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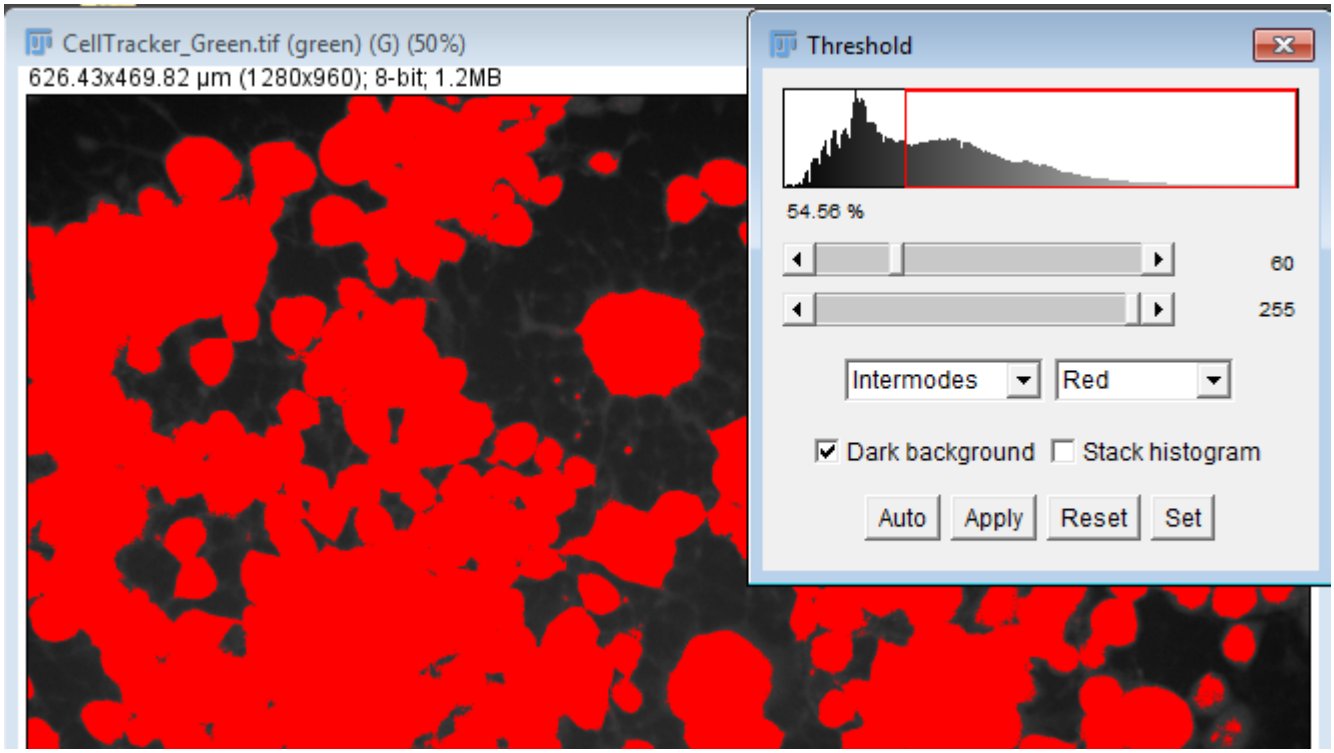
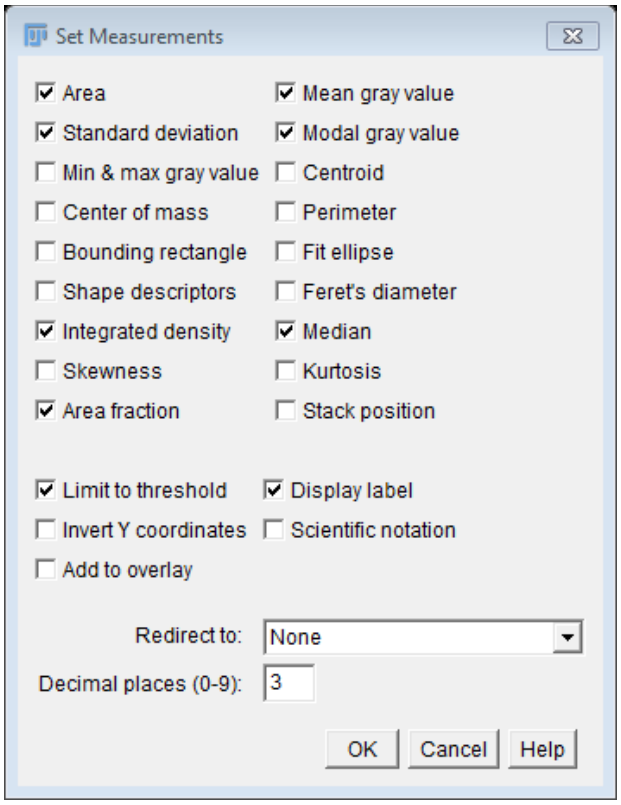
MICROSCOPY NEW ZEALAND INC. CONFERENCE WORKSHOP 2017

