

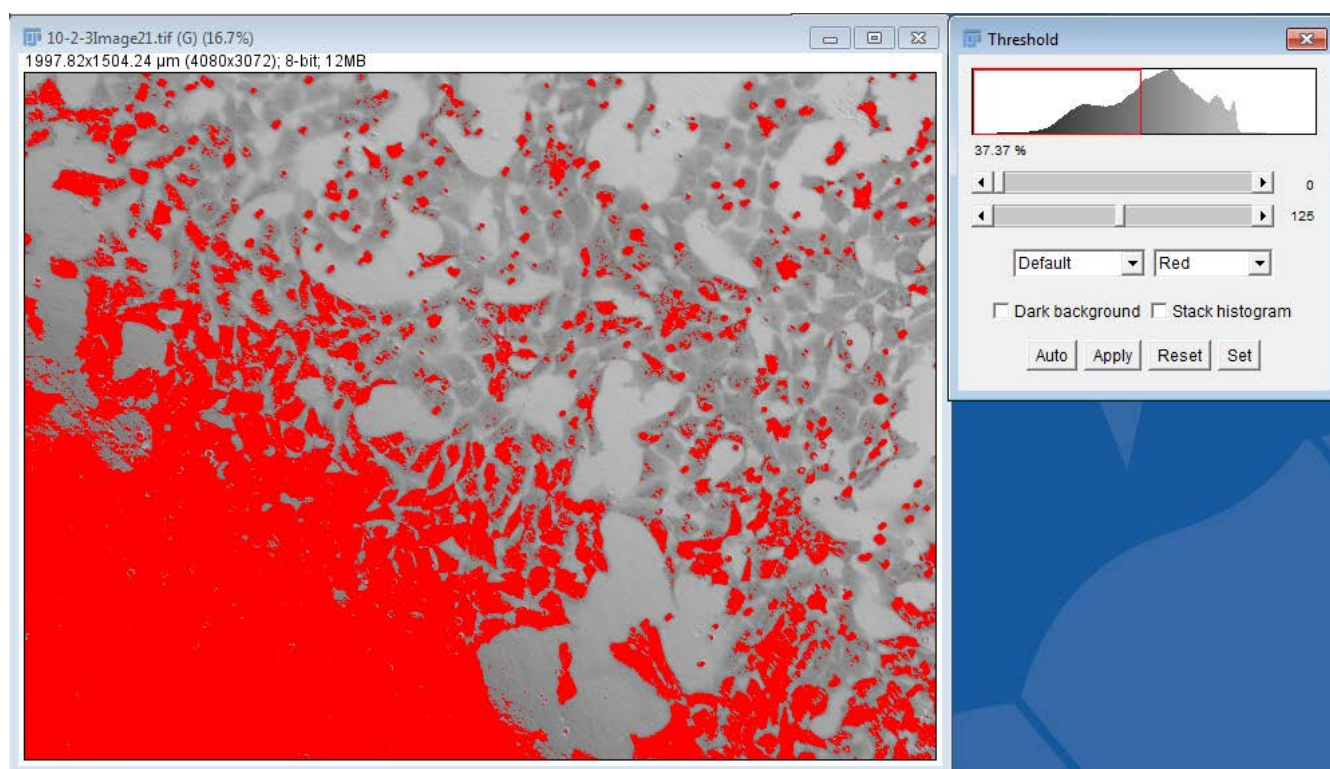
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## MICROSCOPY NEW ZEALAND INC. CONFERENCE WORKSHOP 2017

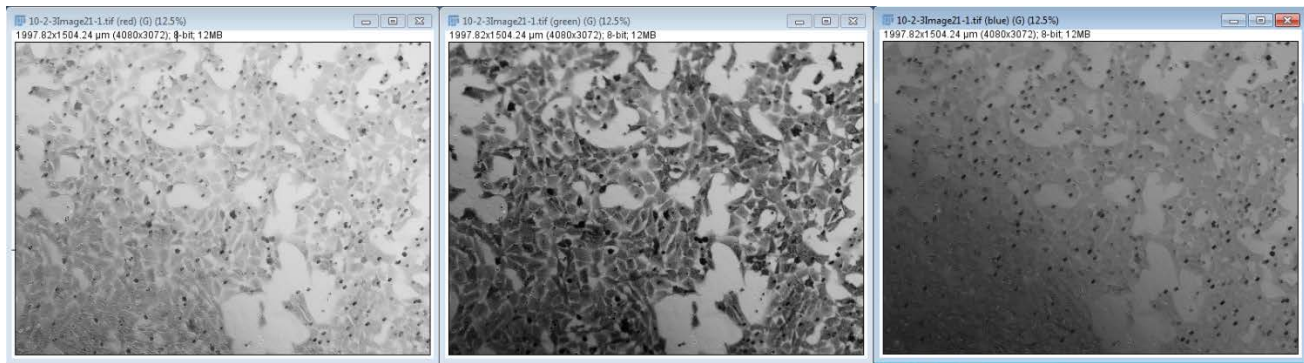
### Measuring area of brightfield images in Fiji

