

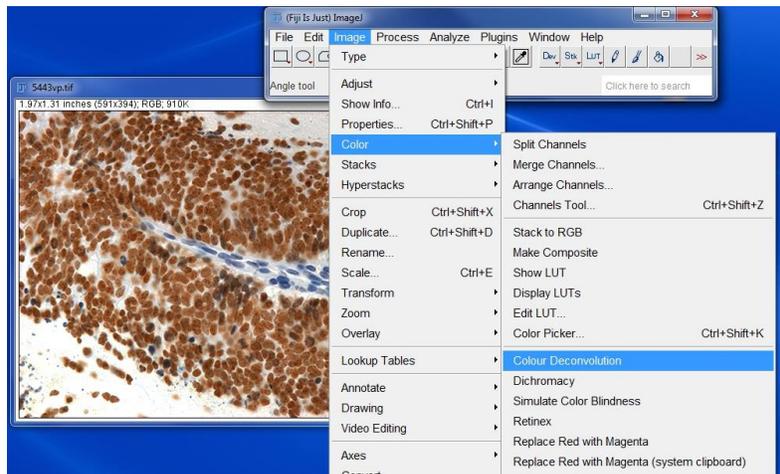
## Analyze Immunohistochemistry images in ImageJ

Please make sure you are using a newer version of **FIJI (Fiji is just Image J)**. FIJI download website: <https://fiji.sc/>

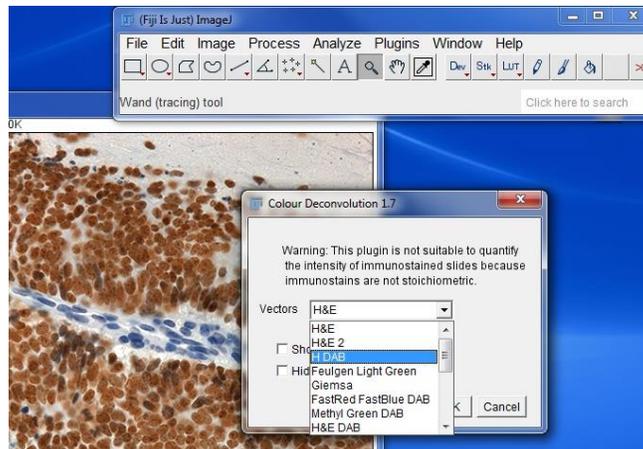
This user guide describes two ways to count cells (& measure area) through adjusting threshold. For manual count, use the point function .

### Threshold Method One

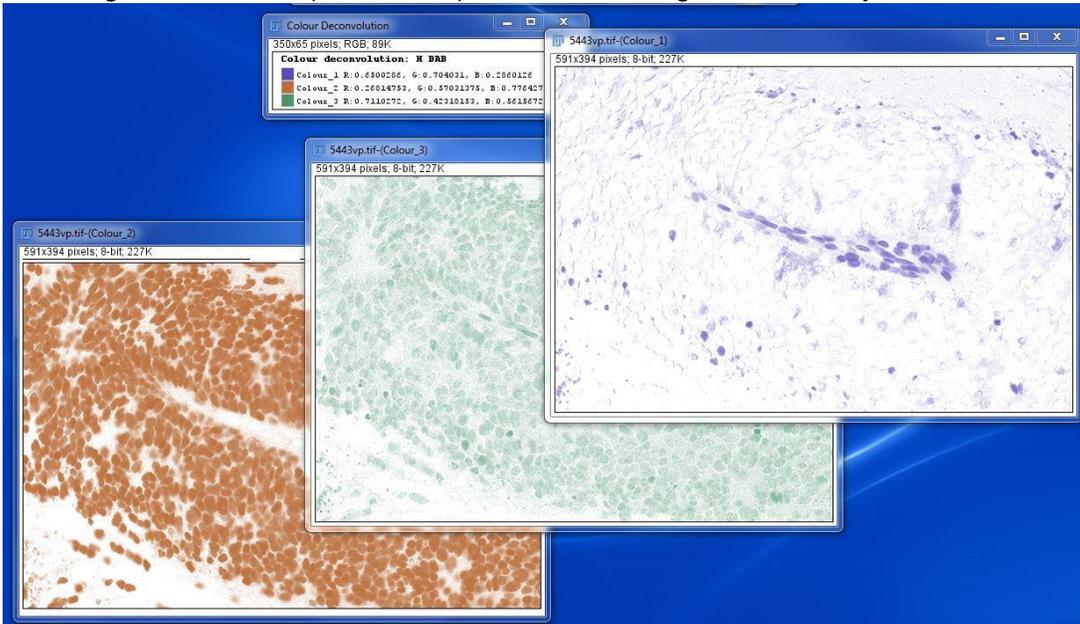
1. Open an IHC stained image through File→Open. (The image below is kindly provide by Dr. Xiuping Yu lab).
2. Click Image→Color→Colour Deconvolution.



