FEB 20, 2019

ImageJ Fluorescence Image Composition

Kenneth Schackart¹, Kattika Kaarj¹

¹University of Arizona

481b Laboratory

Kenneth Schackart
University of Arizona

OPEN ACCESS

DOI:
dx.doi.org/10.17504/protocols.io.yc8fszw

Protocol Citation: Kenneth Schackart, Kattika Kaarj 2019. ImageJ Fluorescence Image Composition. **protocols.io** <https://dx.doi.org/10.17504/protocols.io.yc8fszw>

License: This is an open access protocol distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

Protocol status: Working
We use this protocol and it's working

Created: Feb 20, 2019

Last Modified: Feb 20, 2019

PROTOCOL integer ID:
20608

ABSTRACT

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

MATERIALS

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.