

Using ImageJ to Process and Analyze Images

September 30, 2004

Digital Images

- A matrix of square data points (pixels) containing information about:
 - Brightness (grayscale or color)
 - Location on x, y coordinate system
- Resolution = number of pixels/unit area
 - ↑ resolution = ↑ file size: a trade-off

Digital image file formats

- Uncompressed files contain the most information about the image but are large
 - e.g., TIFF (.tiff or .tif)
- Compressed files can be “lossy” or not
 - Lossy compression “loses” some of the original data
 - JPEG (.jpeg or .jpg) is lossy compression: every time you save a JPEG file, you lose some information

Image file formats

- Which image file formats will ImageJ open?
 - TIFF, JPEG, GIF, BMP, DICOM, FITS, PGM, etc.



Image Type

- Bit depth = how many shades of gray or number of colors
 - 1-bit = binary image, black and white ($2^1 = 2$)
 - 8-bit = 256 colors or shades of gray ($2^8 = 256$)
 - 16-bit = 65,536 colors or shades of gray ($2^{16} = 65,536$), etc.
- RGB color is three 8-bit images in red, green and blue stacked on top of each other (24-bit)

Image Processing with ImageJ

- Changing pixel values (the raw data) to enhance an image for analysis
 - Process/Enhance Contrast
 - Image/Adjust/Brightness/Contrast
 - Process/Find Edges
 - Process/Sharpen
 - Process/Subtract Background...

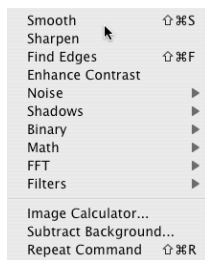


Image Processing with ImageJ

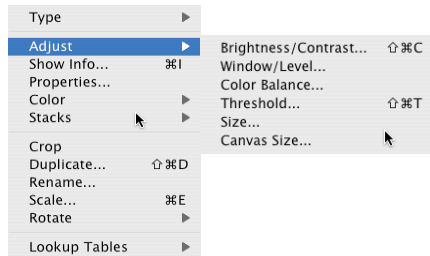


Image Analysis with ImageJ



- Spatially calibrate your image by setting scale:
 - Use the straight line selection tool to select the distance of scale bar
 - Then choose **Analyze/Set Scale...**
 - Type your unit of length in the box indicated; click OK

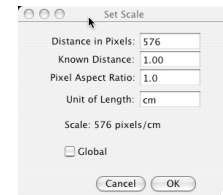


Image Analysis with ImageJ

- Determine measurement parameters by choosing:
 - **Analyze/Set Measurements...**

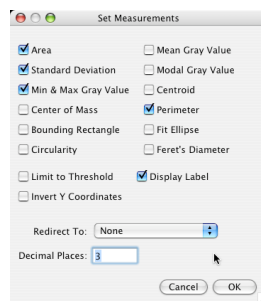


Image Analysis with ImageJ



- Measure structures on your image by choosing a selection tool and then outlining the structure or area to be measured:
 - Use the straight line selection tool for distances; free-hand selection tool for areas; or "magic wand" for thresholded areas
 - Then choose **Analyze/Measure**

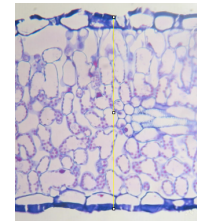
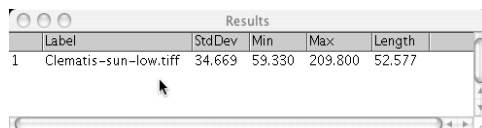


Image Analysis with ImageJ



- Results window will show values for the parameters that you set earlier
 - Clear the **Results** window by choosing **Analyze/Clear Results**
 - When you close the **Results** window, you can save the measurements to a text file