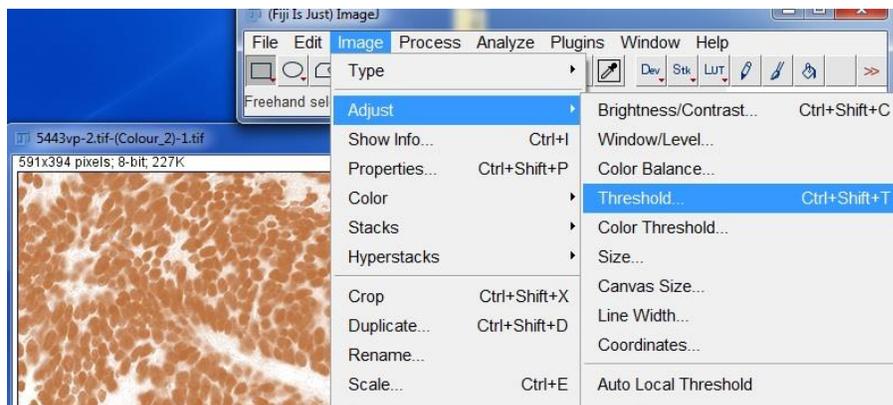
3. In the Colour Deconvolution window, select the type of staining of your image. Then click OK.



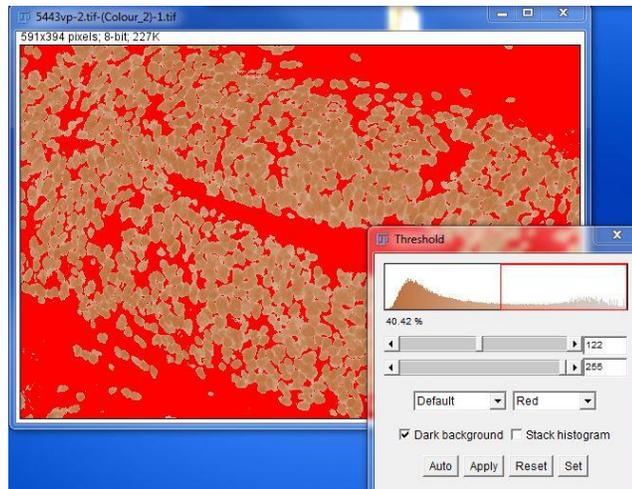
- The original image is then split into 3 single colored images. In the example provided below, only color 1 and color 2 are needed. Close the unwanted image. Save the other two images as Tiff files (File→Save). The saved images are 8-bit by default.



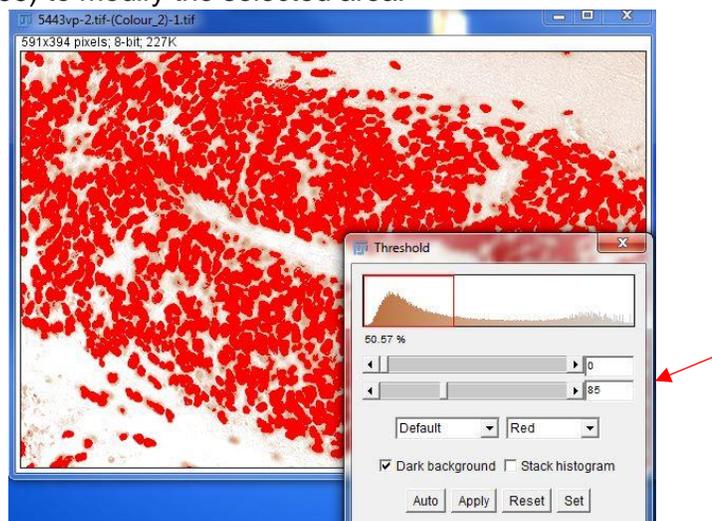
- Open one of the single colored images, go to Image→Adjust→Threshold. (Although it has color, this is not a RGB image).



