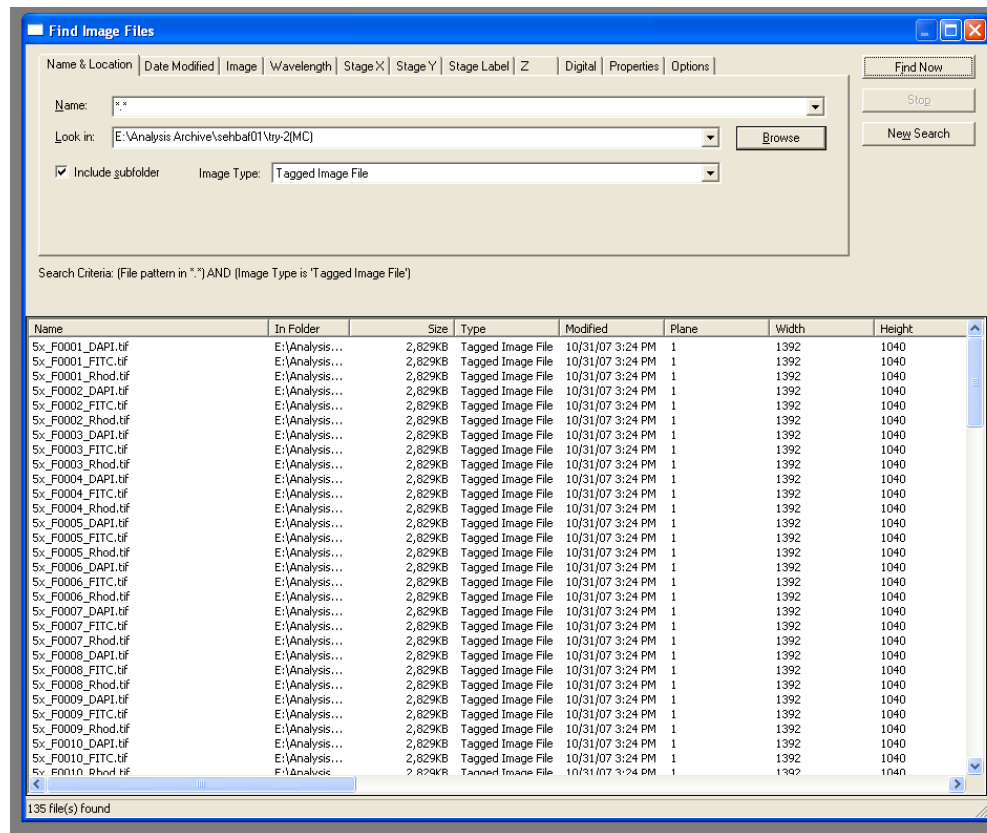


- b. The area shape is a rectangle.
- c. Move the stage to view the top-left corner of the sample. Make sure that part of the sample is visible within the field. Refocus your sample. Log this point.
- d. Move the stage to view the bottom-right corner of the sample. Make sure that part of the sample is visible within the field. Refocus your sample. Log this point.



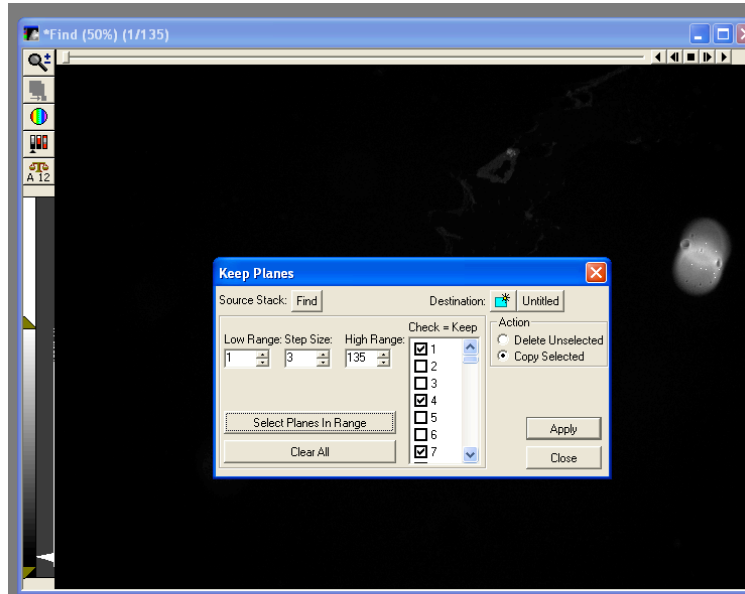
- e. Check “Multi Color” in the bottom left corner.

