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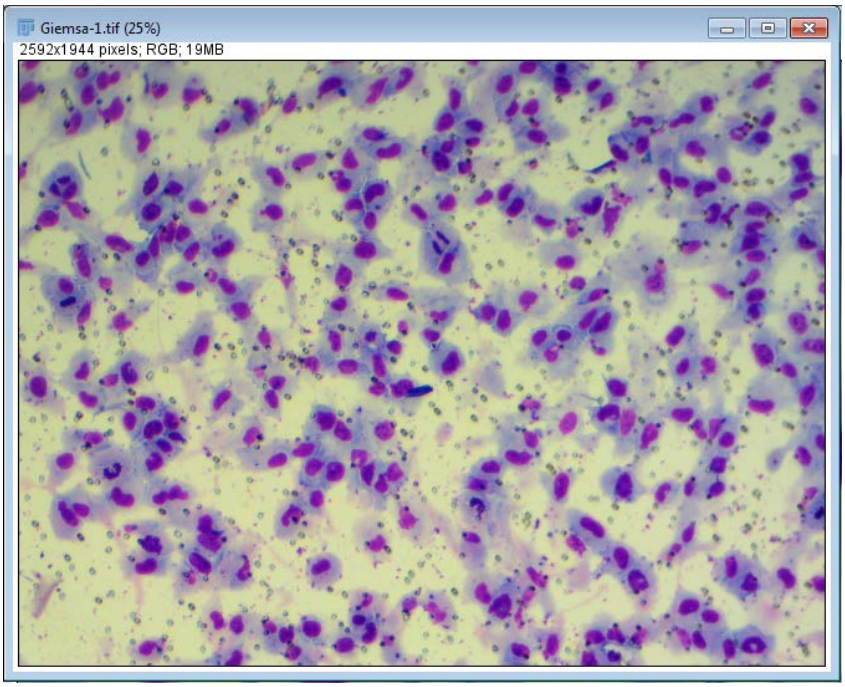
MICROSCOPY NEW ZEALAND INC. CONFERENCE WORKSHOP 2017

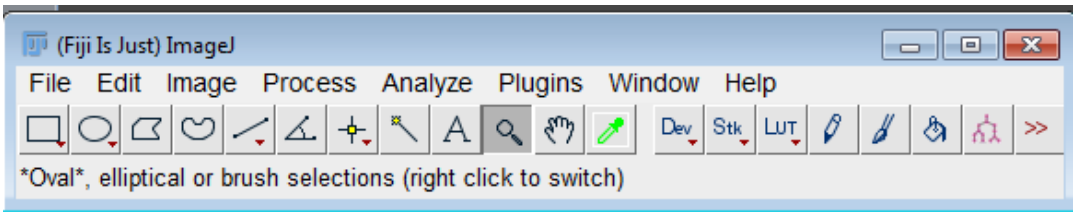
Using Threshold Colour for segmentation in Fiji 31 January 2017

This option of segmenting images based on colours can be useful for segmenting histological stains based on hue where the colours do not separate well using the standard channel split method or where the **Colour Deconvolution** plugin is not working well.

