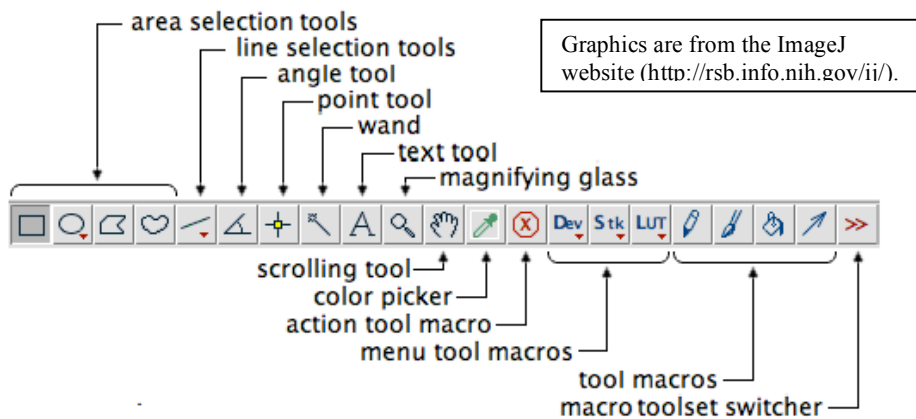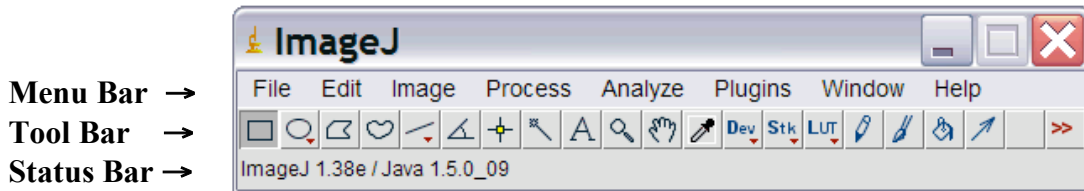# ImageJ Basics
(Version 1.38)

ImageJ is a powerful image analysis program that was created at the National Institutes of Health. It is in the public domain, runs on a variety of operating systems and is updated frequently. You may download this program from the source (http://rsb.info.nih.gov/ij/) or copy the ImageJ folder from the C drive of your lab computer. The ImageJ website has instructions for use of the program and links to useful resources.

Installing ImageJ on your PC (Windows operating system): Copy the ImageJ folder and transfer it to the C drive of your personal computer. Open the ImageJ folder in the C drive and copy the shortcut (microscope with arrow) to your computer's desktop. Double click on this desktop shortcut to run ImageJ. See the ImageJ website for Macintosh instructions.

ImageJ Window: The ImageJ window will appear on the desktop; do not enlarge this window. Note that this window has a Menu Bar, a Tool Bar and a Status Bar.

Menu Bar →
Tool Bar →
Status Bar →

area selection tools
line selection tools
angle tool
point tool
wand
text tool
magnifying glass

Graphics are from the ImageJ website (http://rsb.info.nih.gov/ii/).

scrolling tool
color picker
action tool macro
menu tool macros
tool macros
macro toolset switcher

Adjusting Memory Allocation: Use the *Edit → Options → Memory* command to adjust the default memory allocation. Setting the maximum memory value to more than about 75% of real RAM may result in poor performance due to virtual memory "thrashing".

Opening an Image File: Select *File → Open* from the menu bar to open a stored image file.

Tool Bar: The various buttons on the tool bar allow you measure, draw, label, fill, etc. A right-click or a double left-click may expand your options with some of the tool buttons.

Area Selection Tools: The first four buttons on the tool bar allow you to surround an area on the image with a rectangle, oval, polygon or freehand shape. After selection, these areas may be

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altered, analyzed, copied, etc. using the menu commands. Note that the status bar, below the tool bar, gives information such as the coordinates (xx, yy) of the selection on the frame.

Line Selection Tools: This button allows you to create straight, segmented or freehand lines (right-click to select line type). Information about the line is displayed on the status bar. Double-click on the button to alter the line width, select A*nalyse → Measure* (or Ctrl+M) to record a line length and *Edit → Draw* (or Ctrl+D) to make a line permanent.

Angle Tool: Draws two intersecting lines and measures the formed angle.

Point Tool: When 'Auto-Measure' is selected, this tool allows you to mark locations on an image; with each click the coordinates of the mark (xx, yy) and brightness values (0-255) are recorded in a data window. Color images will have three brightness readings displayed on the status bar, one each for the red, green and blue channels, however only a single grayscale brightness value will appear in the data window.

Wand Tool: This tool automatically finds the edge of an object and traces its shape. It works best with high contrast images (see <u>Thresholding</u>, next page). Place the wand to the left of an edge; click and the algorithm will search to the right for an edge. It will then trace along the edge of the object until it returns to the starting point.

