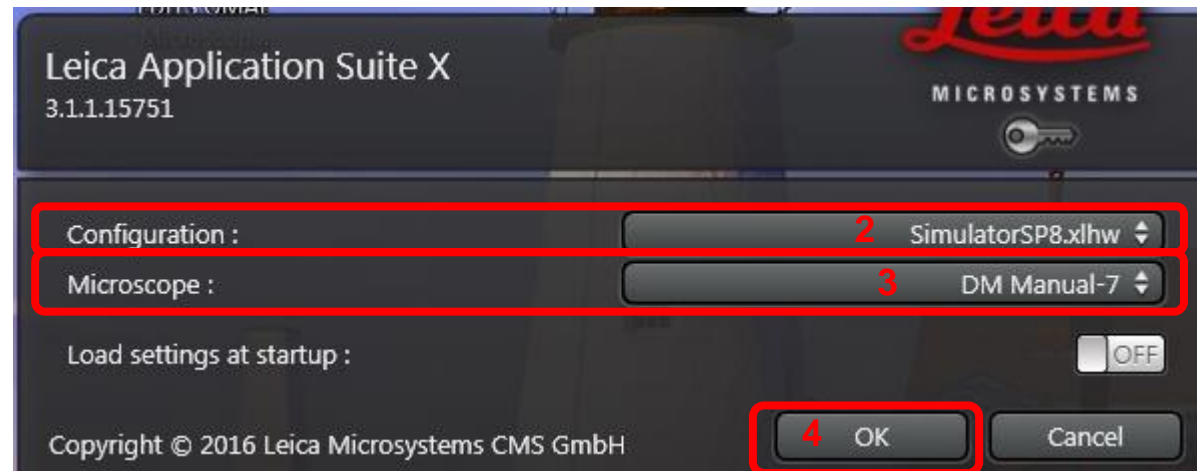
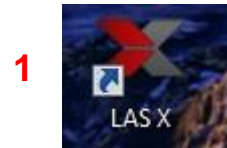


Leica SP8 Analysis

Kim Peifley

02/13/17

1. Click the LAS X icon on desk top.
2. Configuration setting should be SimulatorSP8.xlhw.
3. Microscope setting should be DM Manual-7.
4. Click OK.



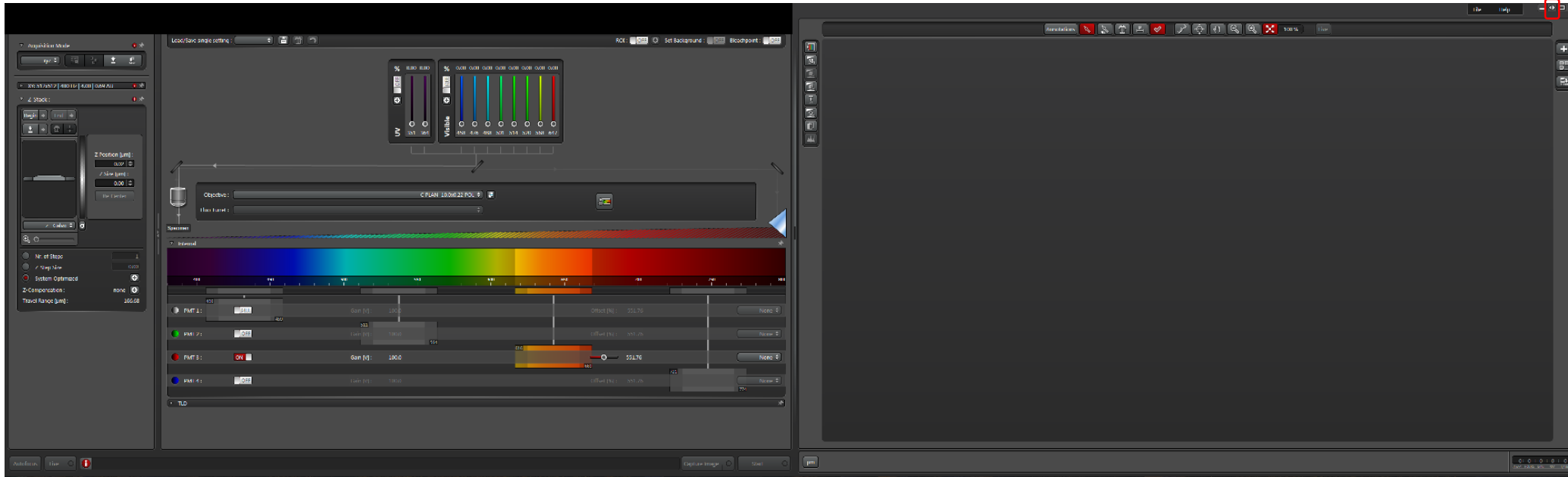
5. Initialize Stage? Click No.



6. If the software opens up taking up the whole screen [A] and you can't see menu selection [B] click the reduce icon [C].

A

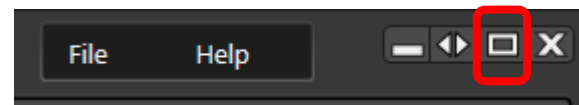
C



B



C

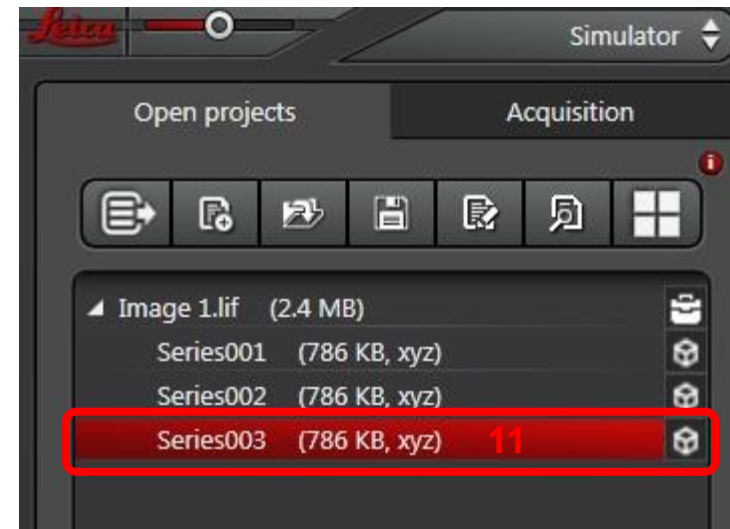
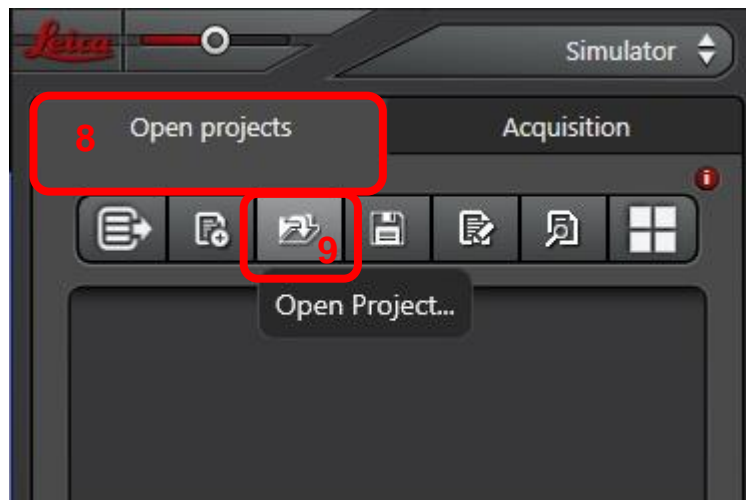


7. This is your analysis work area.

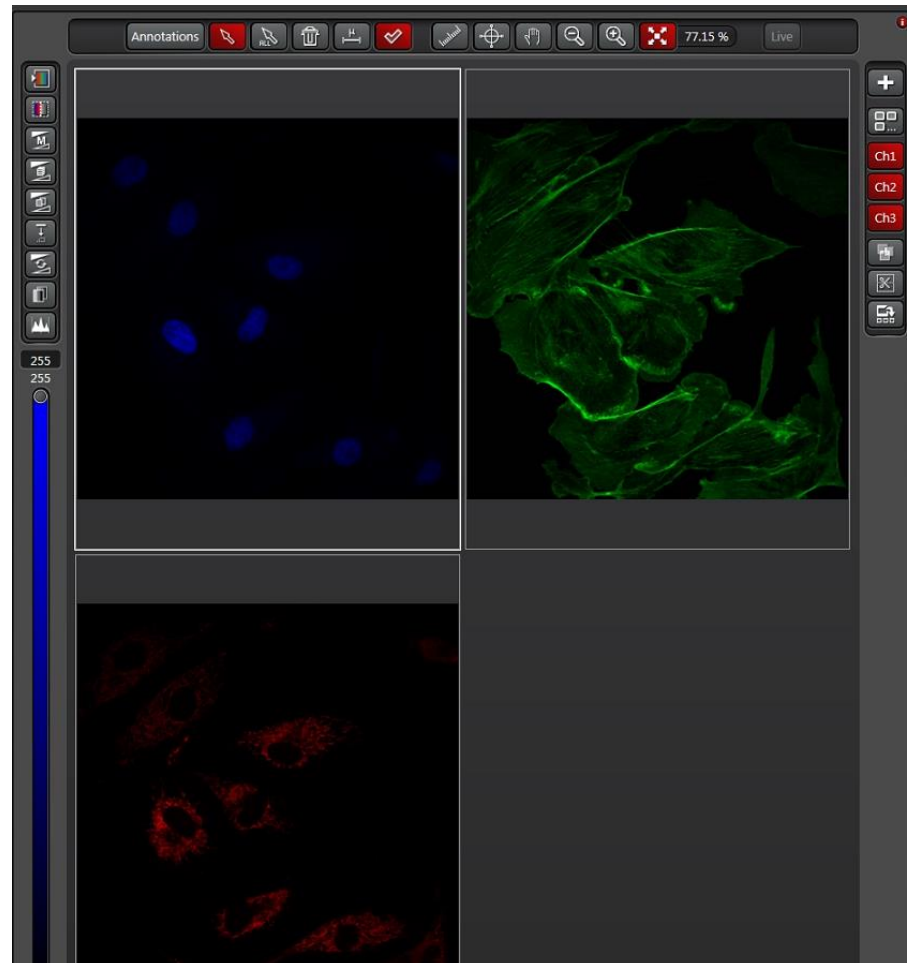
The screenshot displays a microscope software interface with the following components:

- Top Bar:** Includes a menu bar with "File" and "Help", and a navigation bar with "Configuration", "Acquire" (highlighted), "Process", "Quantify", and "Analysis".
- Left Panel (Acquisition):**
 - Buttons for "Open projects" and "Acquisition".
 - "Acquisition Mode" set to "xyz".
 - Parameters: "XY: 512x512 | 400 Hz | 4.00 | 0.69 AU".
 - "Z-Stack" controls: "Begin", "End", "z-Galvo", "Z Position [µm]: -0.02", "Z Size [µm]: 0.00", and "Re-Center".
 - Settings: "Nr. of Steps: 1", "Z-Step Size: 0.00", "System Optimized" (checked), "Z-Compensation: none", and "Travel Range [µm]: 166.68".
- Center Panel (Optics & Specimen):**
 - "Load | Save | Roi" and "Load/Save single setting" options.
 - "UV" and "Visible" light sources with intensity sliders and wavelength lists (UV: 351, 364; Visible: 458, 476, 488, 501, 514, 520, 568, 647).
 - "Objective: C PLAN 10.0x0.22" and "Fluo Turret" selection.
 - "Specimen" area with a color calibration bar and a "TLD" section.
- Right Panel (Analysis):**
 - "Annotations" toolbar with various tools.
 - Large central area for image analysis.
 - Zoom controls: "100 %", "Live", and a "µm" scale bar.
- Bottom Bar:** Includes "Autofocus", "Live", "Capture Image", "Start", and a status bar showing "0 : 0 : 0 : 0" and "Data: 2000x 500x 300x 1000x".