- f. Click on “Start Multi Acquire”. Specify which folder to save in, and enter the filename.
4. Retrieve files through the network on the Image Analysis 2 workstation.
 - a. Double-click on the “Microsrfr” icon on the desktop.
 - b. Double-click on the “DM6000” icon.
 - c. Copy your files from Leica DM6000 onto your folder in the E: drive.
 5. Create the montage using MetaMorph.
 - a. Open the entire sequence of images:
 - i. File -> Find images...
 - ii. Select the data folder through the Browse button
 - iii. Image type is “Tagged Image File”
 - iv. Click on “Find Now”
 - v. Right-click on one of the files from the list and choose “Select all files”
 - vi. Right-click on one of the files and choose “Open as stack”

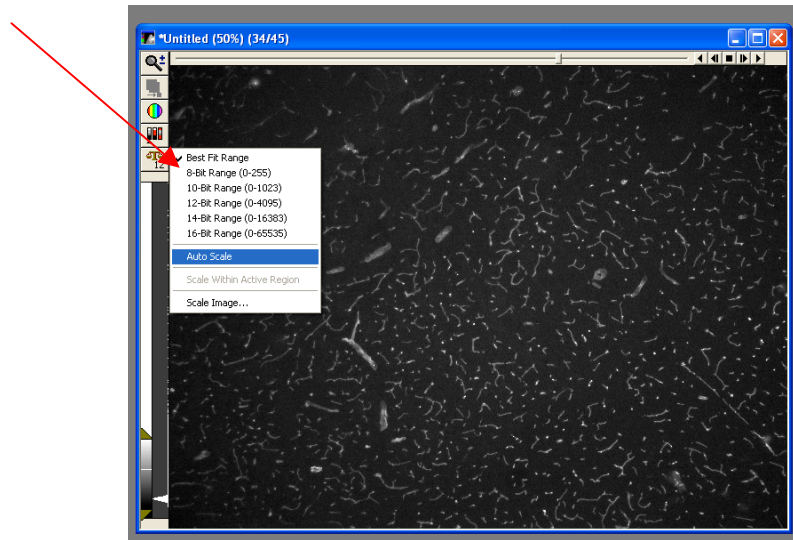


- b. Separate the individual channels from the image sequence:
 - i. Stack -> Keep Planes
 - ii. Ensure that the Source Stack is “Find”

- iii. For the first channel, enter “1” for Low Range. Enter the total number of images for High Range. Enter the number of channels for Step Size.
- iv. Click “Clear All”
- v. Click “Select Planes in Range”
- vi. Click “Apply”
- vii. Repeat steps 7bi-7bvii for all channels. The Low Range value will change depending on the channel.

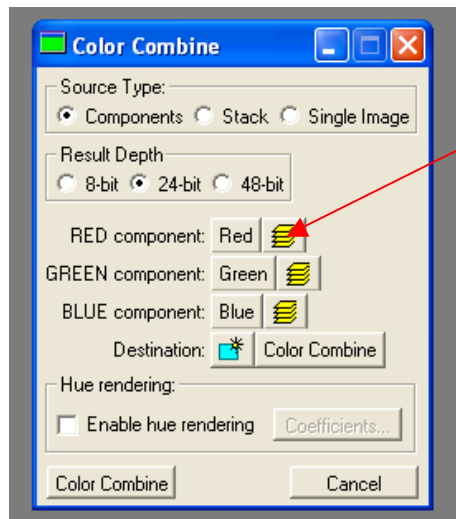


- c. Remove the Auto Scale option for each channel using the icon indicated in the image below.