31 January 2017

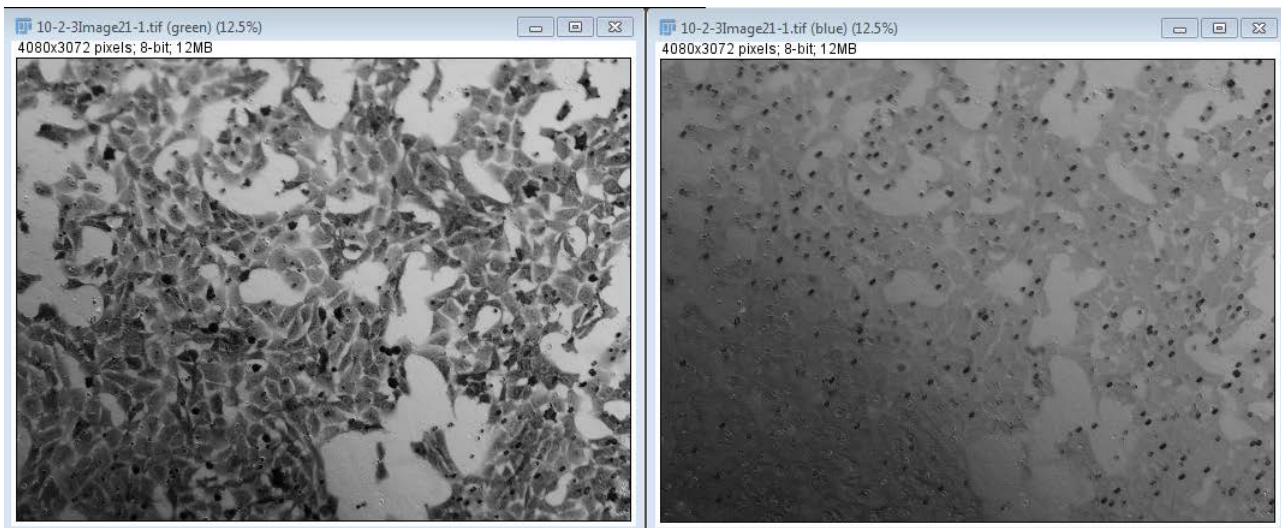
1. Open the image called **10-2-3Image21.tif**.
2. Note that the background areas do not look neutral grey. This could be improved by adjusting the colour balance (**Image – Adjust – Color Balance**). In this case it doesn't matter because we will be working in grayscale for the analysis as it is one colour only and we are interested in measuring the area covered by the cells (migration study).
3. Duplicate the image and convert the copy to 8bit (**Image – Type – 8-bit**).
4. Go to **Image – Adjust threshold**. Deselect **Dark background**. Note that the bottom left-hand corner of the image is darker than the rest of the image. This will cause problems for the analysis.



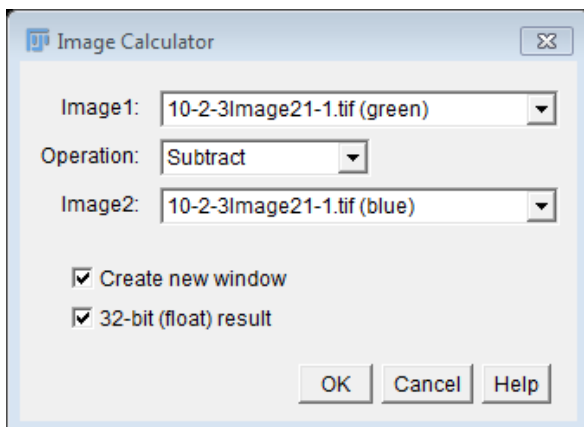
5. Go to the original image and split the channels by going to **Image – Color – Split Channels**. The channel with the best contrast is the green channel. Note that there are a lot of small dark blobs in the **Blue** and **Red** image. These are pores in the filter so we don't want to measure these. These are also seen in the image that we converted directly to 8-bit.



6. Close the **Red** channel image. We will use the **Blue** image to represent the pores.



7. Go to **Process – Image Calculator**. Select the **Green** image for **Image1** and the **Blue** image for **Image 2**. Select **Subtract** for the **Operation**, **Create new window** and **32-bit (float) result**. The 32-bit format is good for mathematical operations as it allows decimal places, not just integers.