8. Click Open Projects Tab.
9. Click Open Project Button.
10. Select project.
11. Click on image "series" to open image.

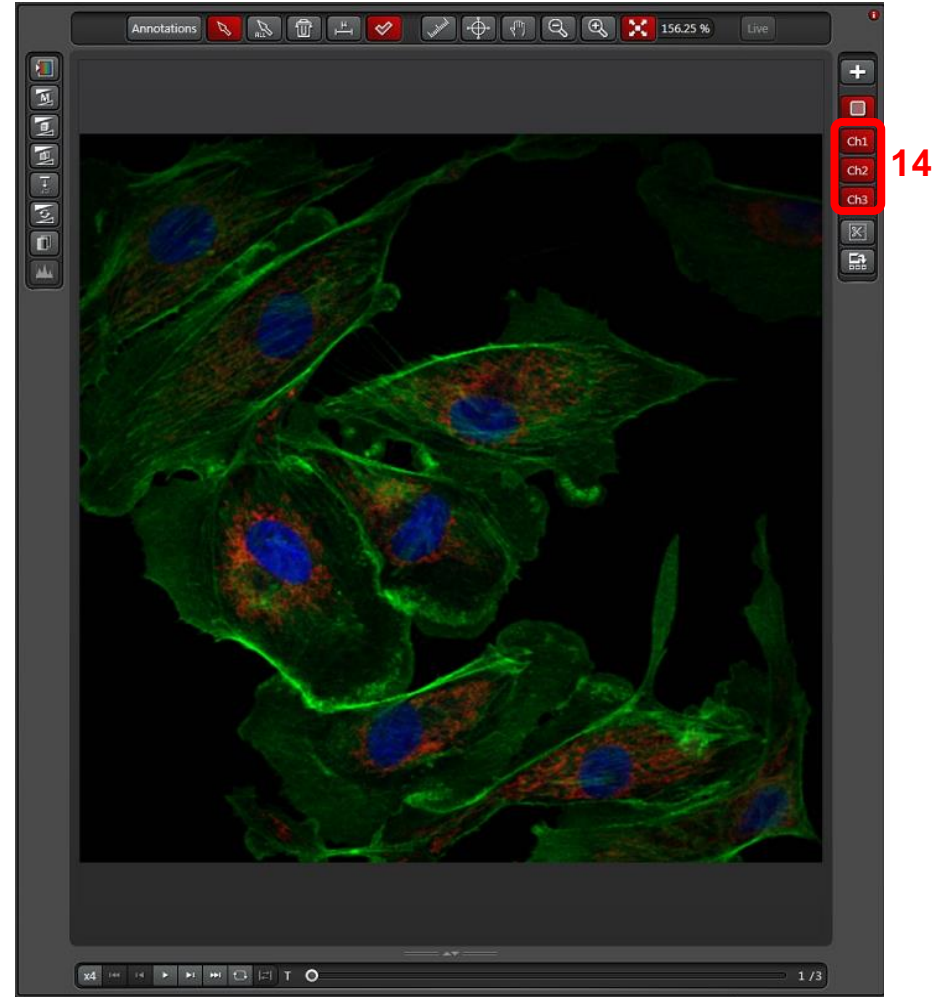
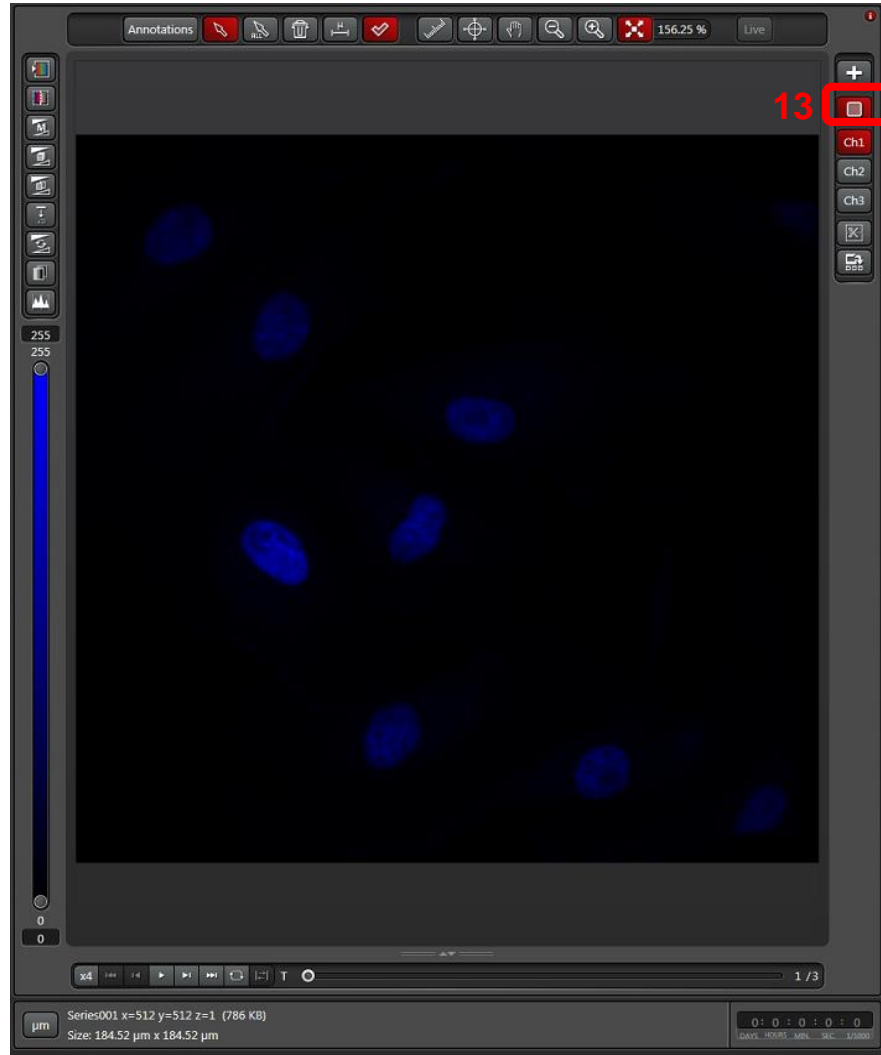


12. When images open up they open in split channel view.



13. If you want to view in merge click the partition icon.

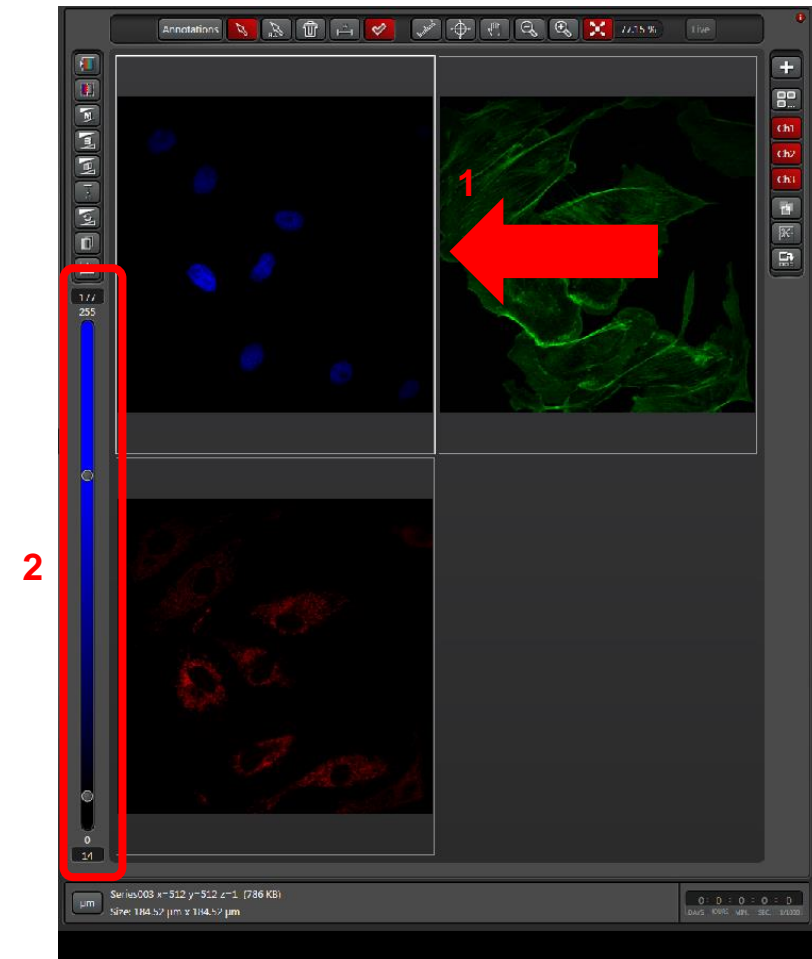
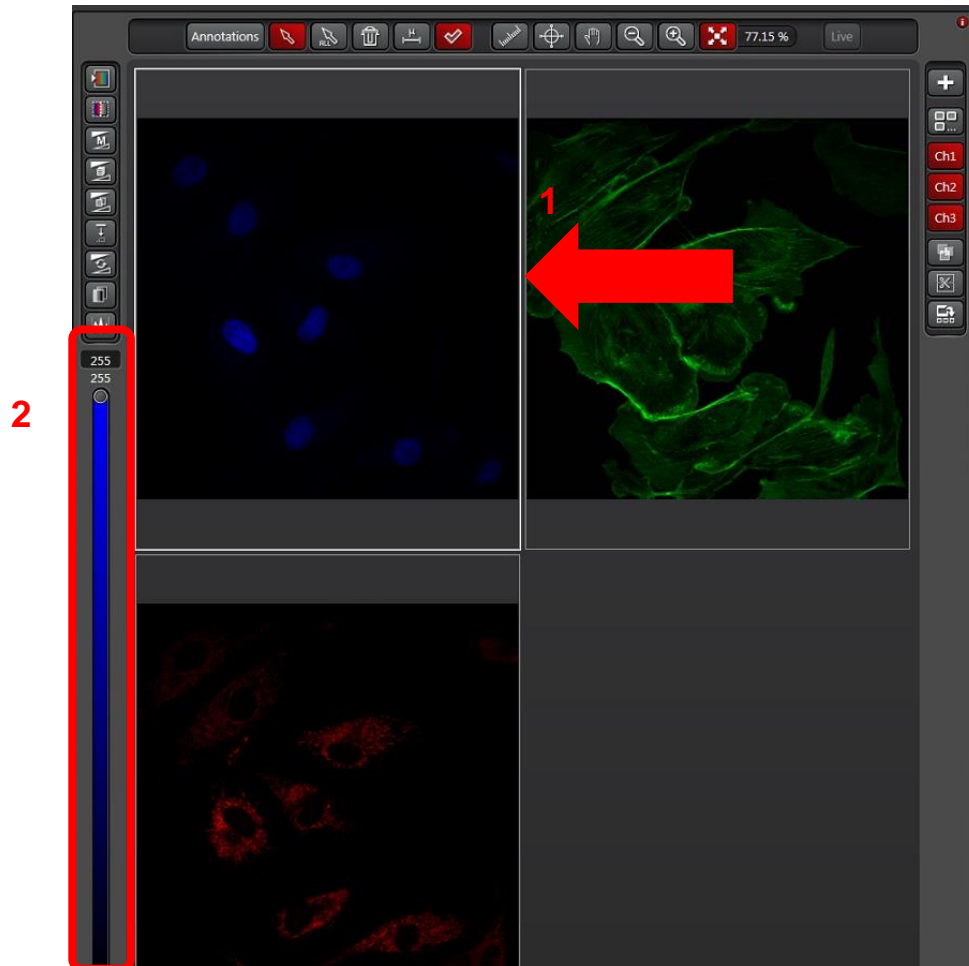
14. The merge will first show only the initially selected channel so click on the other channels to select and view them.