1. Open the image called **Giemsa.tif**;

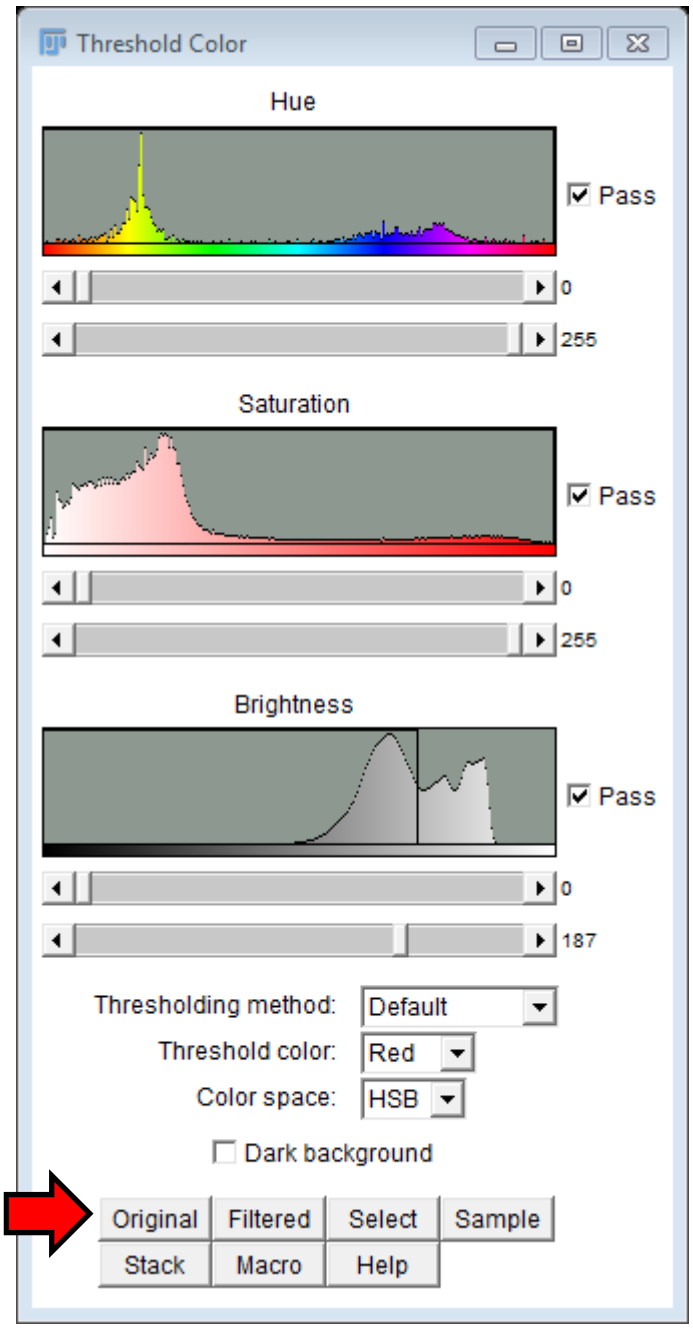


2. Zoom up on the image using the **Magnifying Tool**. Choose a location where you can see the two stain hues quite well, i.e. dark purple/blue.



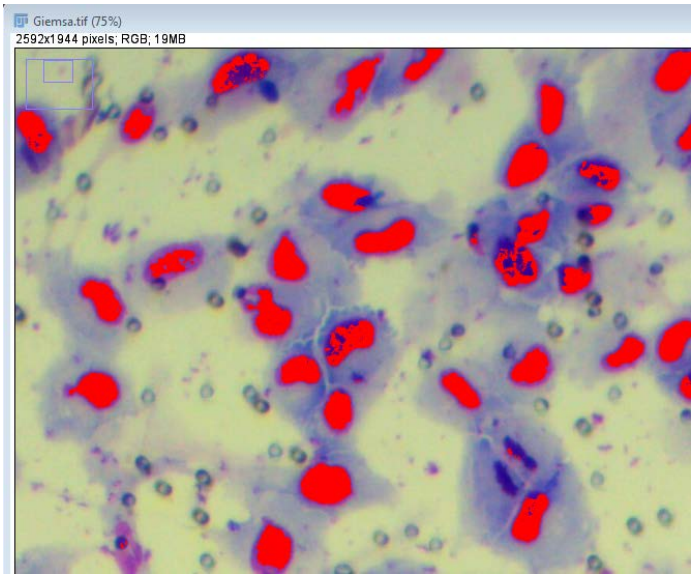
3. Select the **Rectangle Tool**.