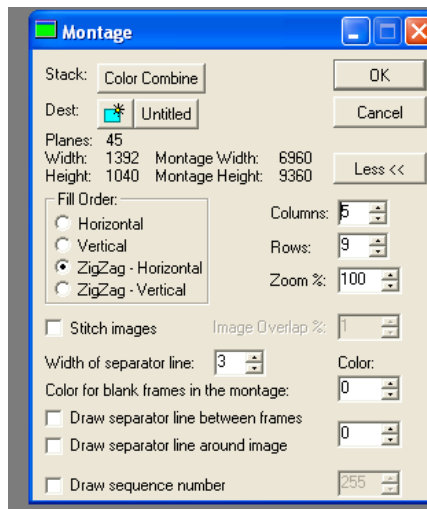


- d. Click on “Color Combine” in the montage taskbar. Select “Components” under Source Type; select “24-bit” under Result Depth; choose the appropriate image stack

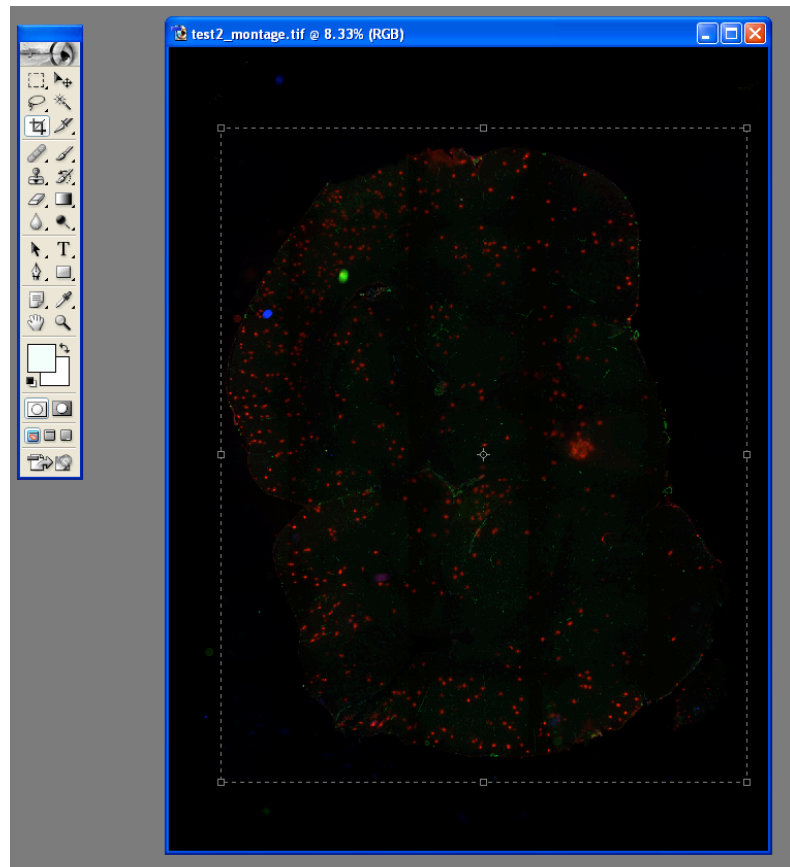
for the corresponding color component; click on the stack icon next to each color component and select “All Planes.” Finally, click “Color Combine” to merge the colors.