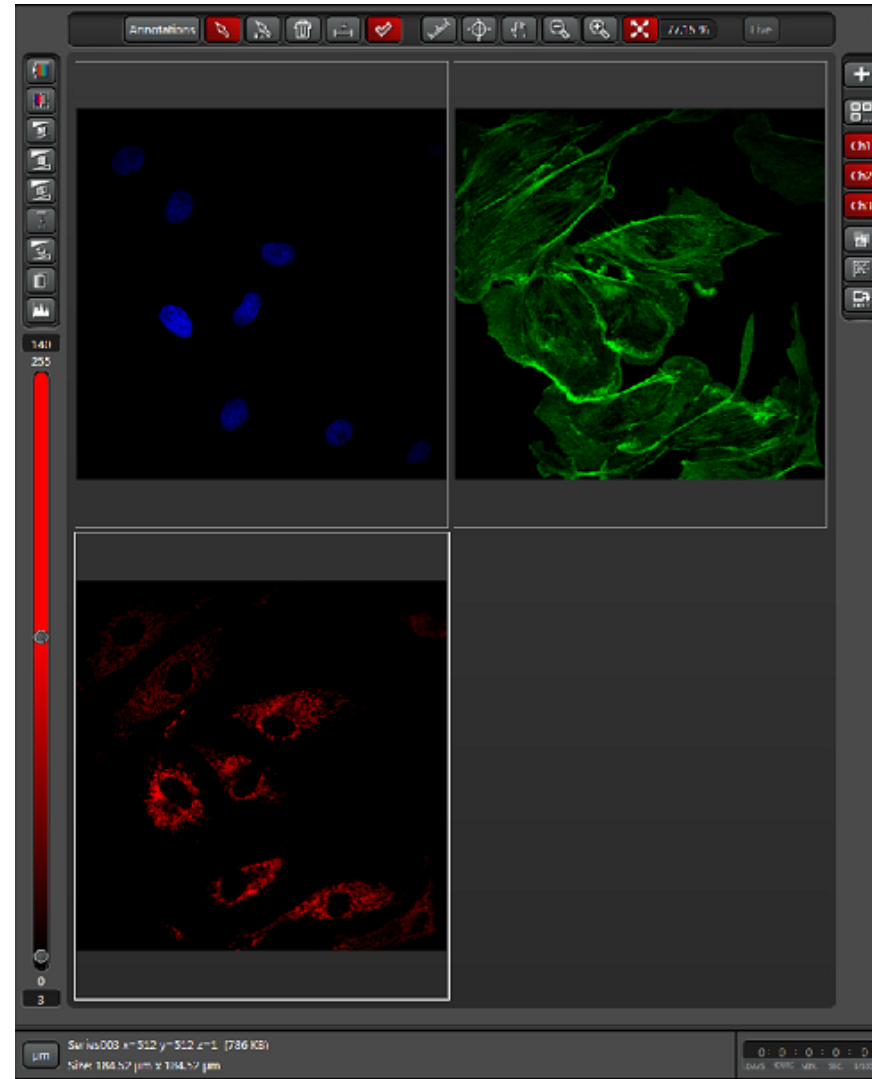
Adjusting Contrast

To Adjust Contrast:

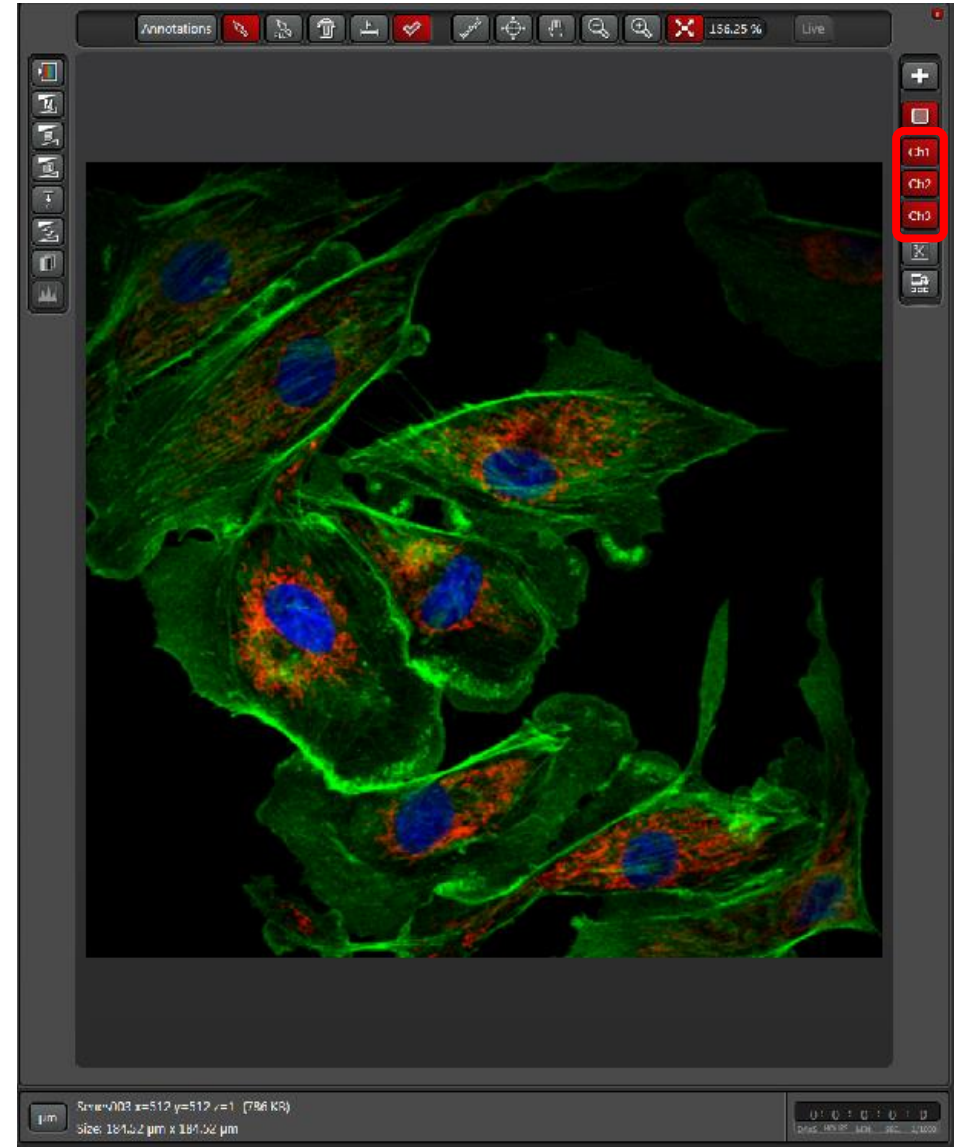
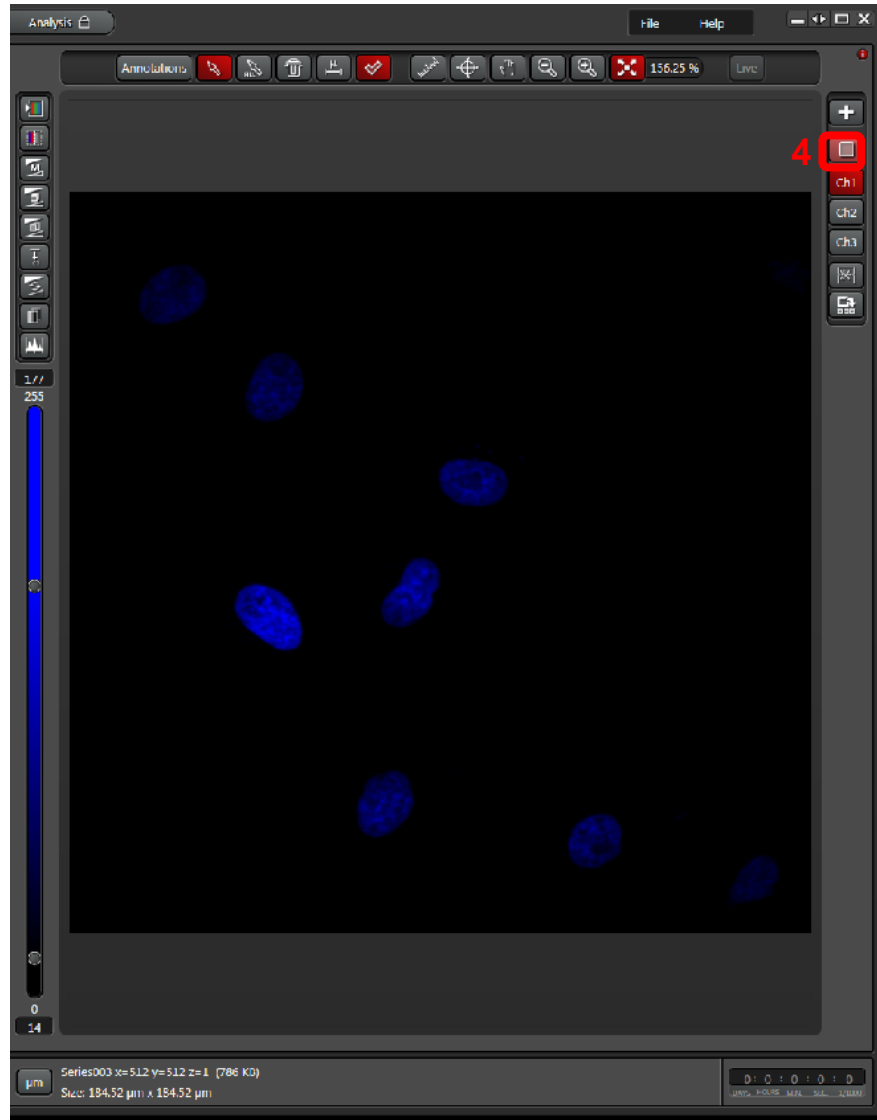
1. Click on window to select it.
2. Adjust the contrast from the bar on the left side of the window.



3. Repeat for the other channels.

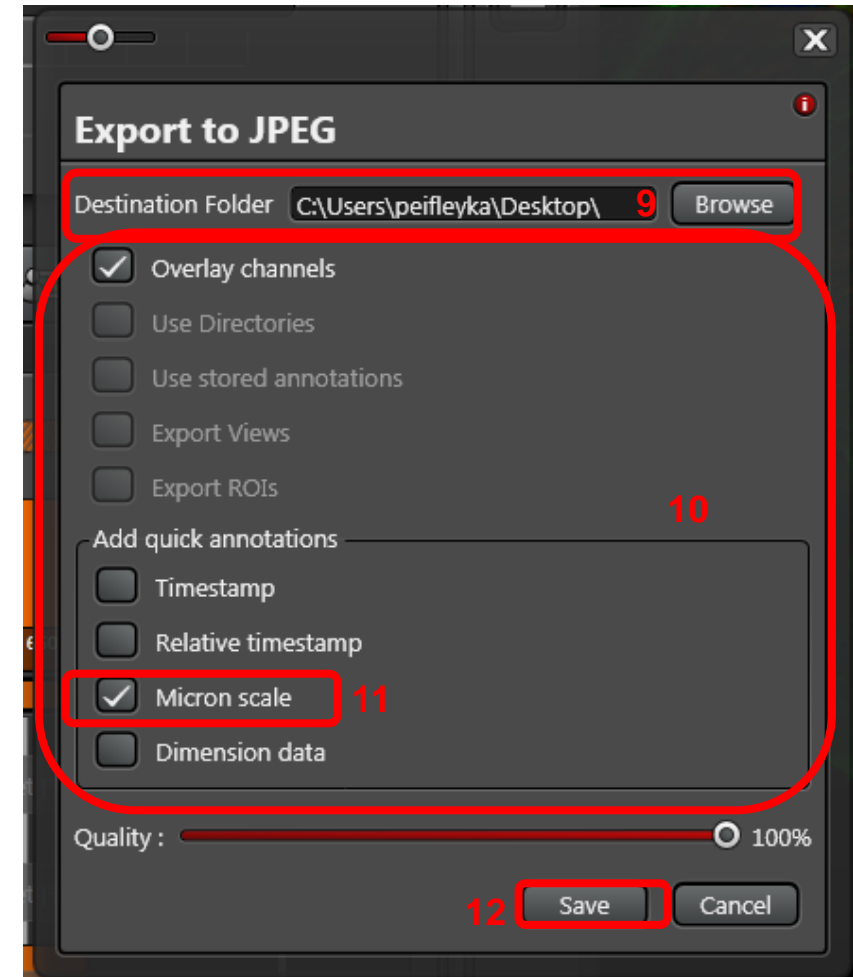
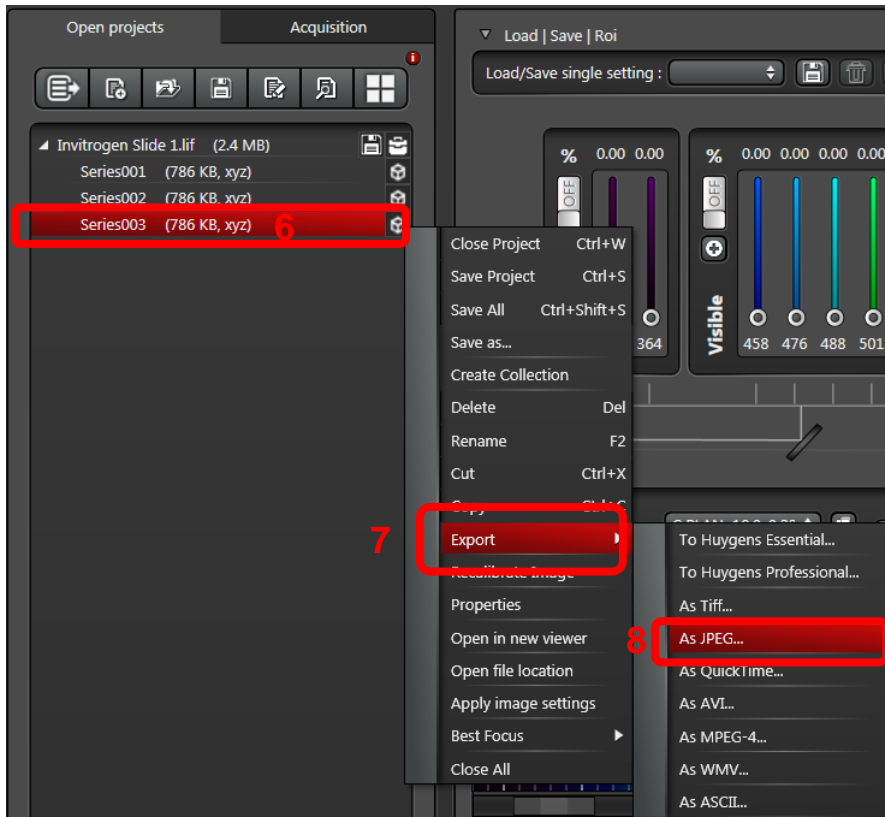