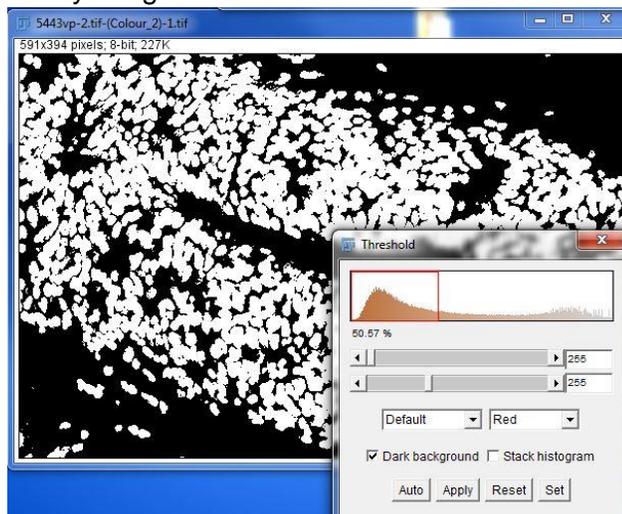
- In the Threshold window, pixels that are within the red box are being selected. Red color in the image indicates selected area. In the example below, the background is selected.



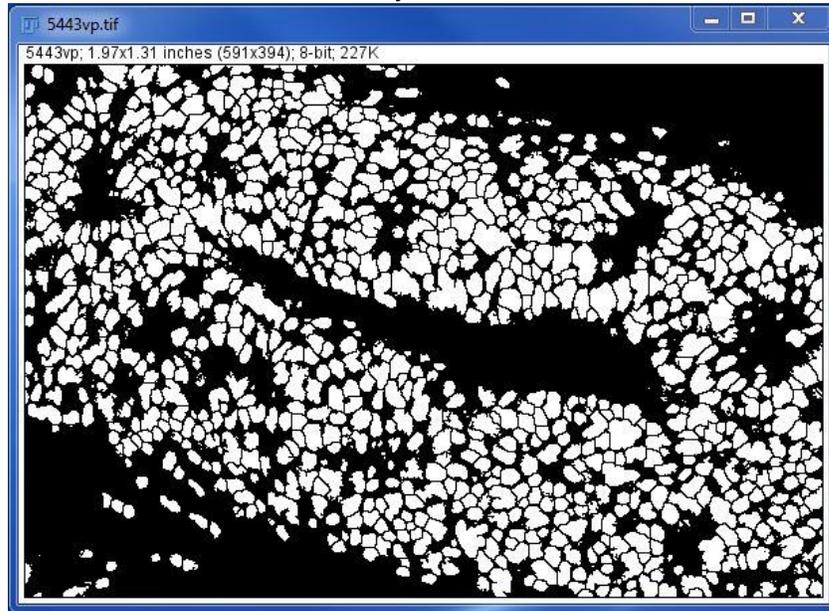
7. Drag the bars (0-255) to modify the selected area.



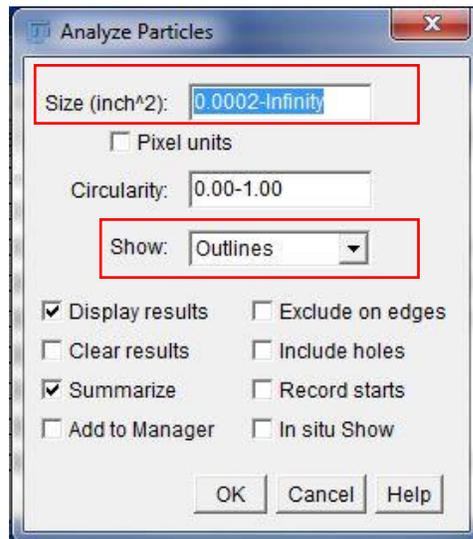
8. Then click Apply. A Binary image is created.



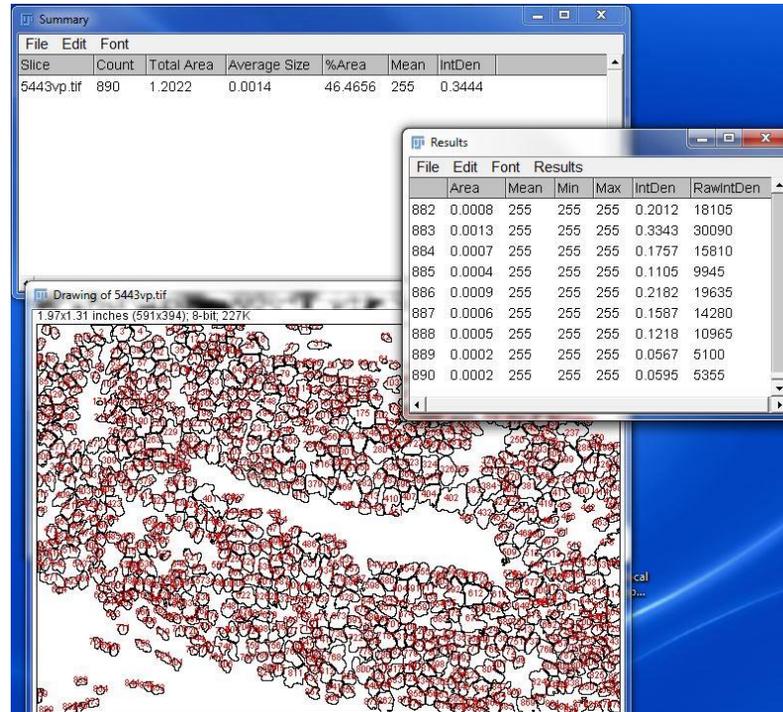
9. Click Process→ Binary→ Watershed. Imagined separation lines among cells will appear. The watershed function is less accurate if your cells are too crowded.