Measuring area and intensity of fluorescence images 31 January 2017

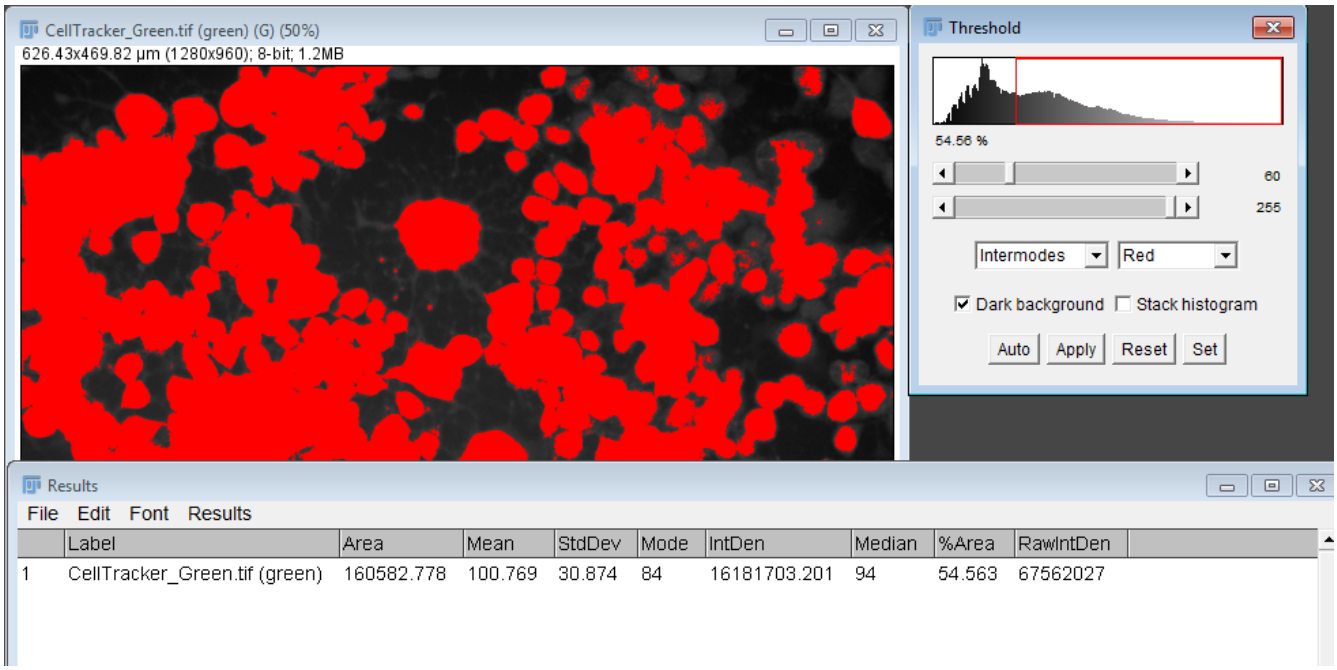
1. Open the image called **CellTracker_Green.tif.**
2. Calibrate the image using the **20x** image of the micrometer slide.
3. Create a grayscale version of the image (e.g. ***Image – Color –Split channels***).
4. Select the **Green** image and go to ***Image – Adjust – Threshold***. Select ***Dark background*** and try out the different methods available until you find the best option. Or go to **Image – Adjust – Auto Threshold** to determine the best option. If necessary, you can adjust the sliders manually but it's better to use an automatic method if possible.