4. If you want to export the image in merge format click the partition icon.
5. The merge will first show only the initially selected channel so click on the other channels to select and view them.

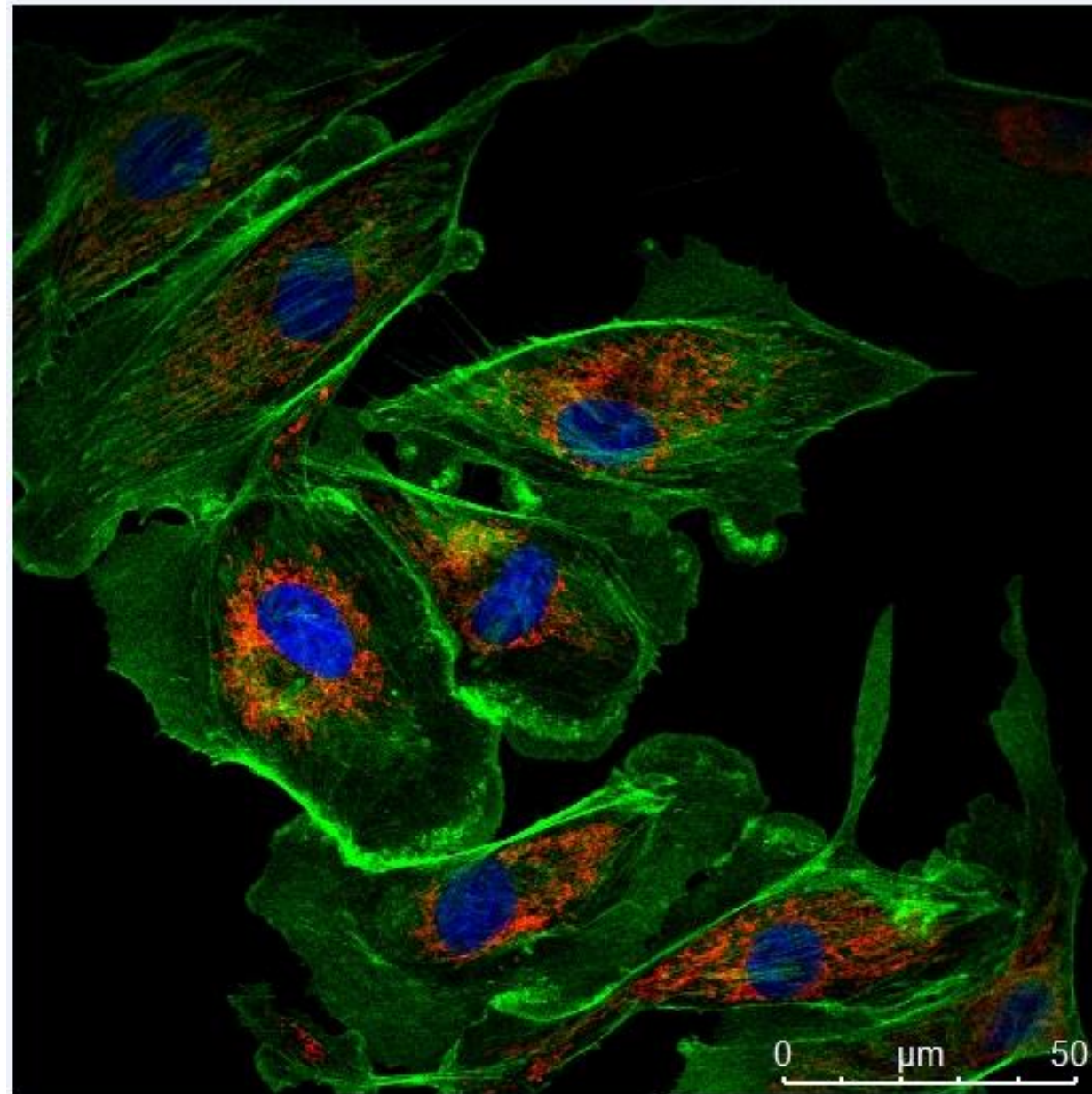


When Image is how you wish it to look and ready to be saved as a .tiff or .jpeg etc.

6. Make sure image name is still highlighted in the Open Projects tab.
7. Right click and select Export.
8. Select file format. In this case “As JPEG”.
9. Select Destination Folder for your image.
10. Check all boxes you wish to have.
11. If you want a scale bar on the image click “Micron Scale” box.
12. Click Save.

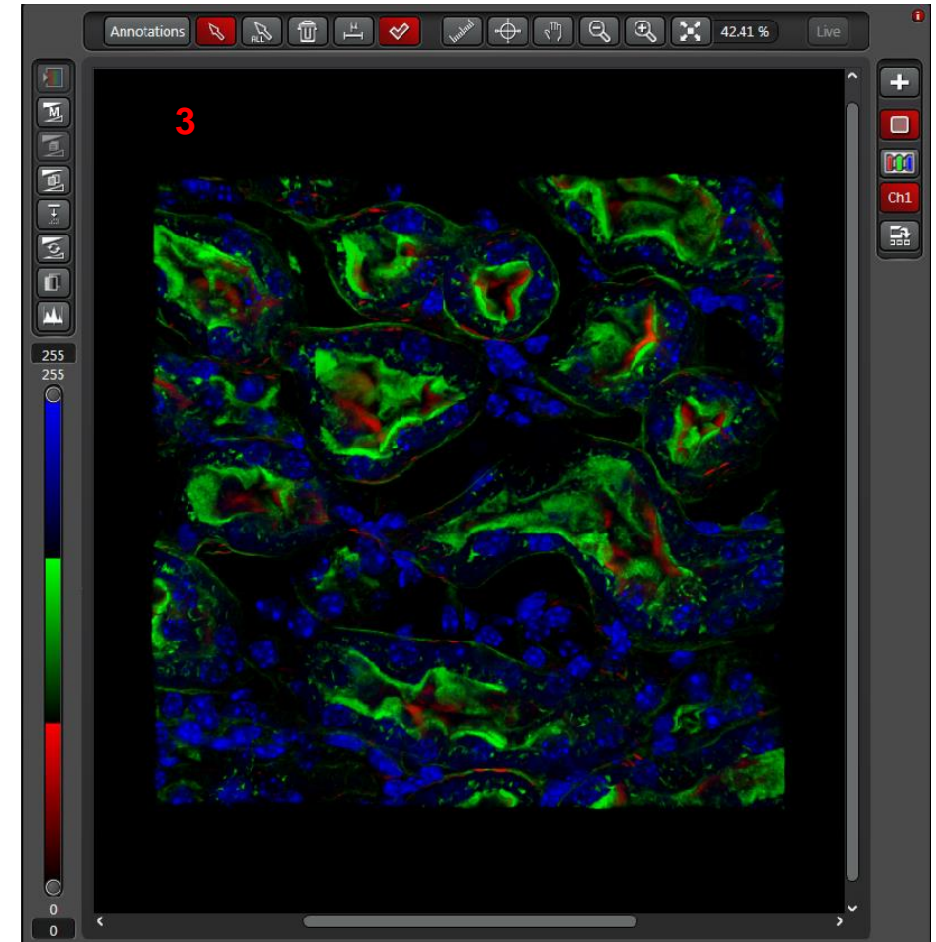
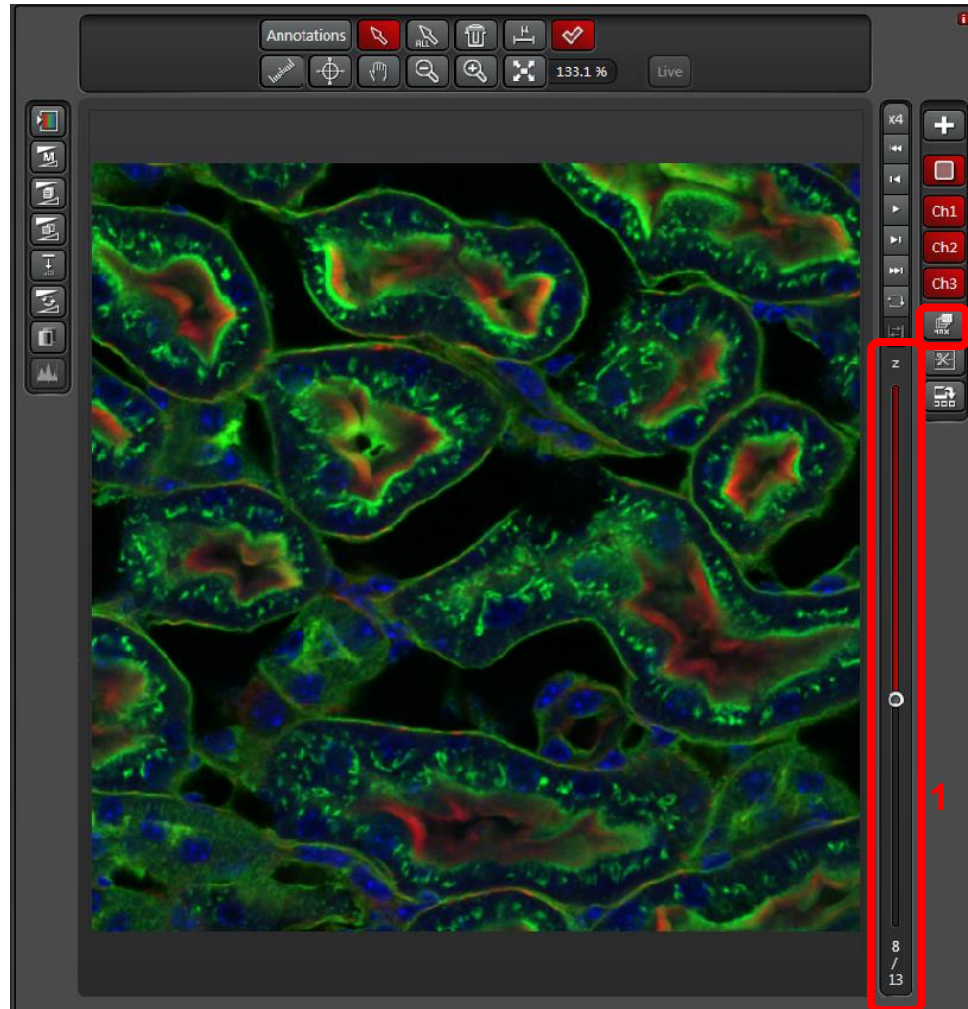


