

ImageJ

(Version 1.24o)

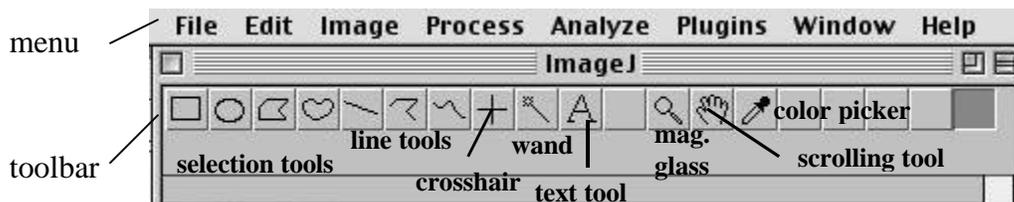
ImageJ is a public domain image analysis program that was developed at the National Institutes of Health. You may copy the ImageJ folder from your lab computer or download this program from the source (<http://rsb.info.nih.gov/ij/>) for use on your home computer. Updates to this program are available at the ImageJ homepage at frequent intervals. Macintosh and Linux versions of ImageJ are also available.

Installing ImageJ on your Home PC (Windows operating system).

Copy the ImageJ folder to your disc and transfer it to the C drive of your computer. Open the ImageJ folder in the C drive and copy the shortcut (microscope with arrow) to your desktop. Double click on this shortcut to run ImageJ. If you are running ImageJ from some location other than the C drive, double click on the 'red apple' icon in the ImageJ folder to launch the program.

Basic Operations.

An ImageJ window will appear on the desktop; do not enlarge this window. Select **File** → **Open** from the menu to open a stored image file.



Selection Tools: The first four buttons on the toolbar are area selection tools; they allow you to surround an area on the image with a rectangle, oval, polygon or a freehand shape. After selection, these specific areas may be altered, analyzed, copied, etc. using the menu commands. Notice that the status bar, below the toolbar, gives the location of the selection (xxx, yyy) and its dimensions in pixels.

Line Tools: The next three buttons are line tools that create straight, segmented or freehand lines. Again note that information is displayed on the status bar as the line is drawn. Double-click on the line button to alter the line width. **Ctrl+D** or **Edit** → **Draw** makes the line permanent.

Crosshair Tool: The crosshair tool allows you to mark locations on the image; with each click the coordinates of the pixel (xxx, yyy) and brightness (0-255) are recorded in the 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 one brightness value will be printed 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 [Thresholding](#), 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 digital camera. Single click the button, click-drag a text box and type the label. Move the box to the desired location and set the text in place with **Ctrl+D** or with **Edit** → **Draw**.

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

Scrolling Tool: This allows you to move the image if the picture is larger than the window.

Color Picker: This tool sets the foreground drawing color or text color by "picking up" colors from images with the eyedropper. Colors also may be picked up from the *Colors* window by double-clicking the color picker button. **Alt-click** in the *Colors* window 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.

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Image Processing

The following is a very small sampling of processing techniques that are possible with ImageJ. See the ImageJ and NIH Image websites for more information. ImageJ is best used for image analysis; I use it in conjunction with more powerful photo editors such as Adobe Photoshop. You may want to open a spreadsheet so that data can be efficiently 'cut & pasted' during image analysis. Also, it is a very good idea to make a backup copy of your image before doing any processing.

Undo. **Edit** → **Undo** reverses the preceding action. Only one back step is possible.

Revert. **File** → **Revert** should revert to the original saved image.

Cropping. Surround the area with the rectangular selection tool followed by **Image** → **Crop**.

Clear Outside. Make a perimeter with a selection tool followed by **Edit** → **Clear Outside**. The technique is useful for clearing extraneous objects near an item of interest. **Edit** → **Clear** clears inside of the perimeter.

Enhancing Brightness and Contrast. **Image** → **Adjust** → **Brightness/Contrast**; click 'auto' or set manually

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 (see above). **Process** → **Binary** → **Threshold** creates a 'thresholded' binary image. 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 thresholds.

Measuring and Counting Objects (also see accompanying handout with ImageJ examples)

Measuring Distance Between Points. Using the straight-line tool draw a line between two points. The status line will show the angle (from horizontal) and the length in pixels. Use the following step to set the scale:

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, should 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 frames.

Measuring Area. Surround an area with a perimeter. This can be done with an area selection tool, the wand (in high contrast images) or with **Analyze Particles** (see below). Go to **Analyze** → **Measure**; the data window will show the area and pixel brightness values for the object in the perimeter. Use **Analyze** → **Set Measurements** to select additional parameters (such as perimeter length) to be displayed.

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 and toggle 'show outlines'. Click on OK and each counted particle will be outlined and numbered in a new window (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. Pressing "S" on the keyboard brings up the *Save as TIFF* window. As you save a file, confirm that the extension '.tif' has been added to the filename.

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 MS Word handout).