<http://rsbweb.nih.gov/ij/docs/guide/146.html>

\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*

#1 rule - MAKE A DUPLICATE BEFORE EACH AND EVERY IMAGE MANIPULATION

#2 rule – CHECK HISTOGRAM BEFORE MEASURING INTENSITIES

\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*

Basics

1. Explore the ‘Image’ bar
2. Start w/ opening an image 🡪 ‘clown.jpg’
3. Save a copy in folder “Raw” folder | Make a duplicate
4. Zoom in and out
5. Cursor information (XY and intensity)
6. Scale
7. Crop
8. Image Info and Properties
9. Analyze>Histogram OR ColorHistogram
10. Image 🡪 Type 🡪 ‘RGB color’

1. **Blobs.gif [BINARY IMAGE]**
   1. SEGMENTATION = Defining Regions of Interest (ROI)
      1. Measurement v Analyze Particles
2. **Dot\_blot.jpg [8-bit/ 256 value GREYSCALE]**
   1. Analyze>Histogram (check for saturated pixels)
      1. “Live” Histogram > List
   2. Look Up Tables (LUTs)
      1. Image Adjust
         1. DO NOT USE “APPLY” FOR QUANT INFO
   3. Analyze dots
      1. Image>Adjust>Threshold
      2. Process>Binary>”Watershed”>”FillHoles”
      3. Analyze particles [Add to ROI Manager]
      4. Click on original gel image
         1. 🡪 from ROI manager “Measure”
         2. OR “Overlay” Add Selection/ from ROI manager
3. **Leaf.jpg [8-bit RGB IMAGE]**
   1. Zoom in to ruler – generate a real-world to pixel correspondence
   2. Separate and stack color channels
      1. Contrast and Brightness
      2. RGB to stack, to 32 and 16 bit gray (see intensities)
   3. Make a stack, montage, animated gif
   4. Practice Thresholding and Analyze Particles
      1. Use HSB color space (hint try Brightness)
      2. \*in Analyze Particles 🡪 Set Measurements, click on ‘limit to threshold’
   5. Using Overlays as Masks **[outlines\_and\_masks.png]**
      1. Analyze particles in thresholded image
      2. Process>Binary>“Fill holes”
      3. Add to ROI Manager
      4. Remove unwanted ROI
      5. Analyze>Overlay
         1. Add from ROI manager
         2. Measure
         3. Analyze Color Histogram v Original Leaf Image
            1. List/ Copy
      6. Results 🡪 (right click) Summarize and Distribution