14. This is the .jpeg image with scale bar.



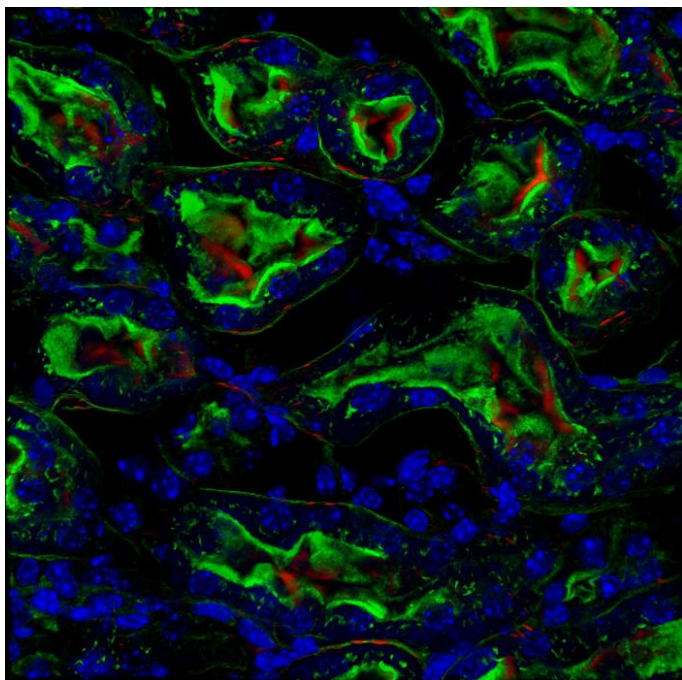
Z Stack Image Processing

1. When viewing your Z stack image you can move through the slices using the bar on the right.
2. To get Maximum Intensity Projection click the box below the last channel button.
3. This is your maximum intensity projection image.

