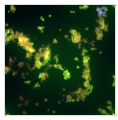

ImageJ Fluorescence Image Composition

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ABSTRACT

This protocol details how to use ImageJ software to create a composite image from fluorescence images taken with a microscope.

PROTOCOL STATUS

Working

We use this protocol in our group and it is working

MATERIALS TEXT

Computer

Fluorescence images

Get ImageJ

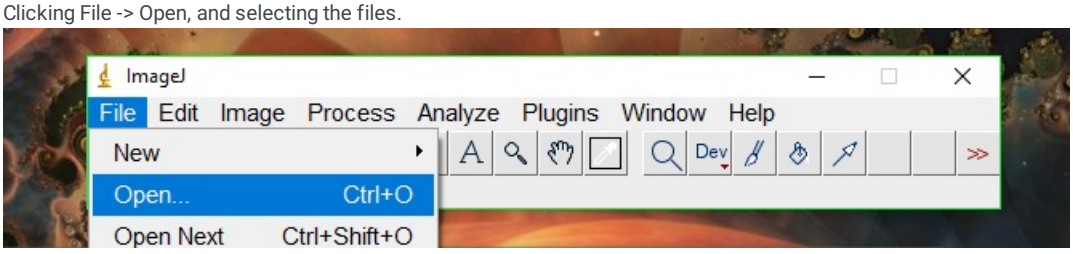
1 Go to https://imagej.nih.gov/ij/download.html and download the software corresponding to your operating system.

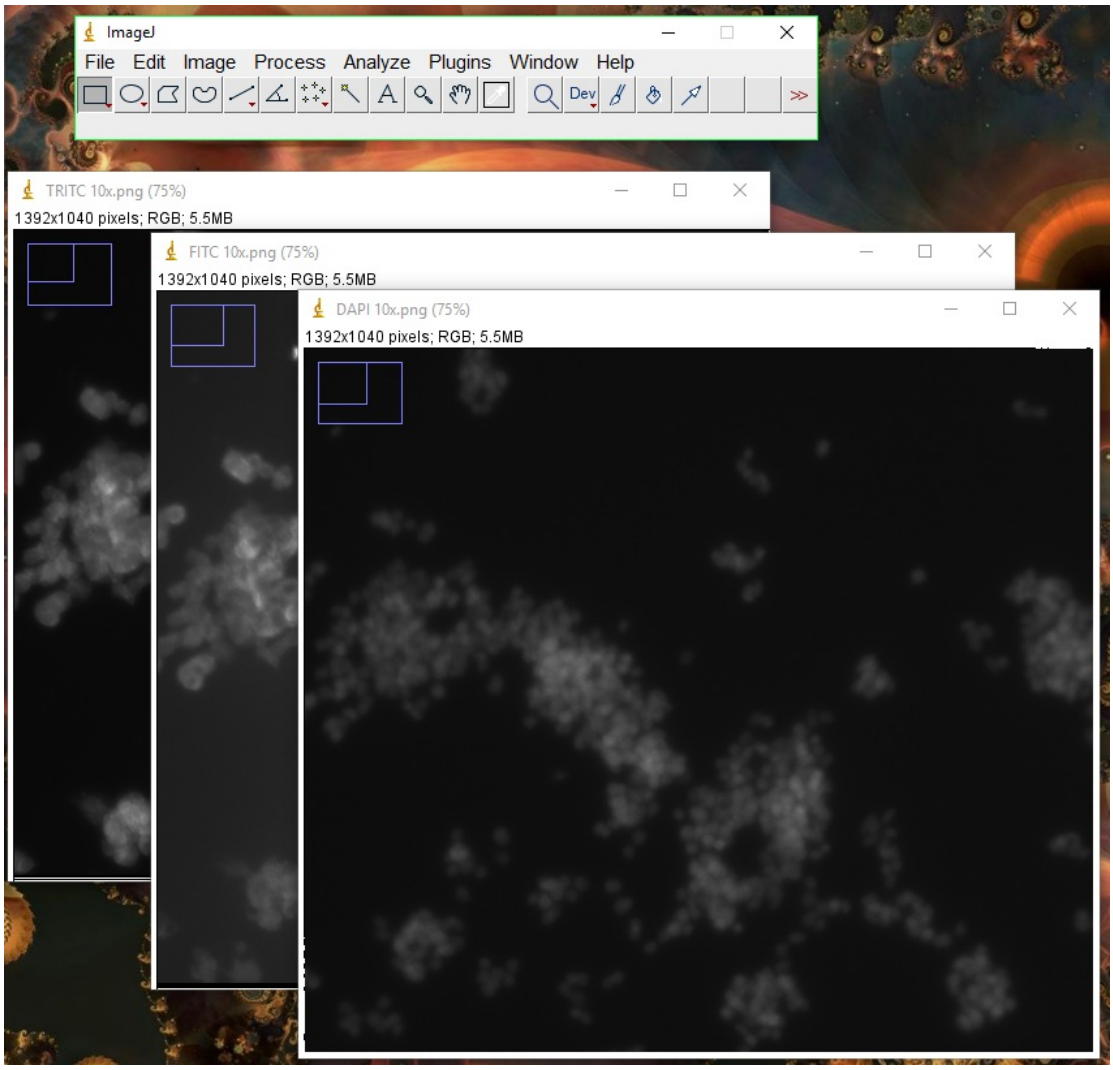
2 Sometimes ImageJ will be hard to find and not show up within your programs. You may have to manually go into your hard drive and open the software from where you downloaded it.

