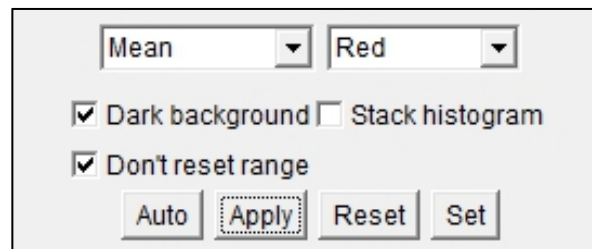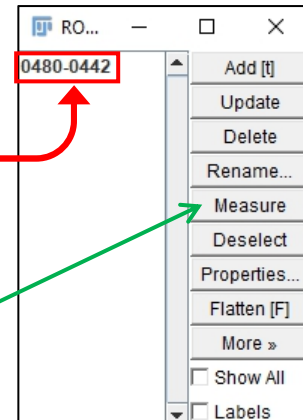# Image Analysis Protocol (Fiji)

1. Open image by dragging and dropping or by **File >> Open...**
2. Remove the scale bar by selecting a rectangle around and pressing backspace. Remember to deselect the area after this step.
3. Turn the image from RGB to 8-bit by **Image >> Type >> 8-bit**
4. Create a copy of this image by **Image >> Duplicate...**
5. Binarize the image by thresholding (**Image >> Adjust >> Threshold...**) Choose the best filter and check **Dark background**, then hit **Apply**

6. Fill the holes in the ring by **Process >> Binary >> Fill Holes**
7. For safety, dilate the shape to include the spots in the border of the ring by **Process >> Binary >> Dilate**

8. Select the wand tool and click on the ring

9. Press "t" to add the selection to the **ROI Manager**. A window like this should appear ⟶

10. Select the original picture and then click on the ROI
11. Before measuring, you must decide what to measure in **Analyze >> Set Measurements...** In your case **Area** and **Mean gray value** are enough (you just need to do this step once)
12. Now you can measure either by clicking here or by going to **Analyze >> Measure**