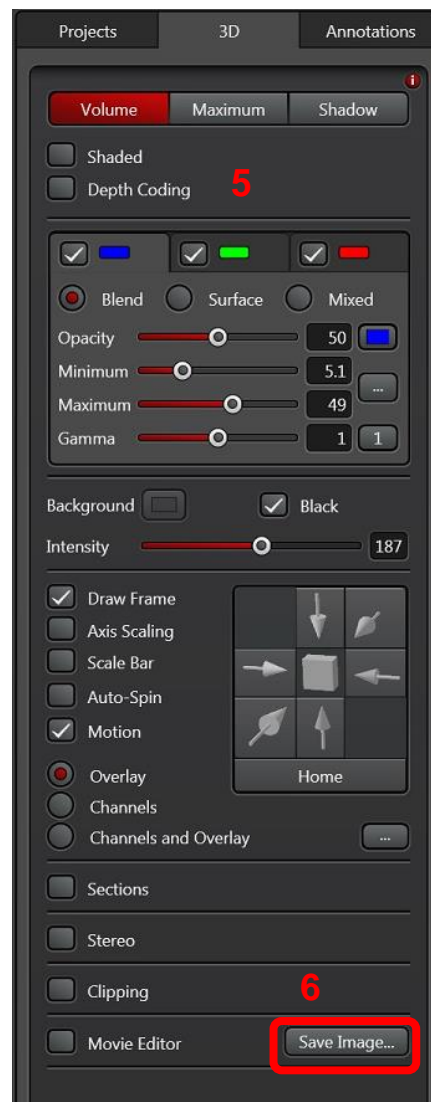


4. To rotate image click the 3D button. This will open a whole new window.
5. Set your parameters.
6. Click Save Image.

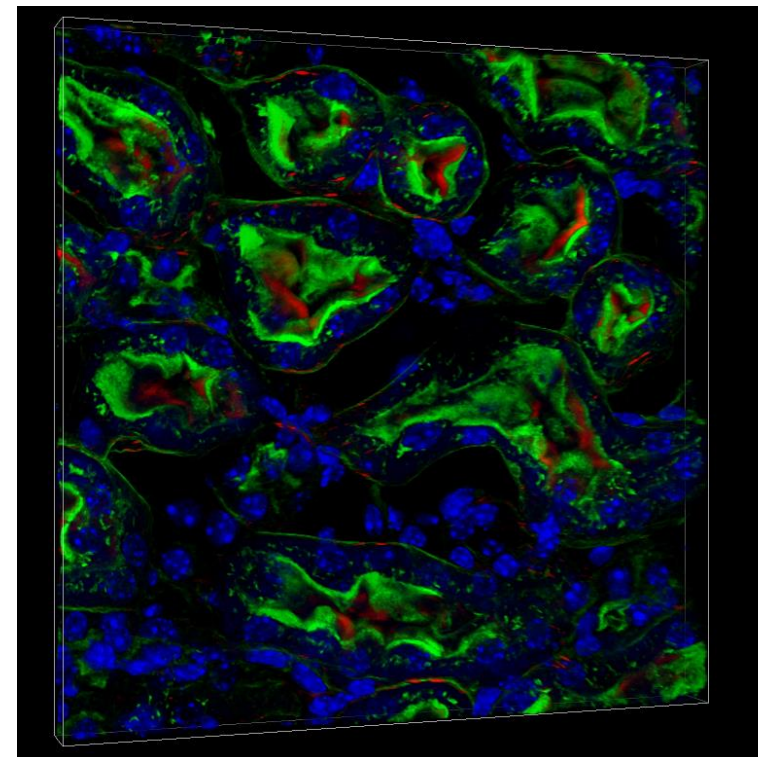
Maximum Intensity Image



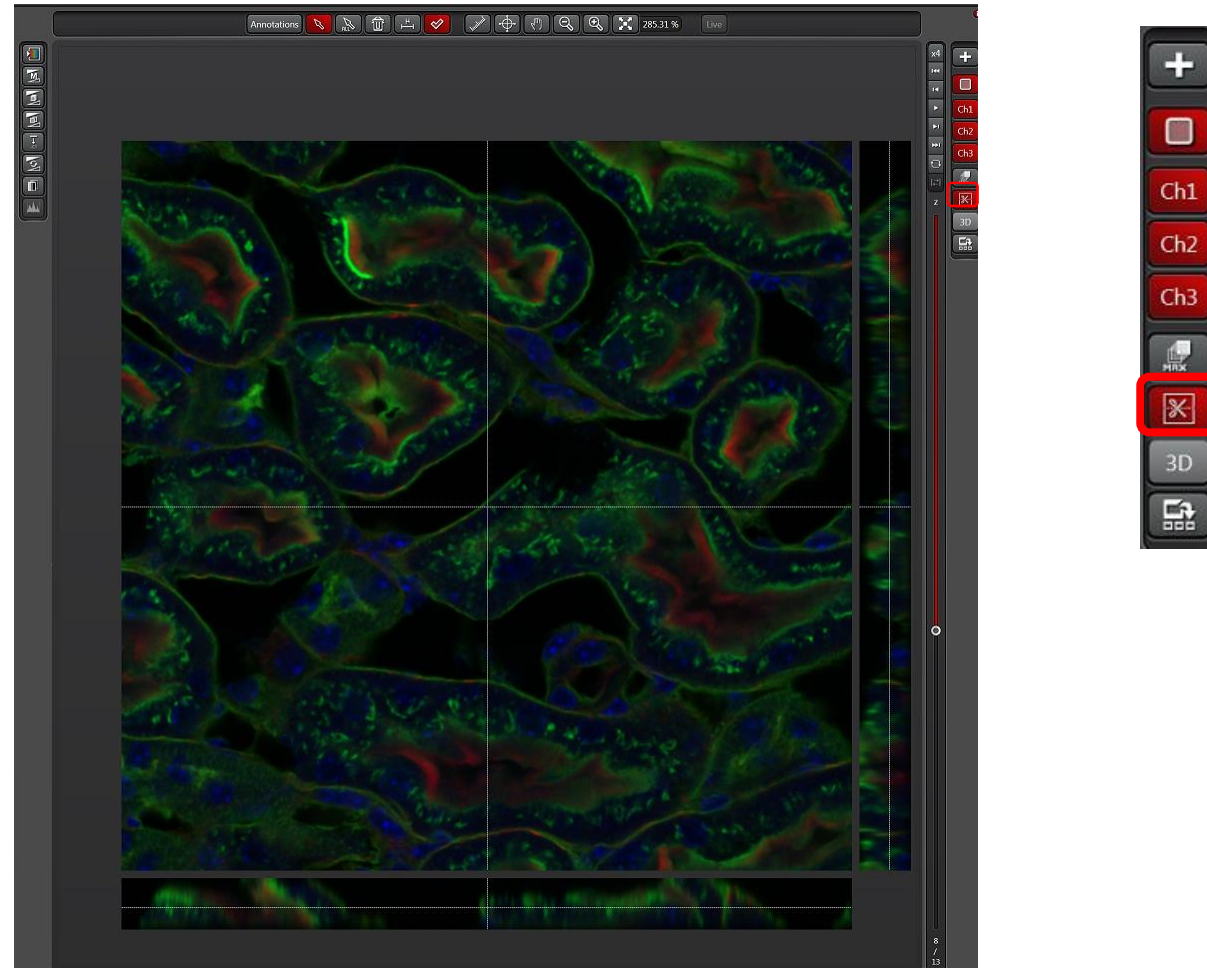
4



Rotational Image

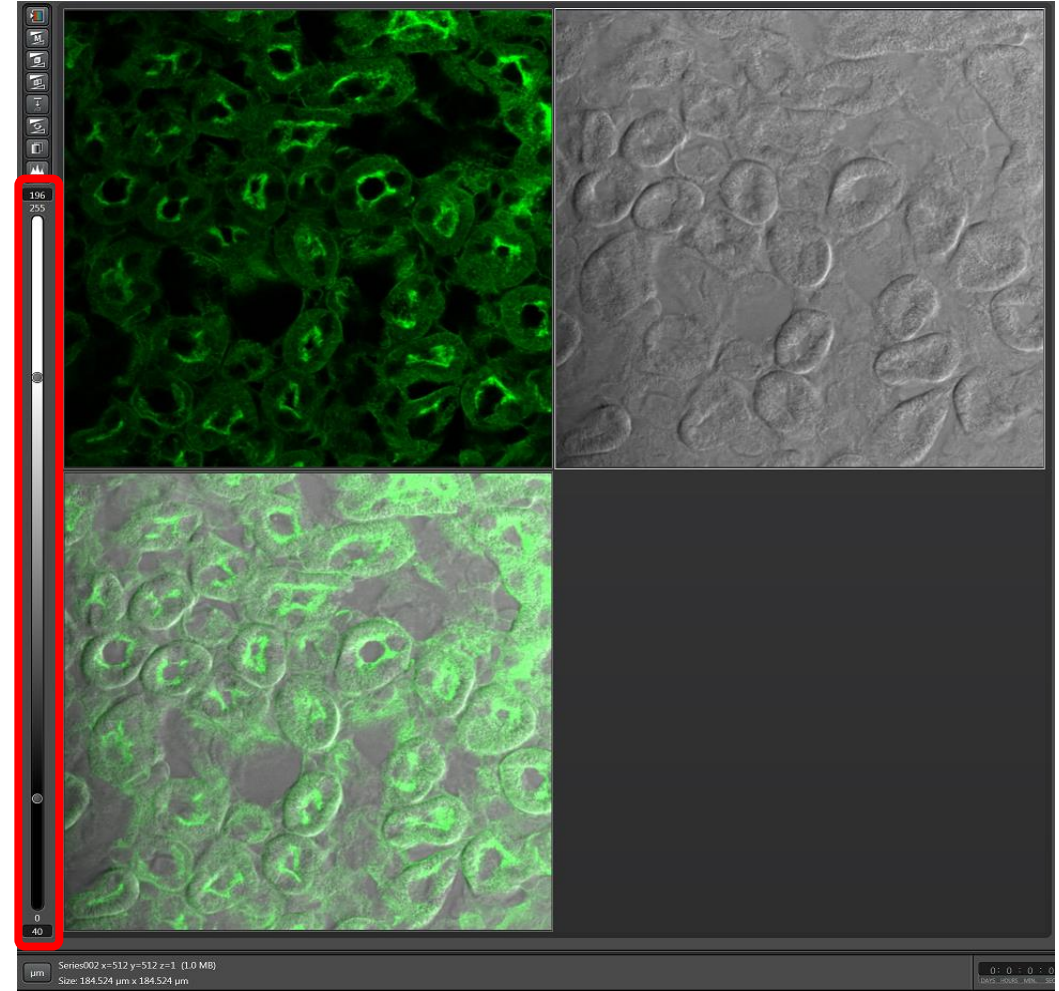
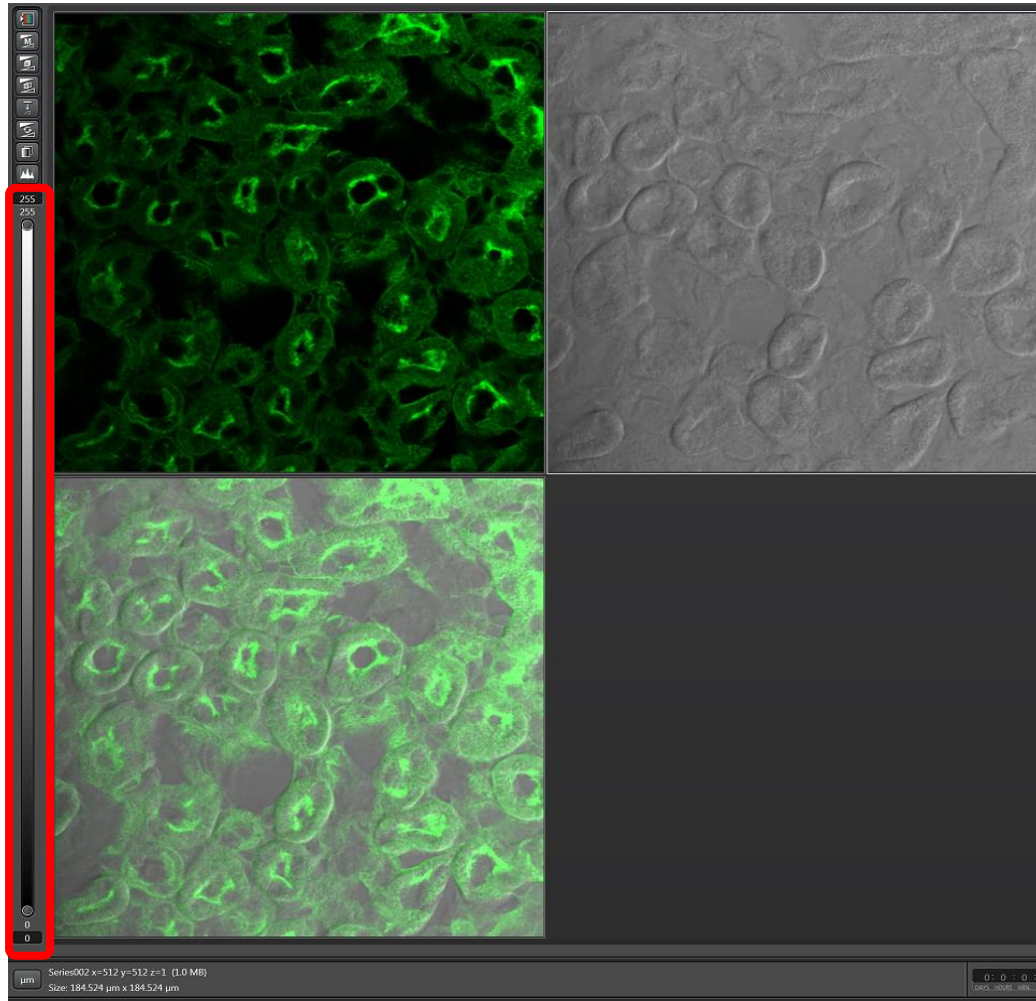


7. For orthogonal view click the Scissors Icon to the right of the image.



DIC Image Processing

DIC Images need to be contrast adjusted. Highlight the DIC image box and adjust the contrast.

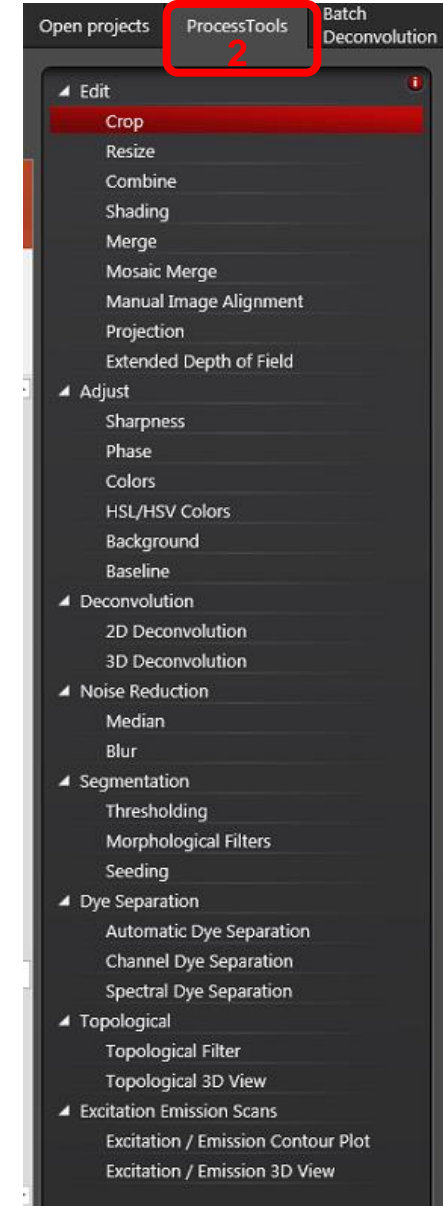


Process Tab



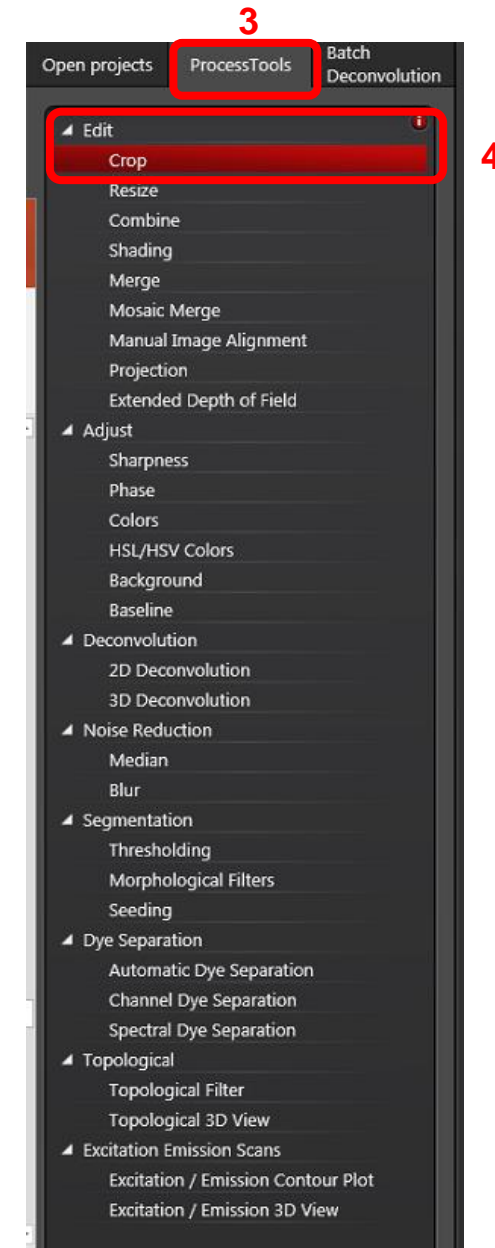
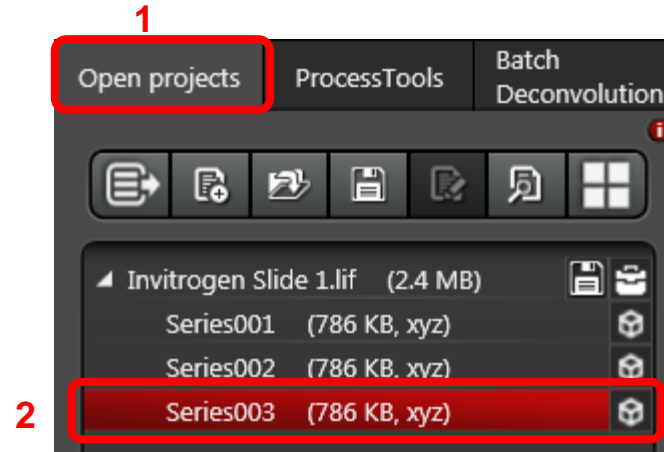


1. If you click the Process tab in the main menu you will now see a ProcessTools Tab.
2. Clicking on Process Tools tab opens up a menu of options you can use.