Open images

3 Open your images that are to be superimposed in ImageJ. This can be done by either:

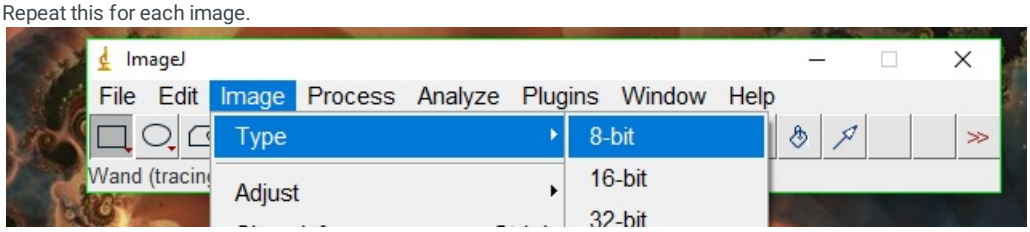
- Dragging the file onto the ImageJ window.
- Clicking File -> Open, and selecting the files.



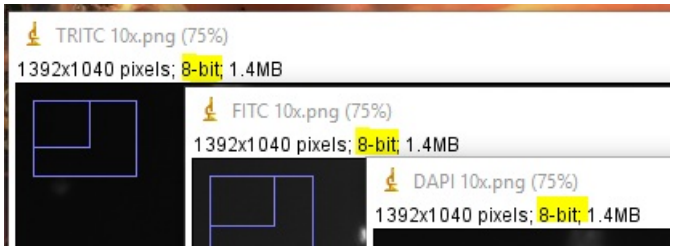


Convert images

4 Convert each image to 8-bit by clicking on the image, then going to Image -> Type -> 8-bit.
Repeat this for each image.