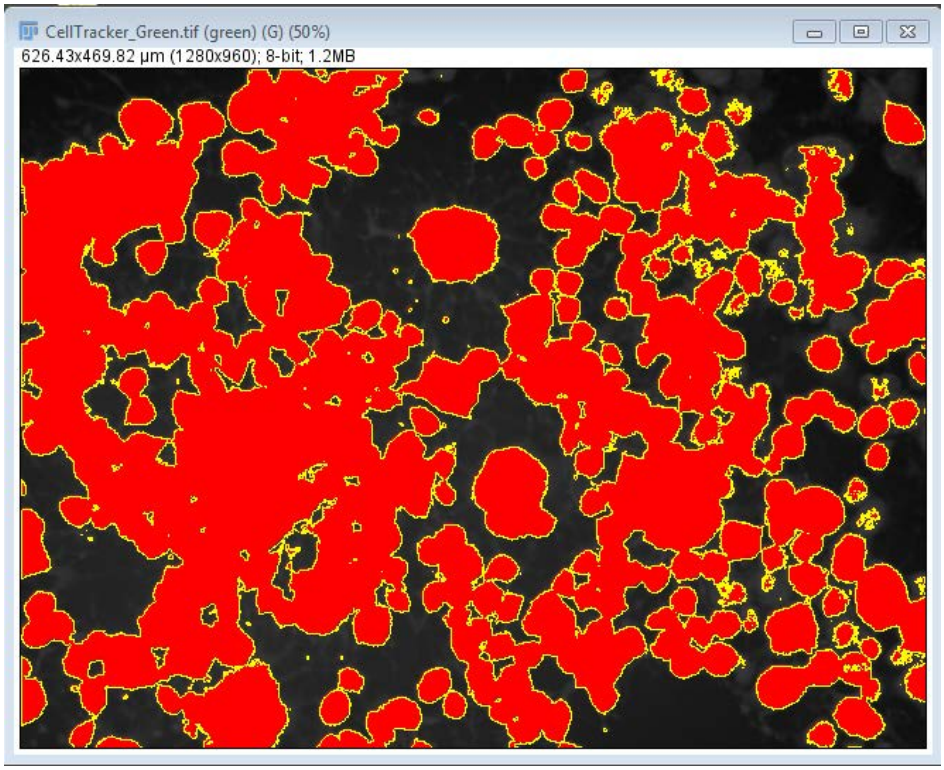
5. Go to ***Analyze – Set Measurements*** to select the parameters you want to measure.
6. Make sure you select **Limit to Threshold** and **Display Label**. If you are interested in intensity values, select all of the gray value measurements.



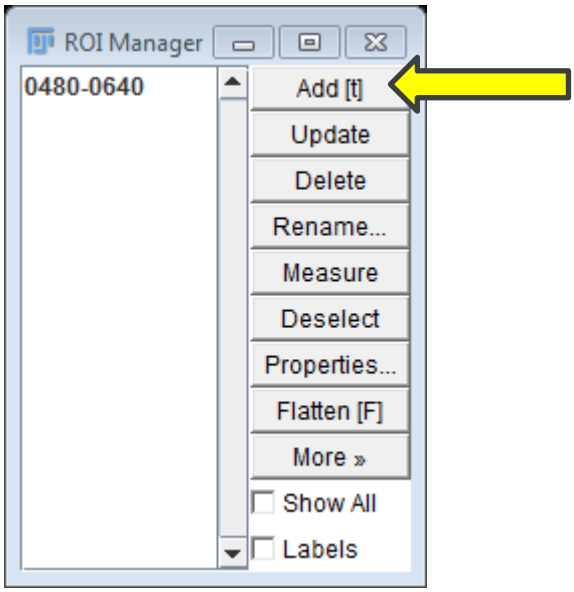
7. Click **OK** and then go to ***Analyze – Measure***. The **Results** table will appear. You can save the **Results** table and open it in Excel or any other software which supports tab-delimited or comma-separated text.