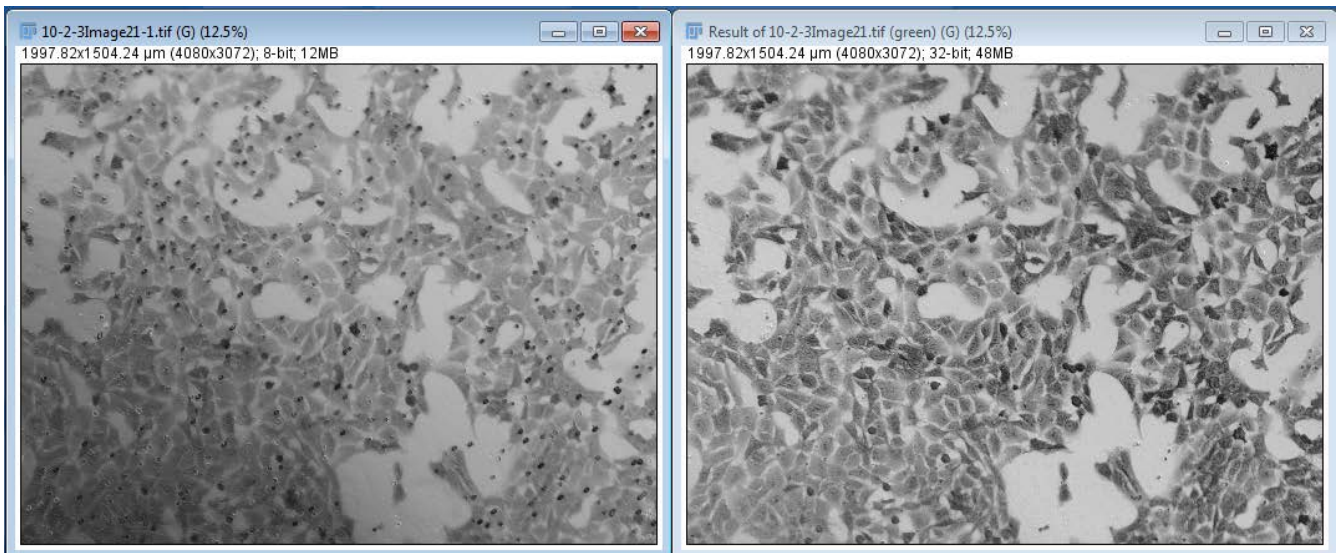


8. Note that the resulting image no longer shows the pores and the dark shadow in the lower left-hand corner has disappeared.