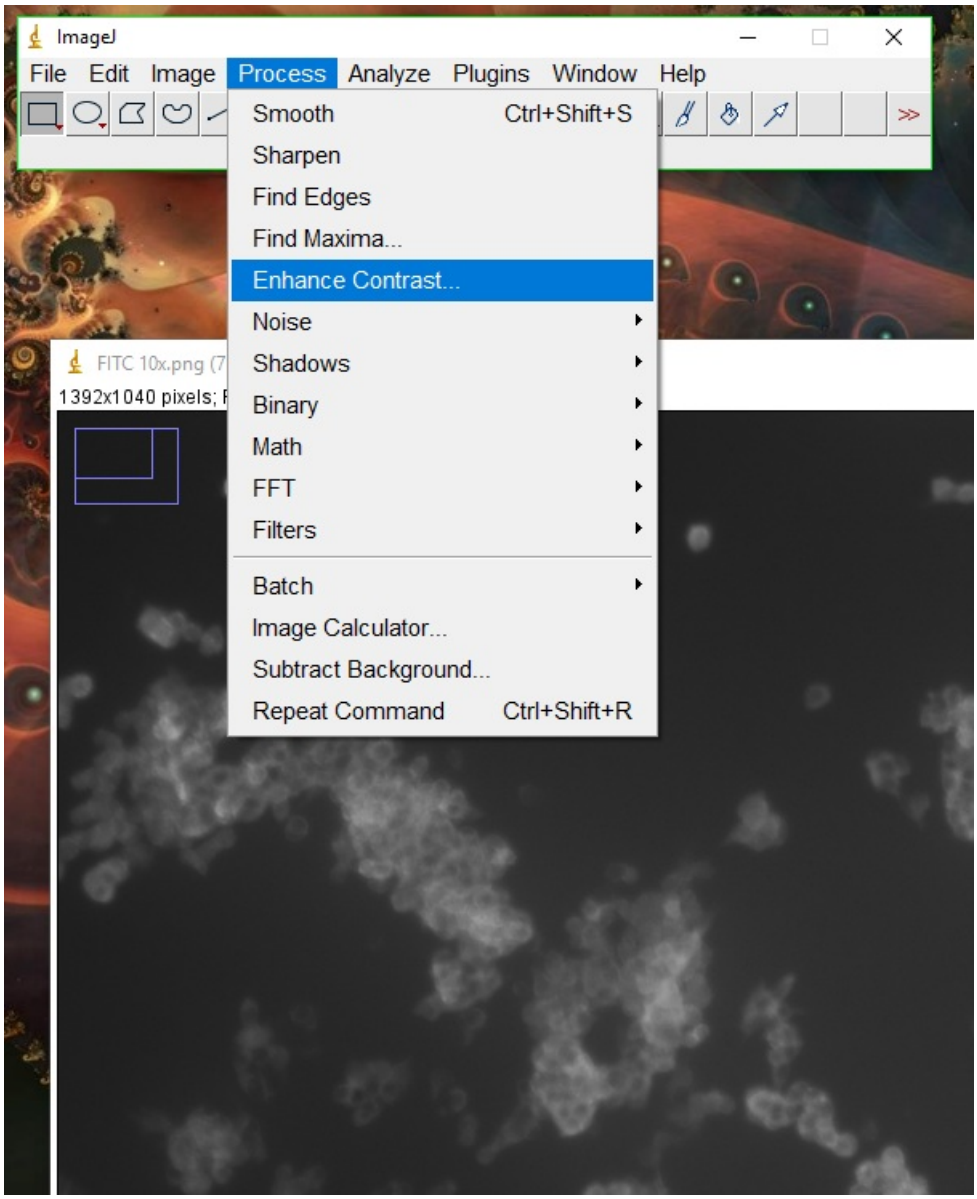
Changing image type to 8-bit



Make sure each image is now 8-bit. You can see where I have highlighted the image type.

5 Adjust the image to increase contrast, etc. An easy way to enhance the contrast without much effort is to select Process -> Enhance

Contrast...