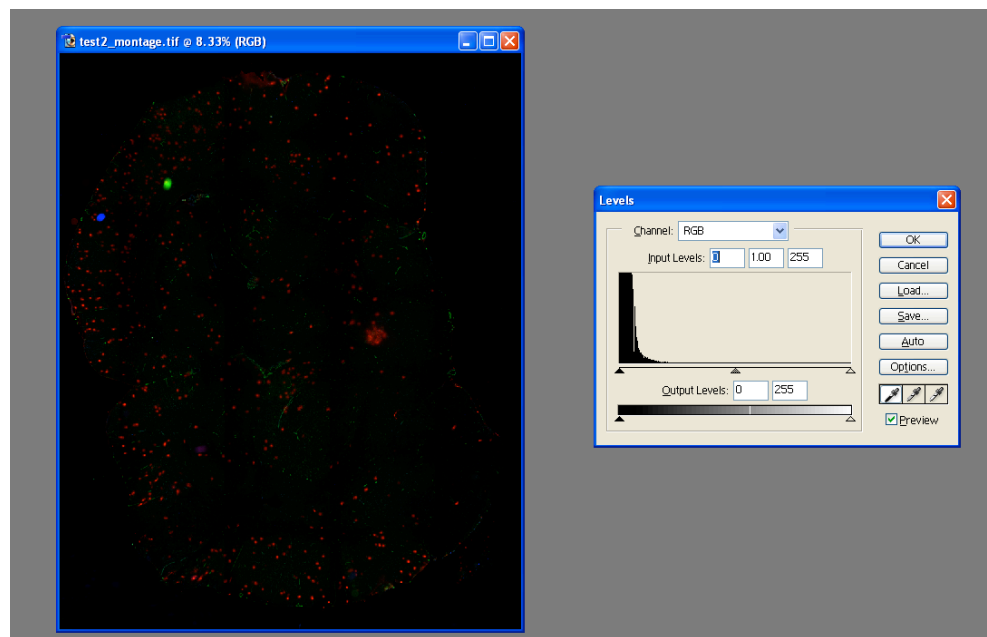
- e. Click on “Montage” in the montage taskbar. Choose the Color Combine image sequence as the source stack. Select “ZigZag – Horizontal” under Fill Order and enter the appropriate number of columns and rows. Uncheck “Stitch images”. Leave every other option at the default setting. Click OK.



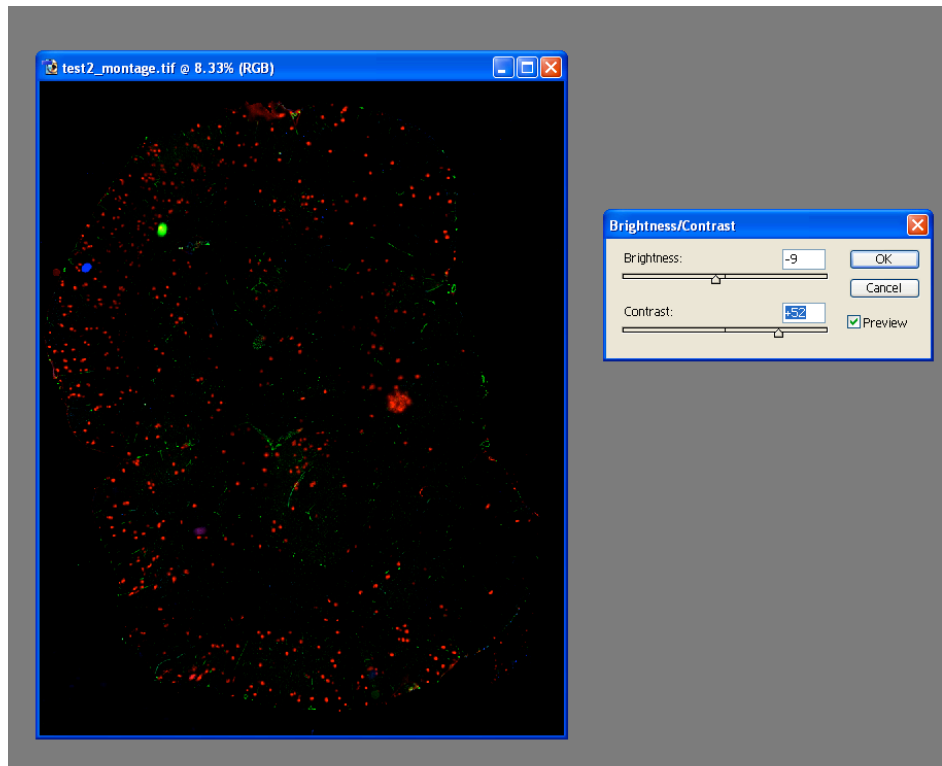
- f. Save the montage as an rgb-tiff.
6. Edit the image using Photoshop.
 - a. Open the image.
 - b. Crop out the field of interest: Select the crop function under the “Tools” toolbar or simply press “c”. Highlight the field of interest and then double-click on the image to crop.



- c. Correct the background: Image > Adjustments > Levels
Click on the black point dropper icon on the bottom-right corner of the window.
Click on a part of the image that is supposed to be black.



- d. Adjust the brightness and contrast: Image > Adjustments > Brightness/Contrast
Modify the brightness and contrast appropriately for the best results.



e. Save your changes.