10. Next, click Analyze→Analyze Particles. In the Analyze Particles window, the size of particles is important if you have tiny dots that are selected within the threshold, but they are not cells. Set the minimum particle size to exclude them. In the example below, 0.0002 inch<sup>2</sup> is used (do the measurement for your image). It is better to select Outline in the Show option. Then click OK.

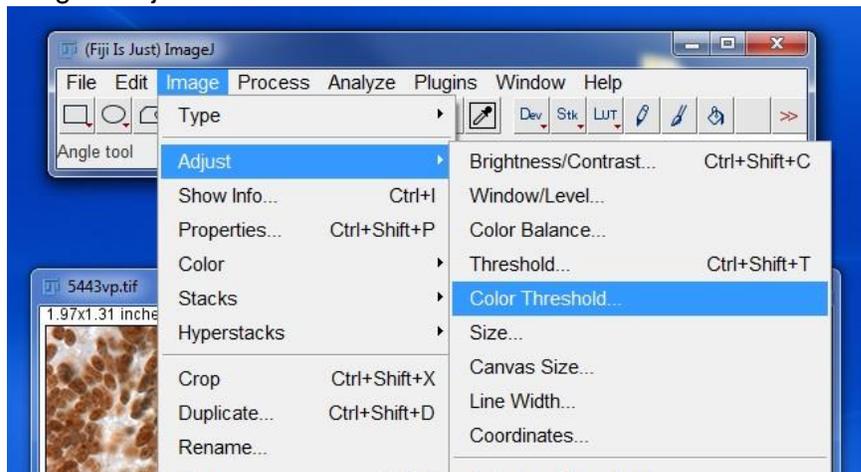


11. For results, you will see 3 windows as shown below. In the counting map, you can clearly see which cells are being counted. The unwanted dots are eliminated.