4. Go to ***Image –Adjust - Color Threshold***;

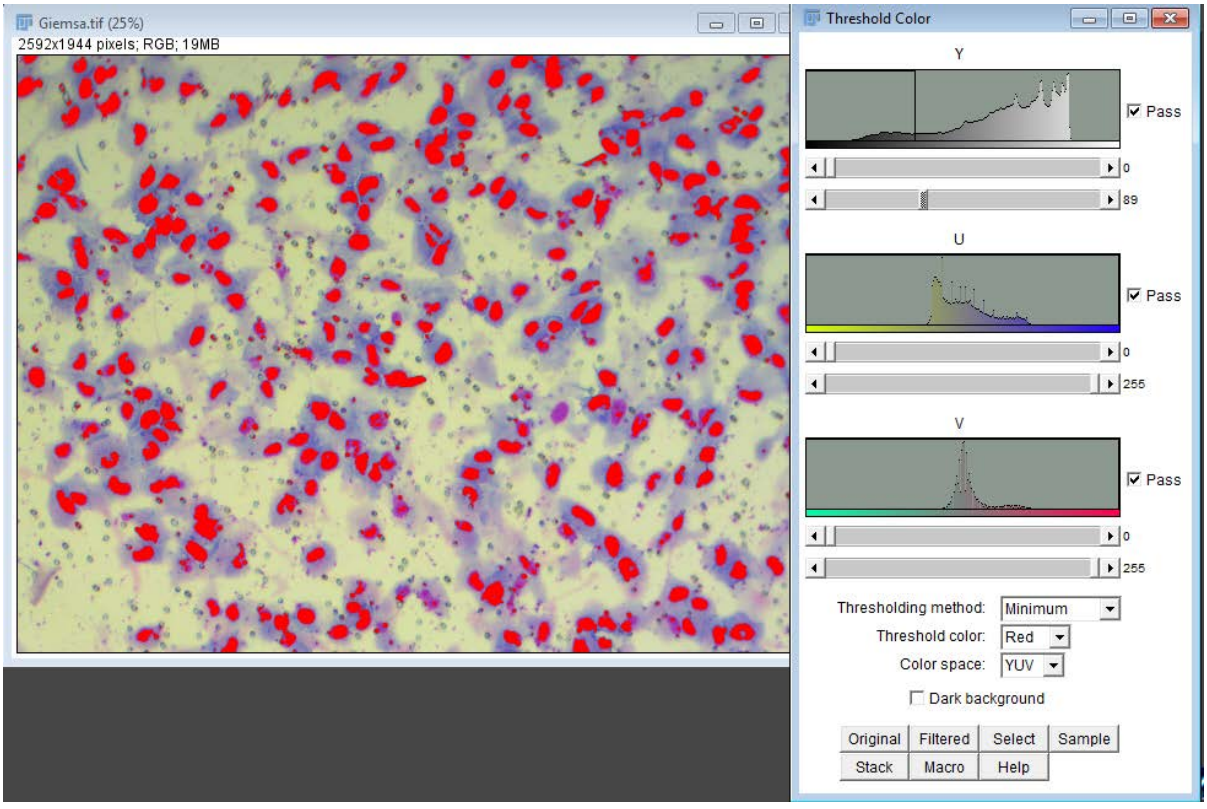


5. Turn off **Dark background** if it is selected and click on the **Original** button to remove the automatic threshold and then draw a **Region of Interest** over the stain you want to segment, e.g. the dark purple nuclei. Note that you have the same thresholding algorithms that are available for grayscale images.
6. The default setting for the **Color space** is **HSB** – **Hue**, **Saturation**, **Brightness**.

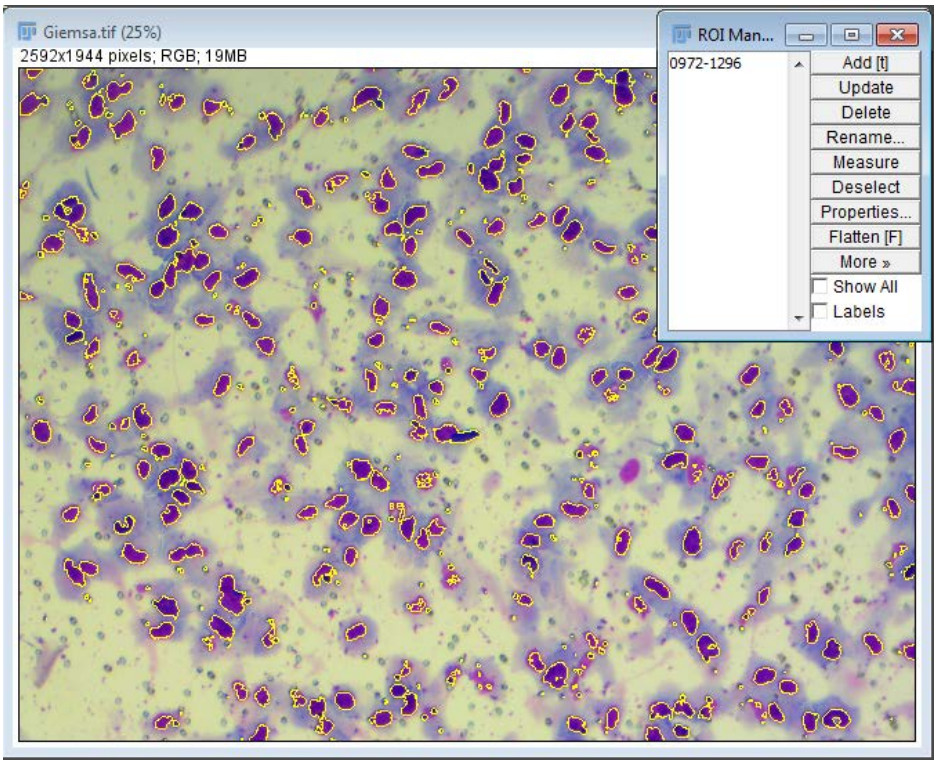
8. Click the **Sample** button;



9. Try altering the sliders to include a wider range or changing the **Thresholding method**.
10. You can also try a different **Color space**, e.g. **YUV** as below. This works quite well to select almost all of the nuclei although you can see a couple of them are not selected. The blue pores are not selected.



12. You can create a selection from the threshold by clicking **Select**. This can be measured directly or added into the **ROI Manager**. You can also run the **Color Threshold** on a stack or call it in a **Macro**.



13. Once you are satisfied by the thresholding, you can go to ***Process – Make Binary*** to create a binary image and then continue with making measurements, e.g. **Analyze – Set Measurements** followed by **Analyze – Measure** or **Analyze – Analyze Particles**.
14. Make sure that you calibrate the image before making any measurements unless you are only counting objects.

Acknowledgement – image kindly supplied by Stacey D'Mello, Auckand Cancer Society Research Laboratories