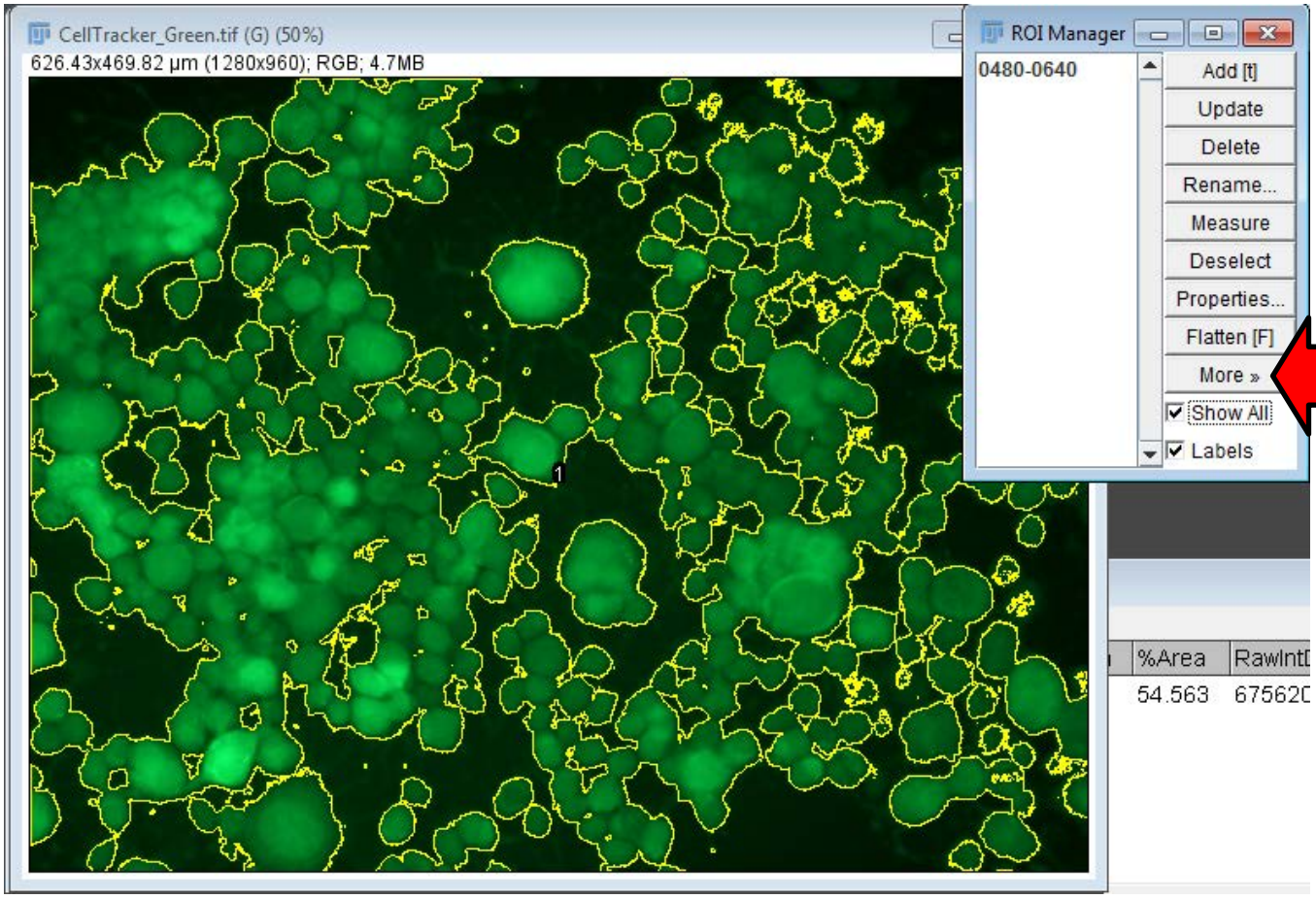
8. You can create a selection from the red overlay by going to ***Edit – Selection – Create Selection***;



9. If you want to **Save** the selection (e.g. for future reference or to apply to your original image), you can add it into the **Region of Interest Manager** (or **ROI Manager**). Go to ***Edit – Selection – Add to Manager*** (or open the **ROI Manager** from ***Analyze – Tools – ROI Manager*** and click ***Add*** to add the selection).



10. Go to ***More – Save*** to save the **ROI**. To see the ROI displayed on your original image, open the image and highlight the ROI to display the selection.



11. If all of your images are similar, then all of these steps can be written into a **Macro** for batch processing.