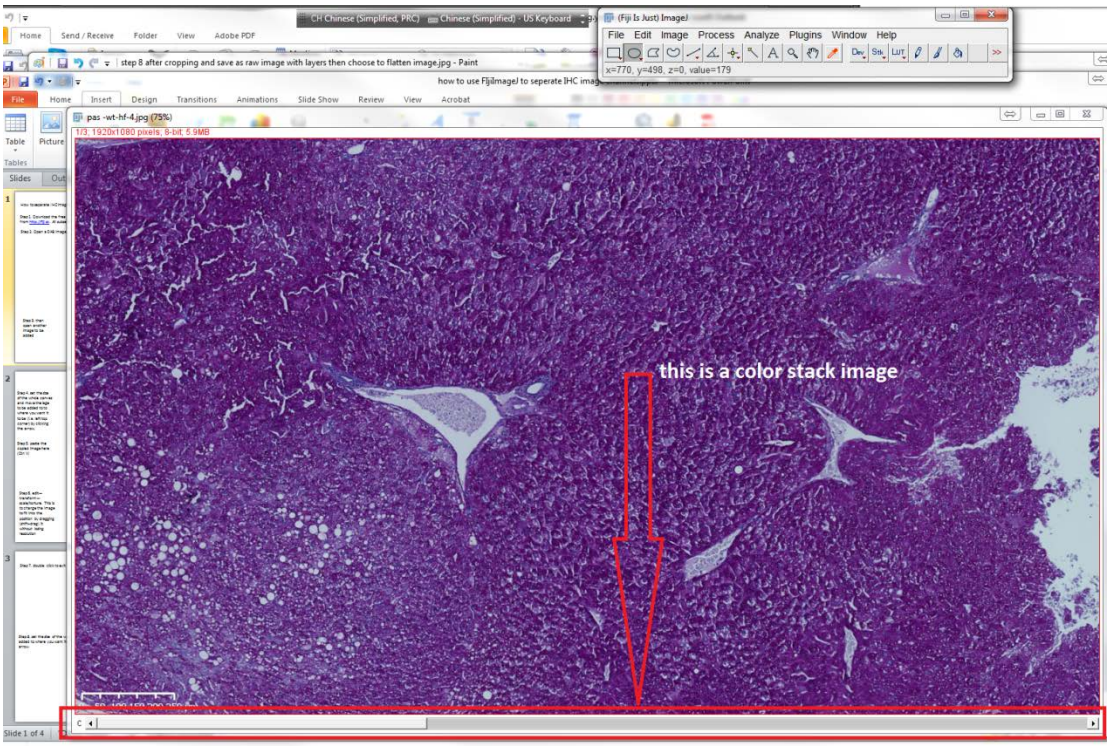
How to separate IHC images using color deconvolution in FIJI ImageJ

Step 1. Download the free "Fiji" version of ImageJ from http://fiji.sc. All subsequent steps are performed in Fiji

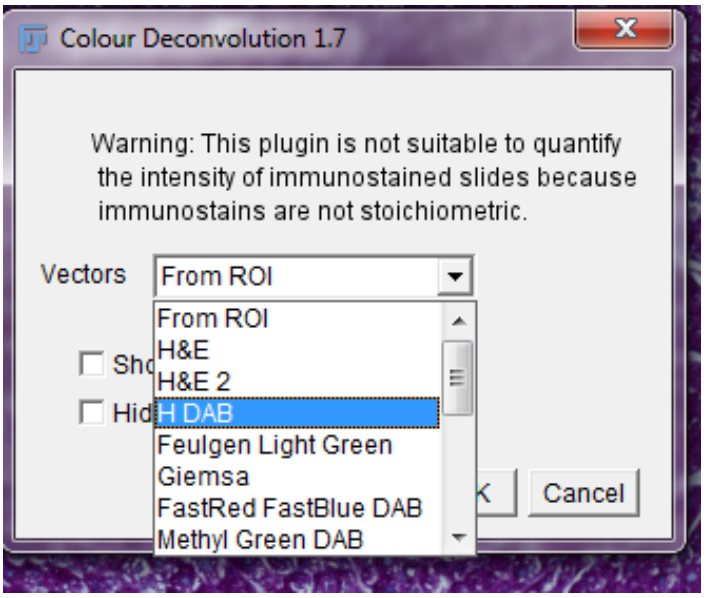
Step 2. Open a DAB image. If it is a stack image, then step 3 otherwise jump to step 4.



Step 3. run Image—color---stack to RGB image

Step 4. run Image > Color > Colour Deconvolution.