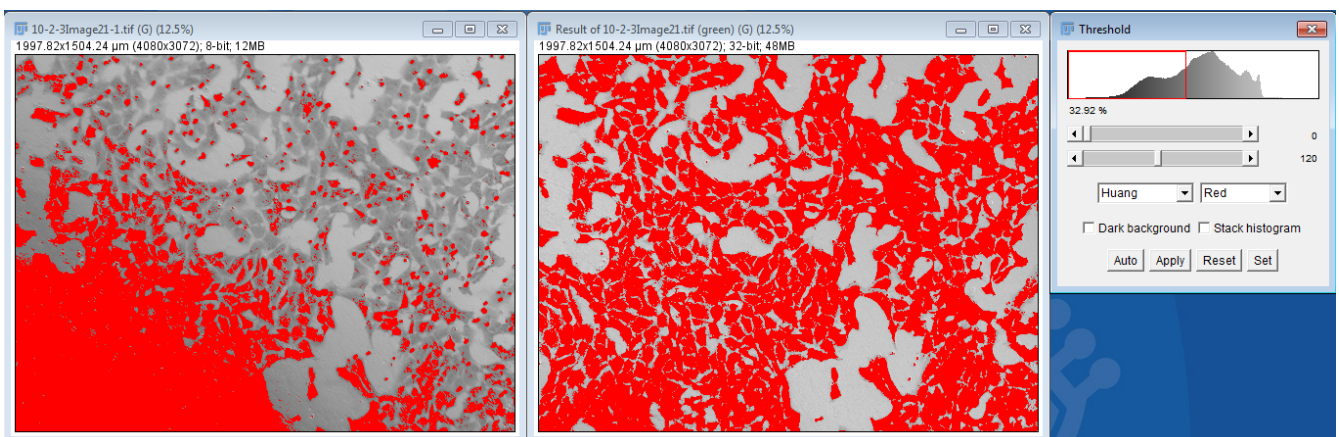


## 8-bit grayscale copy of original

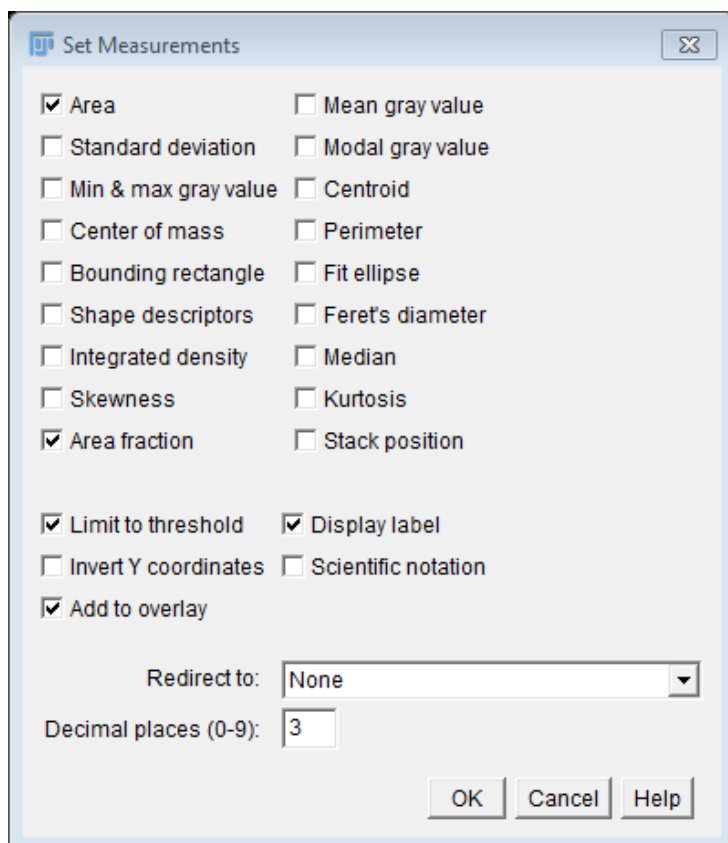
## Result image



9. Go to **Image – Adjust – Threshold** for both images. Note that the corrected image is thresholding better than the 8-bit grayscale copy because the **Blue** image has also acted as a **Background** image. Close the 8-bit copy.



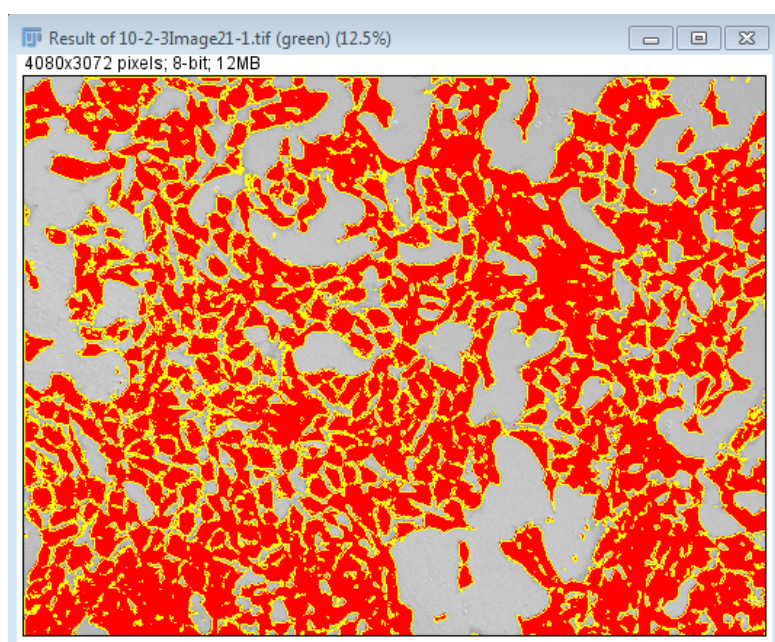
10. Convert the **Result** image to 8-bit by going to **Image – Type -8-bit**.
11. Go to **Analyze – Set Measurements** to choose the parameters you want to measure. This image is already calibrated. We are most interested in **Area** and **Area Fraction**, which will represent the percentage of cells that have migrated. Please note that intensity/density measurements are not appropriate for brightfield images.



12. Then go to **Analyze – Measure**. The **Results** appear in a table as below. You can save this and open it in Excel.

Results				
File Edit Font Results				
	Label	Area	%Area	
1	Result of 10-2-3Image21-1.tif (green)	7422287	59.218	

13. To see how well the area measured represents the original staining, create a selection by going to **Edit – Selection – Create Selection**;



14. Open the original image and show the selection on it by going to **Edit – Selection – Restore Selection**.
15. If you want to save the selection, add it to the **ROI Manager** by going to **Edit – Selection – Add to Manager**. Then go to **More – Save** in the **ROI Manager**.
16. You can save the image with the ROI drawn on it as a record of what was measured.