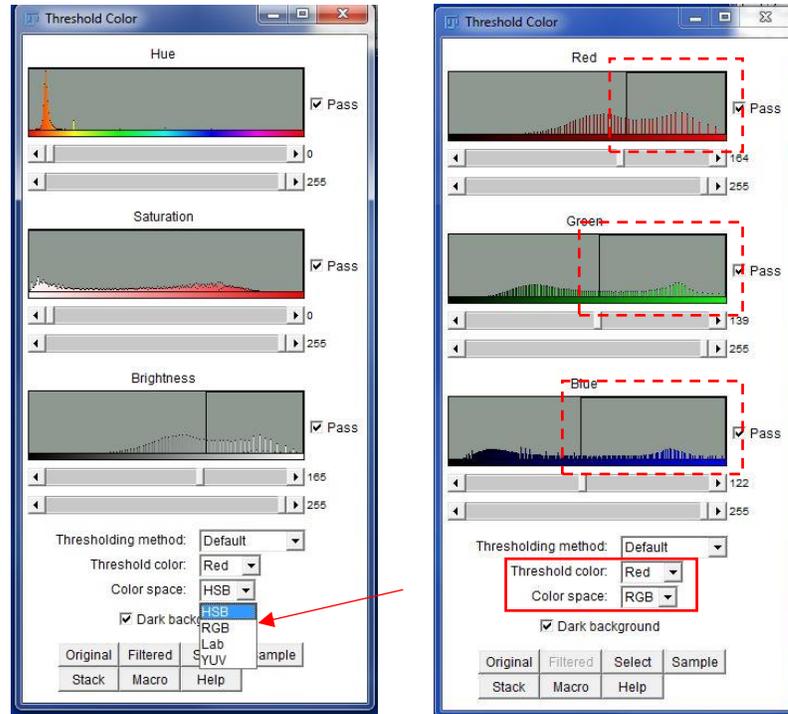


### Threshold Method two

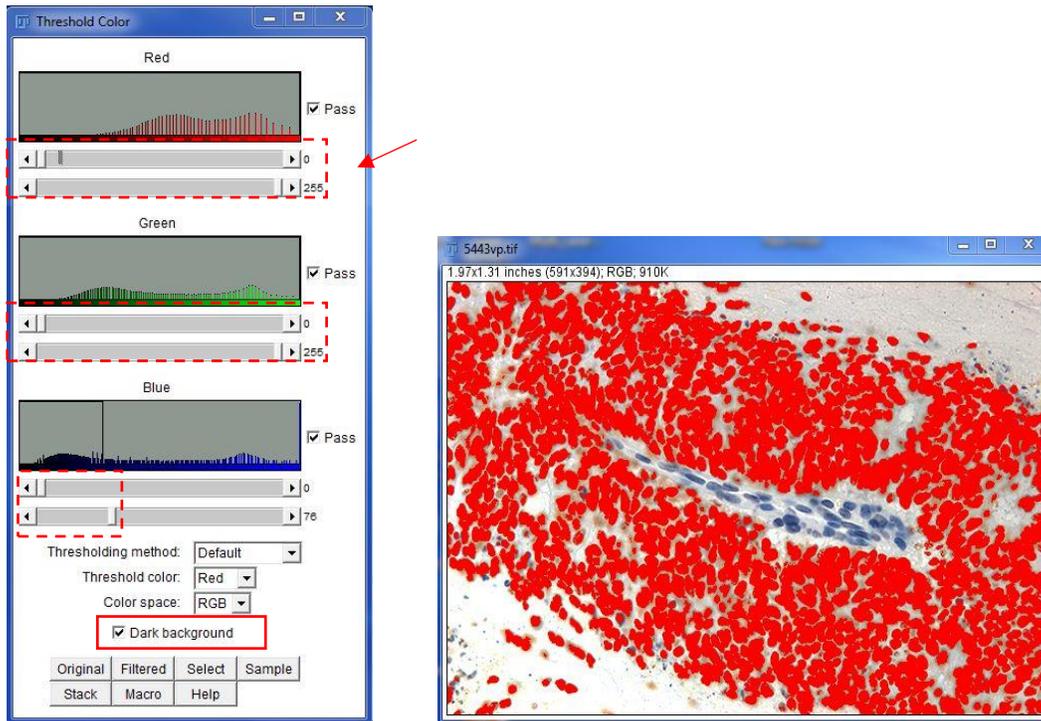
1. Open an image through File→Open.
2. Then click Image→Adjust→Color Threshold.



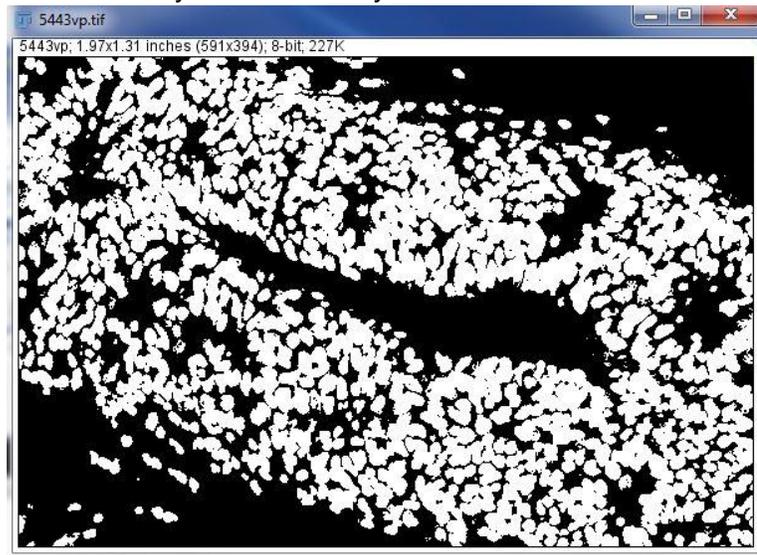
3. In the Color Threshold window, you can adjust the threshold through RGB or HSB mode. In the example below, RGB mode is used (For other images, it may be easier to use the HSB mode).



4. Drag the bars (0-255 grey scale) under each color to modify the selection. In the image below, brown staining is selected. To select brown, allow more red and green, and less blue to pass. Select Dark background.



5. Next, go to Process→ Binary→ Make Binary.



6. After a Binary image is created, follow steps 10-12 as in [Method One](#).