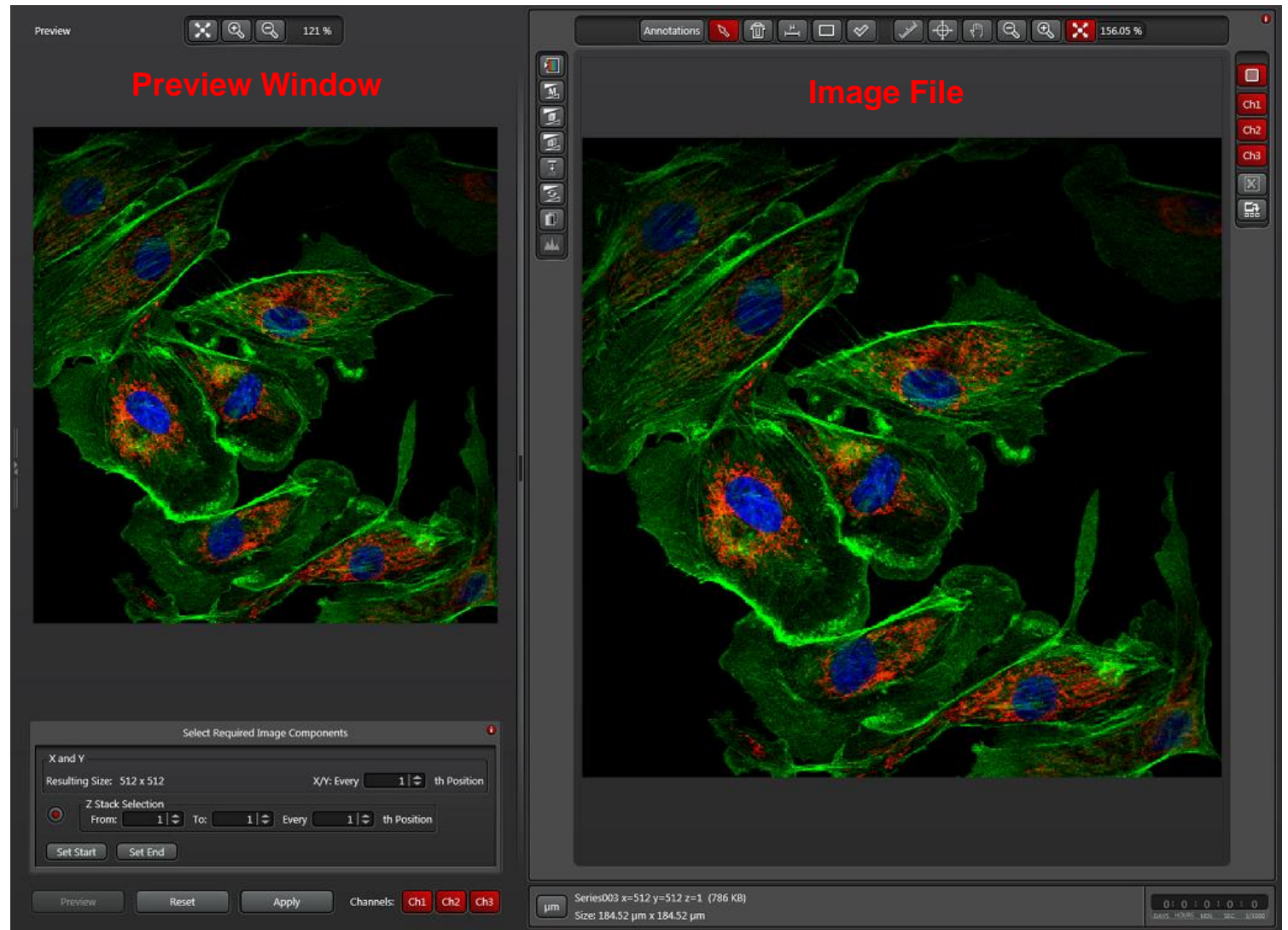


Crop

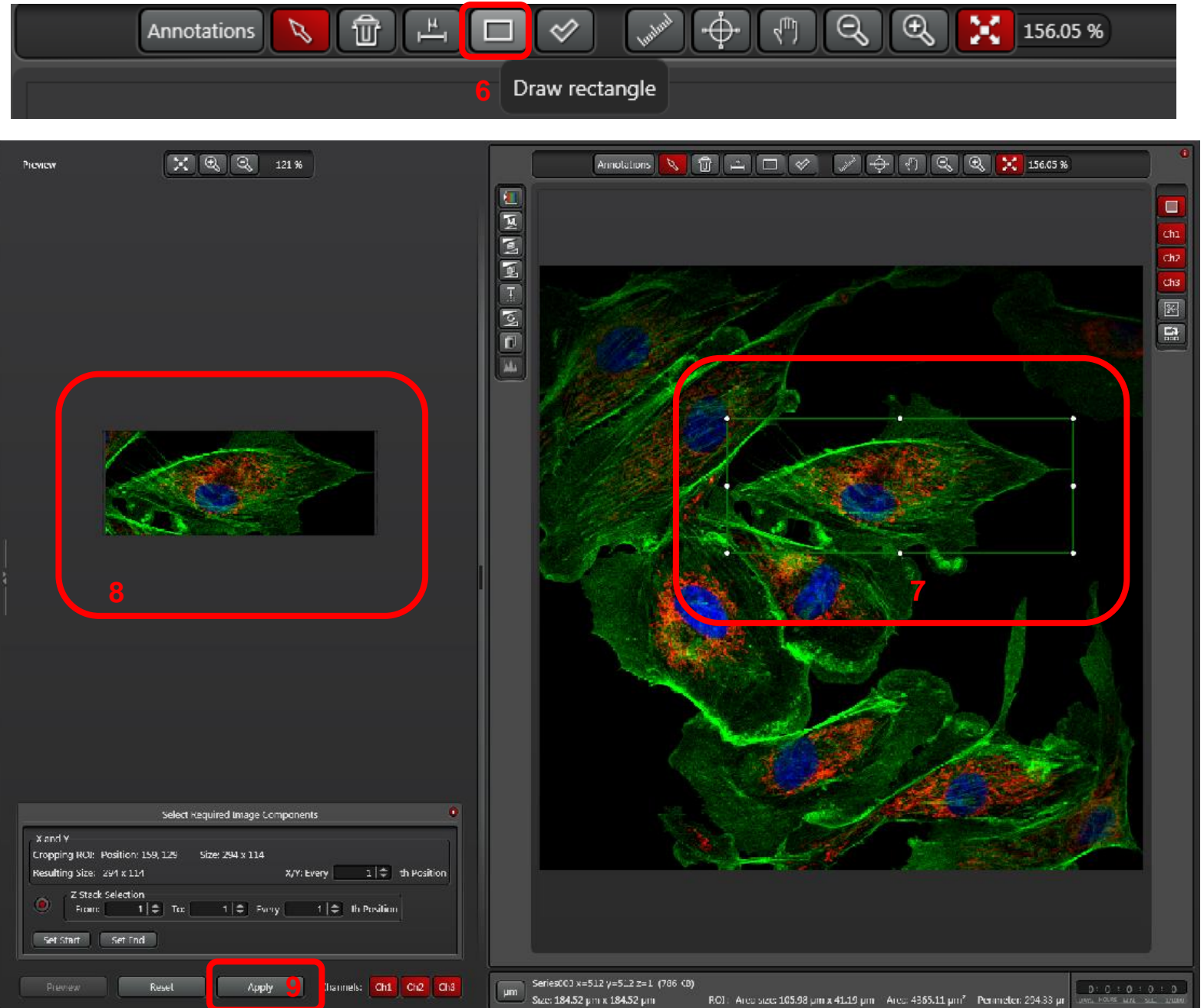
1. Select Open Projects tab.
2. Highlight the image you wish to crop.
3. Select ProcessTools.
4. Under the Edit section click on Crop.



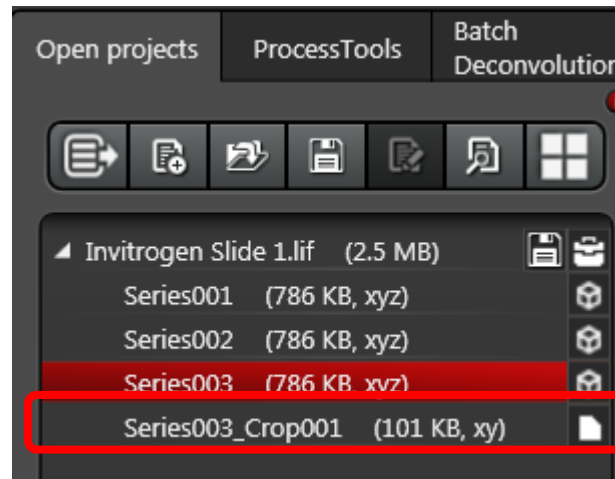
5. You will now see your image twice. In the Image window [right] and the Preview window [left].



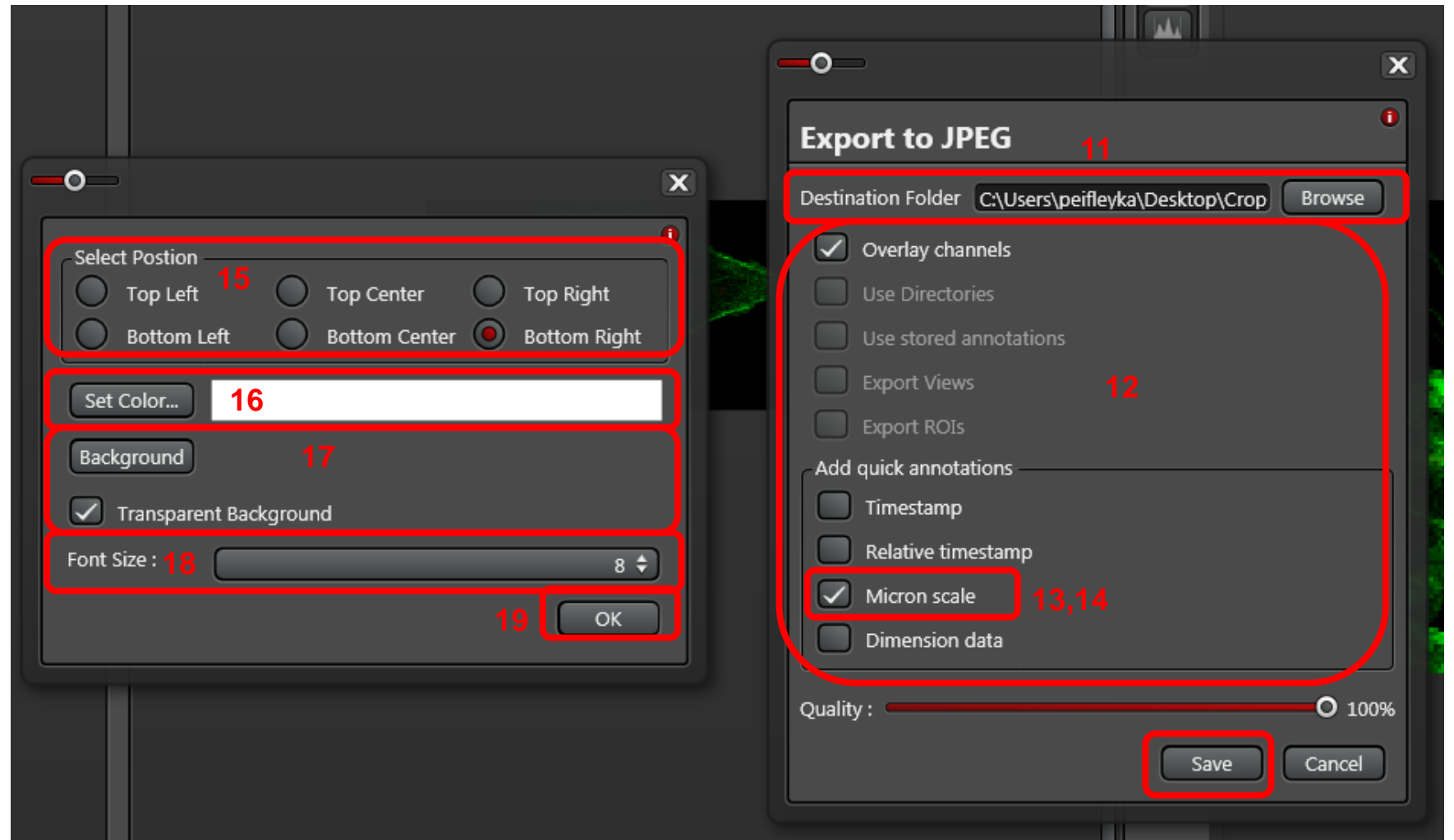
6. Click Draw rectangle button.
7. Draw the area you wish to crop.
8. You will now see the crop version in the Preview area on the left.
9. Click Apply



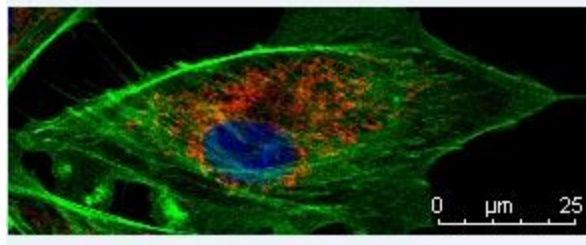
10. To export highlight and right click on crop image.



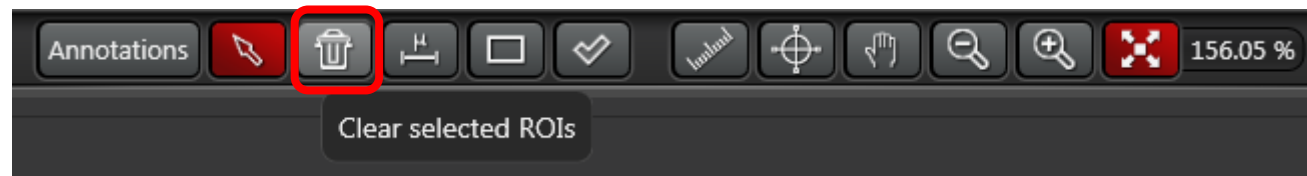
11. Select destination folder.
12. Select other options you may want.
13. If you want a scale bar check “Micron scale”
14. Double click on “Micron scale” to get options window.
15. Set Position of scale bar.
16. Select color of scale bar.
17. Select background color or check Transparent background.
18. Select Font size of scale bar.
19. Click OK.
20. This is your saved .jpeg image.