Text Tool: Double click on this button to select a font and size. A large font size will probably be required for an image from a high resolution digital camera. Single click the button, click-drag a text box and type the label. Move the box to the desired location and permanently set the text in place with *Edit → Draw* (or Ctrl+D)**.** Use the color picker tool to select font color.

Magnifying Glass: Left-click on the image to magnify; right-click to reduce the image size.

Scrolling Tool: This button allows you to move an image if it is larger than the window. Pressing the keyboard space bar is a shortcut that temporarily activates this tool.

Color Picker: This tool sets the foreground drawing/text color when a color on an image is 'picked up' with the eyedropper. Colors also may be selected from the *Colors* window by double-clicking the color picker button (or via *Image → Color → Color Picker*). Use Alt+click to change the background color. The icon for this tool (eye dropper) shows the current foreground color while the frame around it shows the background color.

Other Tools: The remaining tool buttons are similar to those found in drawing programs (spray can, flood fill, etc.) and can be easily used after a bit of experimentation.

<u>Image Editing and Analysis</u>

The following is a small sampling of processing techniques that are possible with ImageJ. I often use ImageJ in conjunction with a photo editing program such as Adobe Photoshop. During analysis, measured values can be easily transferred to a spreadsheet with a 'cut and paste'. It is a very good idea to make a backup copy of your image before doing any processing. Refer to the ImageJ and NIH Image websites for more information

<u>Undo.</u> *Edit → Undo* reverses the preceding action. Only one back-step is possible.

<u>Revert.</u> *File → Revert* converts all changes back to the original saved version.

<u>Cropping.</u> Surround the area with the rectangular selection tool followed by *Image → Crop*.

<u>Clear Outside.</u> Make a perimeter with an area selection tool followed by *Edit → Clear Outside.* This technique is useful for clearing extraneous objects near an area of interest. *Edit → Clear* clears inside of the perimeter.

Enhancing Brightness and Contrast. *Image → Adjust → Brightness/Contrast*; click 'Auto' or set manually with the sliders. Also try **Process → Enhance Contrast**.

Removing Noise**.** *Process → Noise → Despeckle* or try *Process → Filters → Median*

Rotating an Image. *Image → Rotate* and select type of rotation

Converting to Grayscale. *Image → Type → 8-bit* converts the image to 256 shades (8-bit) of gray. In this scale 0 = pure black and 255 = pure white…. a grayscale reading of 128 would be a medium gray.

Thresholding (Binary Contrast Enhancement). This is commonly used when detecting edges, counting particles or measuring areas. A grayscale image is converted to binary (a.k.a. halftone or black & white) by defining a grayscale cutoff point. Grayscale values below the cutoff become black and those above become white. The procedure: First convert the image to 8-bit grayscale as described above (*Image → Type → 8-bit*). Create a 'thresholded' binary image by selecting *Process → Binary → Make Binary*. A less automated procedure involves: *Image → Adjust → Threshold*; use the slider to adjust the threshold. The red areas will become the black portions in the binary image. Click 'Apply' to complete the conversion. 'Brightness slicing' is a similar procedure that uses both upper and lower threshold values.

Measuring and Counting Objects

Setting Measurement Scale. Draw a line between two points of known distance such as a ruler on the photograph. Go to *Analyze → Set Scale*. In the *Set Scale* window the length of the line, in pixels, will be displayed. Type the known distance and units of measure in the appropriate boxes and click OK. Measurements will now be shown using these settings. If the pixel:length relationship is known from a previous measurement you may directly type this information in the *Set Scale* window. Check 'global' to apply this scale to other image frames.

Set Measurements. Choose parameters to be measured via *Analyze → Set Measurements*.

Measuring Distance Between Points. Draw a line between two points. The status bar will show the angle (from horizontal) and the length. *Analyze → Measure* (or Ctrl+M or simply type M on the keyboard) transfers the values to a data window.

Measuring Area. Surround an area with a perimeter. This can be done with an area selection tool, the wand (for high contrast images) or with *Analyze Particles* (see below). *Analyze → Measure* (or keyboard M) transfers the area measurement to a data window.

Counting Particles. As described above, convert the image to 8-bit grayscale and then 'threshold' the image. Go to *Analyze → Analyze Particles*, type the upper and lower limits for the particle size, toggle 'show outlines' and check 'Display Results'. Click OK and each counted particle will be outlined and numbered in a new widow (numbers may be very small). The data window contains measurements for each particle.

Saving Files. Images from digital cameras are usually saved as JPEG files. JPEG is a type of memory compression that results in the loss of some data. A JPEG image degrades each time it is opened, edited and resaved. It is best to save a file in a 'lossless' format such as a TIFF during the editing process (*File → Save As → Tiff*).

Printing. Should you encounter printing problems, save the processed image and print with a photo editor. Saved images also may be inserted into MS Word for printing (see "Using Microsoft Word as a Simple Photo Editor" in lab manual).