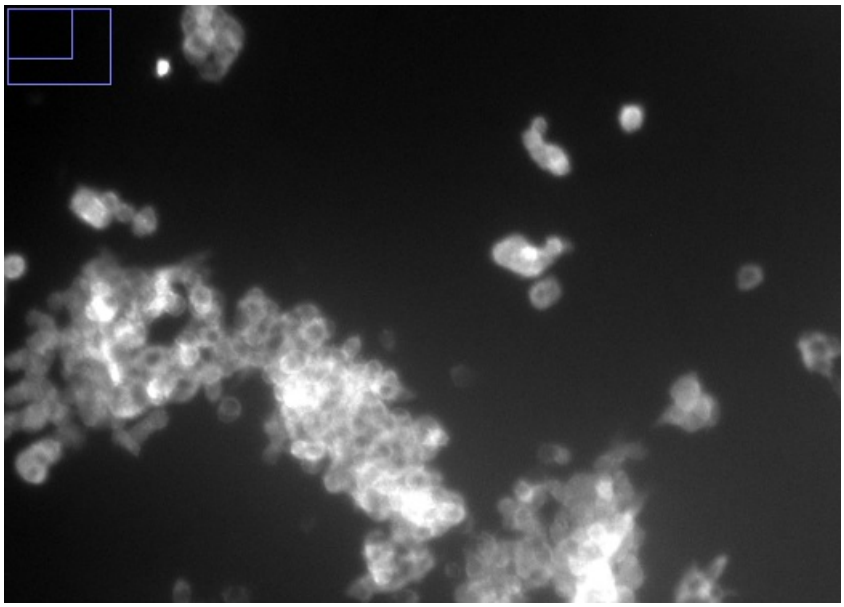
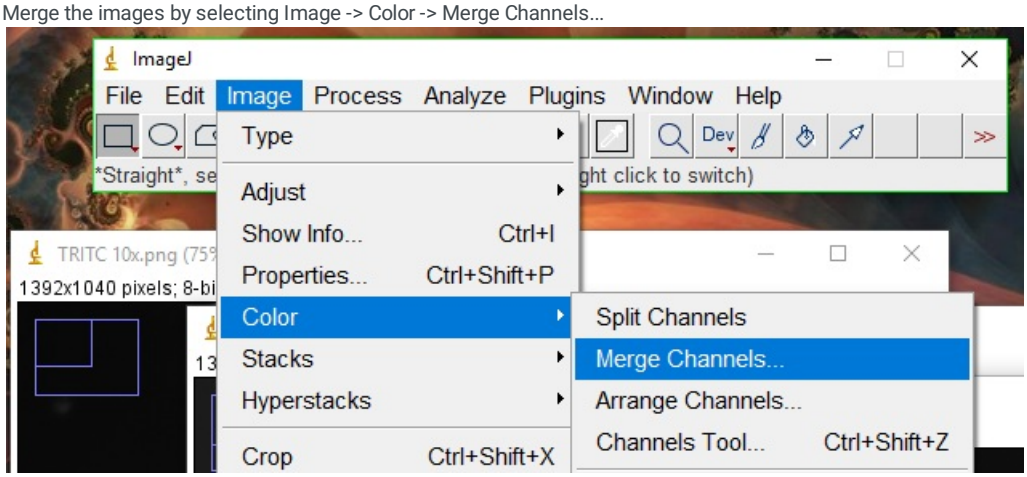


Image after enhanced contrast

Merge images

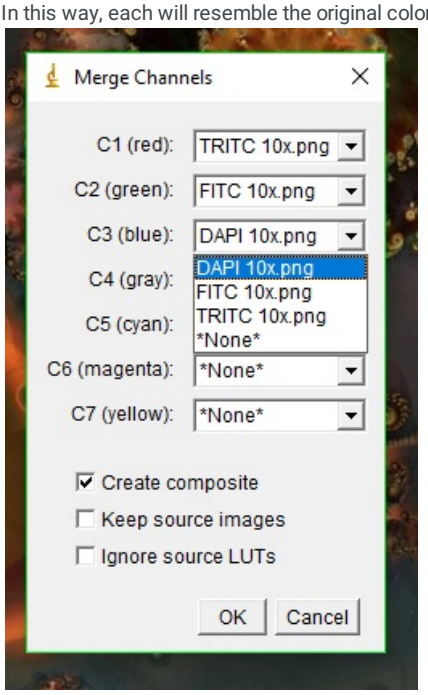
6 Merge the images by selecting Image -> Color -> Merge Channels...



7 For the various channel colors, choose the image from the drop down menu. Typically, we would do:

- Red: TRITC
- Green: FITC
- Blue: DAPI

In this way, each will resemble the original color image.



8 Press ok, and you will have a merged image.