20

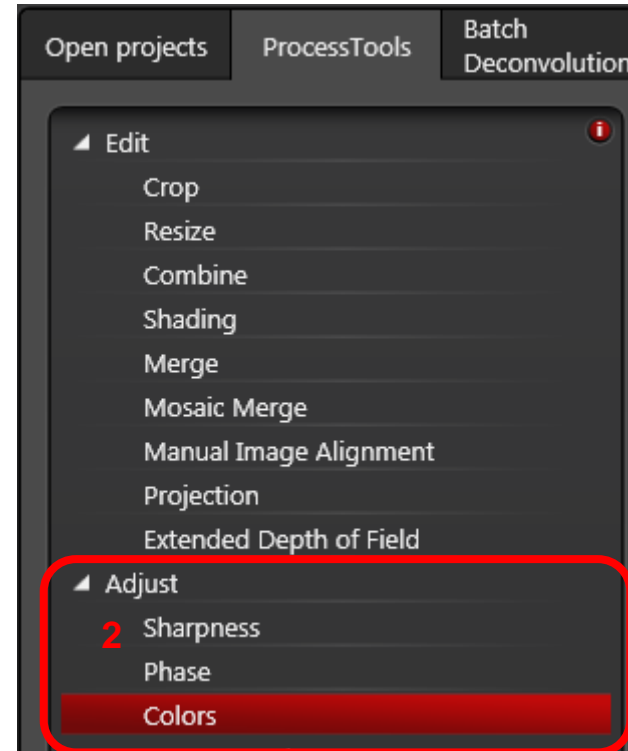
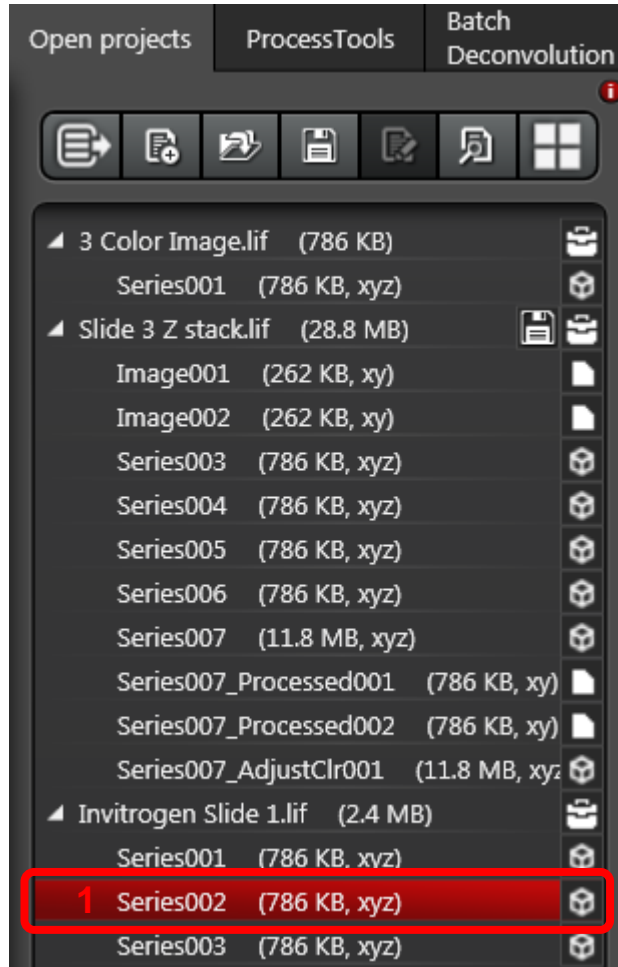


21. To clear the crop click on the Trash can icon.

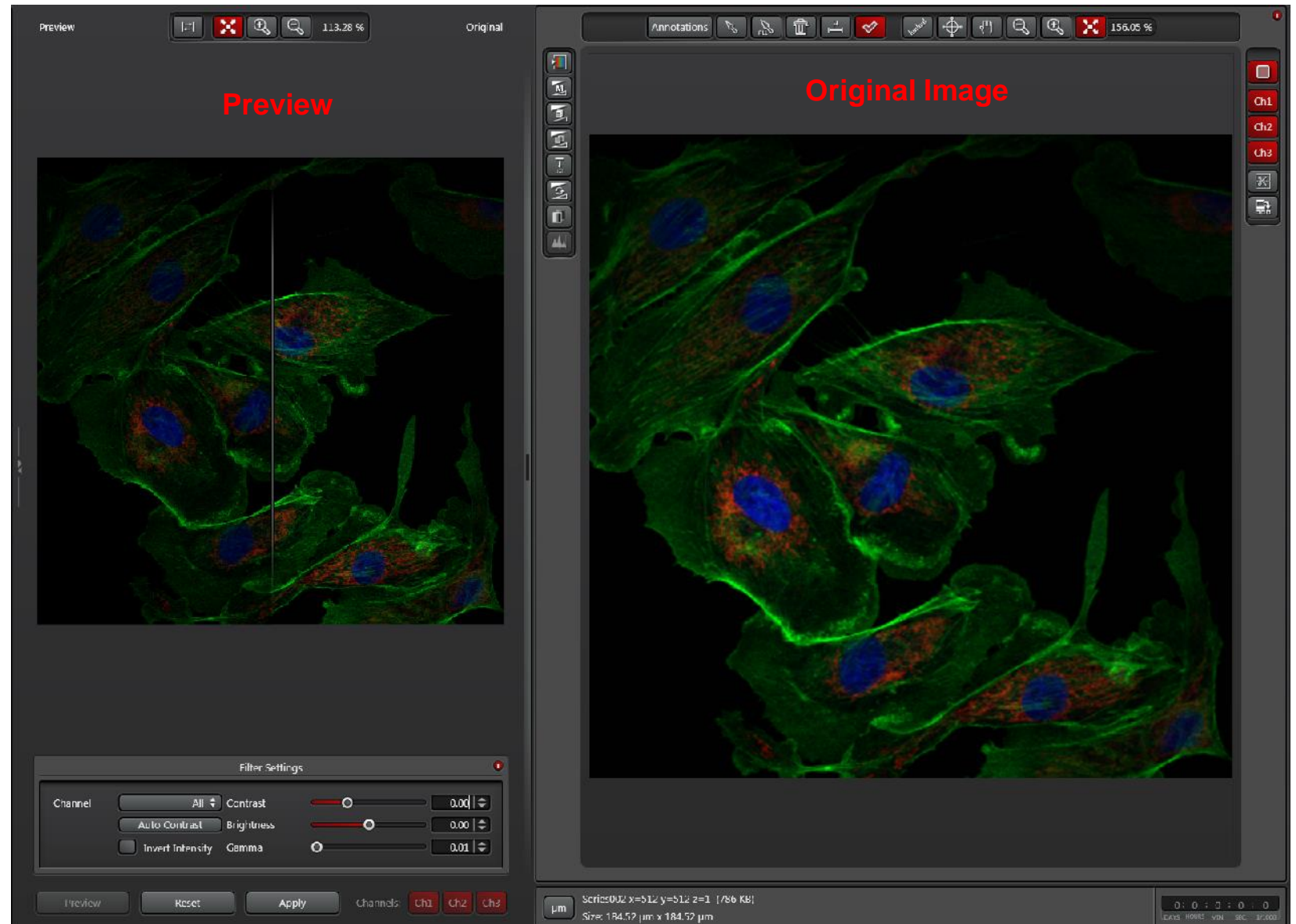


Adjust Colors

1. In Open projects highlight image you want to select it.
2. In ProcessTools under Adjust select Colors.



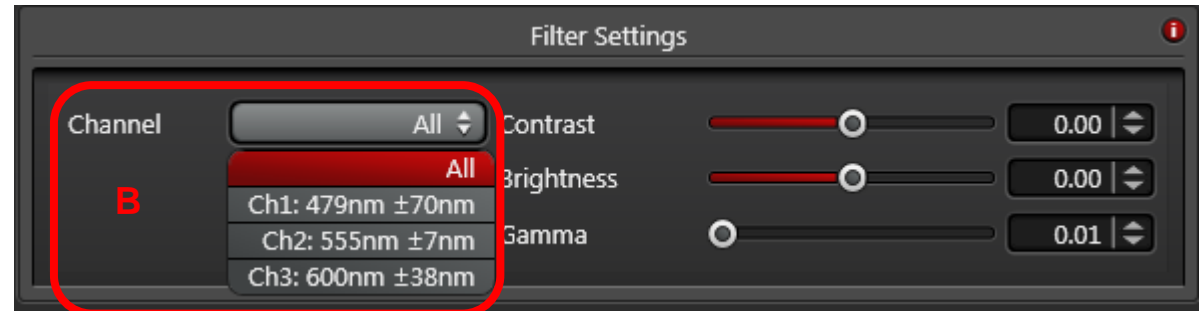
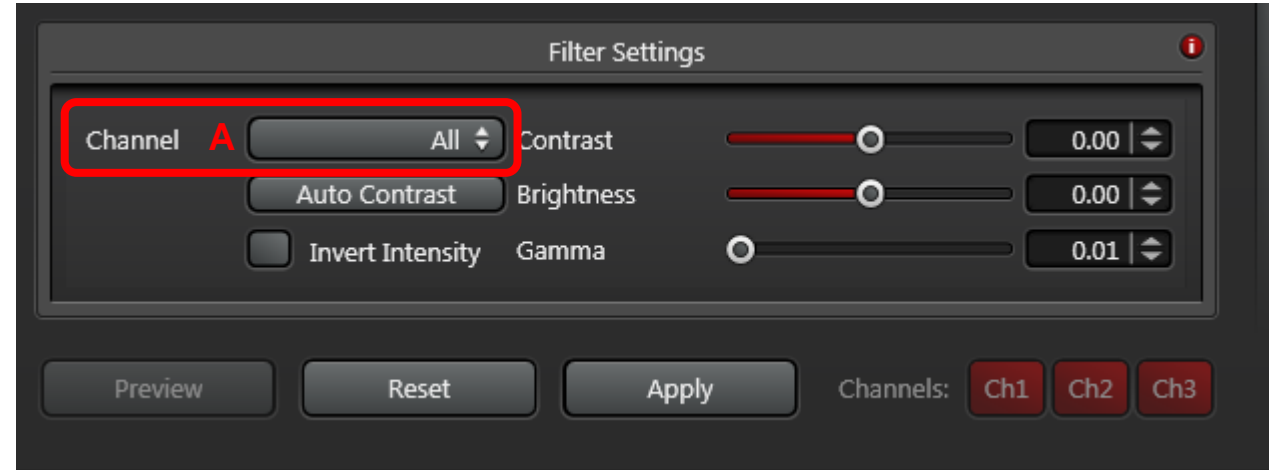
3. The left window is the preview window. The right window is the original image.



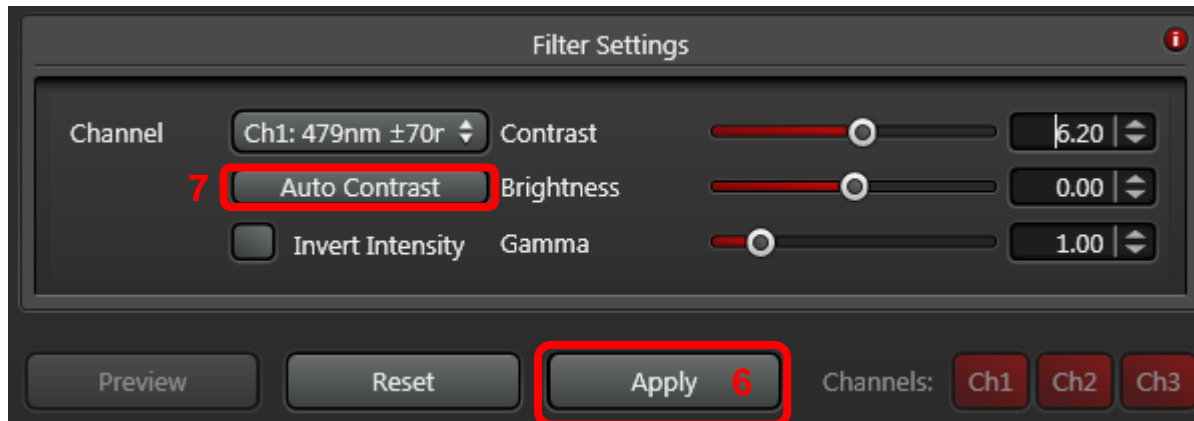
4. You can select the channel you want to contrast from the drop down menu or contrast all at same time.

A. Selecting the All channel allows you adjust all the channels at once.

B. The drop down menu allows you select individual channels to adjust.



5. When you adjust the filter settings you will see the adjustment on the left side and original on the right.
6. When everything is adjusted as you wish click Apply.
7. You could also click Auto Contrast.



8. The word `_AdjustClr001` and so on is added to the original file name to indicate contrasted image.