Step 5. From the Vectors pull-down, choose "H DAB" as the stain (this assumes your images are correctly white-balanced, otherwise you have to define your own colors by choosing "From ROI" for the vector then drawing your own Regions Of Interest to define the stain colors)

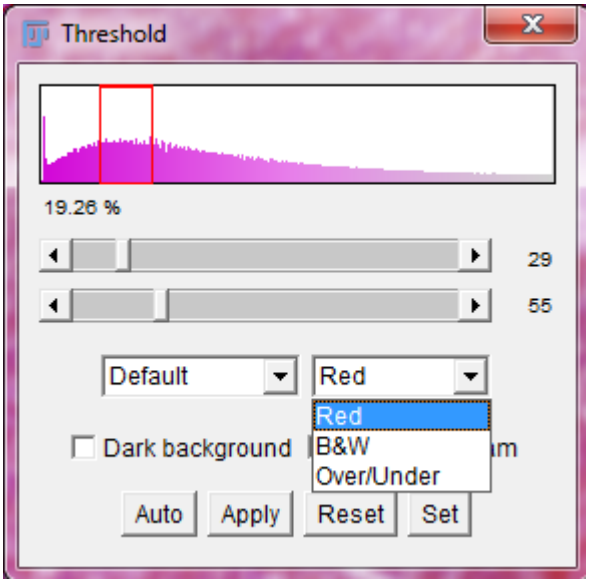


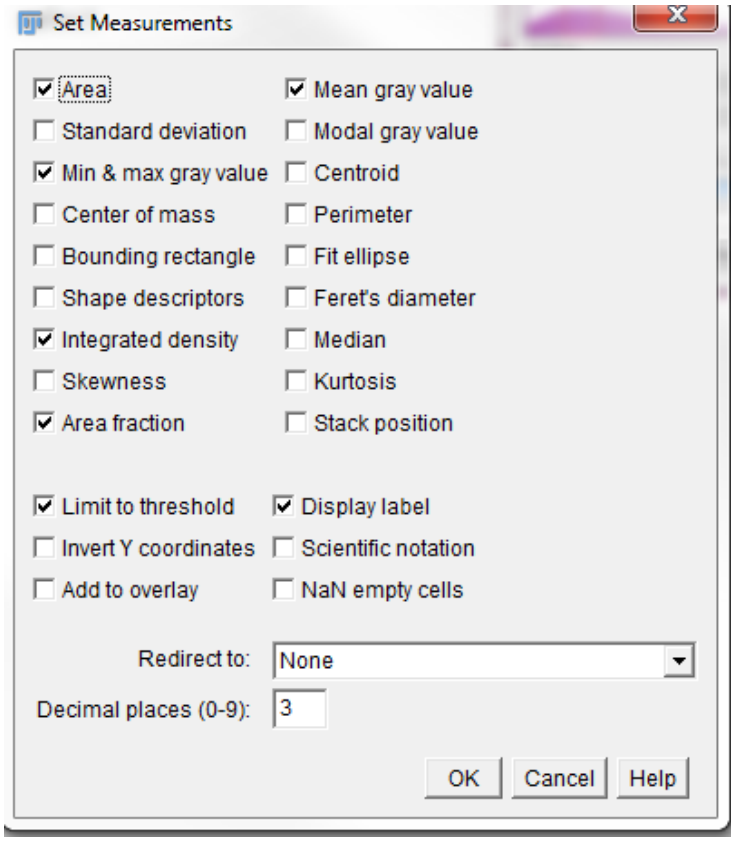
Step 6. Click OK in the Colour Deconvolution window, you will get three new images. The one with "Colour_2" in the title is the DAB image (Colour_1 is the hematoxylin image), you will quantify the Colour_2 image.

One way to measure: 6. Run Analyze > Set Measurements and select "Mean gray value" and "Display label".
7. Select the Colour_2 image window.
8. Run Analyze > Measure (or press Ctrl-m); a "Results" window will pop up with the quantification in units of intensity.
9. You need to convert the intensity numbers in the Results window to Opitcal Density (OD) numbers with the following formula:
OD = log(max intensity/Mean intensity), where max intensity = 255 for 8-bit images.
This will quantify the average darkness of the image due to DAB signal. If you have areas of the image without tissue it will bias your results because it will bring the average OD down. If that is the case we need to discuss further. Also, you may need to use Integrated Density rather than Mean

Step 7. another way to measure is to threshold and measure.

Note 1: “red” display way is much easier to see what region you select. Make sure that “Dark Background” is not checked.





Note 2: when set measurement, tick “limit to scale”, the area will then be limited to selected (thresholded) area rather than the whole image