

## Raster Images

This tool is for scientific analysis of “Raster” images. A raster image is a collection of square or rectangular colored dots, called pixels. Any image seen on a monitor is a raster image, regardless of how it was created. Any image created with a camera is a raster image.

Raster images can be saved in many formats: (jpg, gif, bmp, tif, or a million others). Each has advantages and disadvantages:

- a. Jpg: the default for cameras. Can store hi-resolution (many, many pixels) colored images without using a lot of memory. The images are generally compressed and “lossy”, meaning that the saved image, when re-opened, is not exactly the same as it was before you saved it. Colors are 24 bit (meaning there are  $2^{24} = 16777216$  unique possible colors for each pixel) The colors are internally stored as 3 separate 8-bit values ( $2^8 = 256$  possible unique colors), representing Red level, Green level, and Blue level (RGB). These 3 numbers are then combined into a total pixel color.
- b. Gif: the default for raster images created from vector graphic source data (like a word drawing). Can store very hi-resolution colored images using almost no memory. Colors are stored in a color table, which is a list of 256 separate RGB triplets. So, GIF images can contain any RGB color, but can only contain 256 unique colors at one time.
- c. Bmp: (bitmap). Similar to a jpg, but without compression or loss.

## Creating Raster Images

Of course, cameras always create Raster images. Also, you can make a raster image from anything you see on your computer screen using “Print Screen” or the “Clipping tool” program located in the Windows accessories. Also, you can create or “paint” a new raster image using Photoshop or an equivalent program (like GIMP).

If you create a raster image from a vector graphics original, you are making it less accurate. This becomes most apparent when you zoom in on something. In many cases, you can’t tell the difference in quality until you zoom in.

Rule of thumb: NEVER use a raster image when it is possible to use a vector graphics image instead.

## Using ImageJ (made by NIH). Tools I use:

**File/Open**: Function is obvious. You can also drag an existing image onto the toolbar.

**File/New/ System Clipboard**: Imports an image from the operating system (such as those made using the “Print Screen” button, or any copy-and-paste from another program.

**Magnifying Glass**. Default is zoom in one “level” perclick. Control-click – zoom out.  
Shift = undo all zooms/restore to original.

**Select: *Straight Line.*** Used to choose a linear region of the image for analysis. Good for setting the scale (i.e., lightyears per pixel), or making a profile (Volts as a function of lightyears), or making a histogram (a map showing the likelihood of various colors to appear in the image).

**Analyze/Set Scale:** If you have a line selection, you can set the scale (like lightyears per pixel) so that all future results and calculations made by ImageJ are reported in lightyears instead of in pixels (which is otherwise the default for measured distances on an image). The distance in pixels can be typed in by hand, but is auto-typed whenever you select a straight line. So, you draw a “straight line selection” on the image, spanning a known distance (in lightyears, or volts, or meters, or whatever), and type that known distance into the “Set Scale” window.

**Select: *Segmented Line.*** Right-click on “Straight line” to see this option. Used to click on a series of points that are not necessarily linear. So, if you have a picture of a coiled rope, and you know the rope is 10m long, you can use this to set the meters per pixel scale by clicking many points along its length.

**Select: *Freehand.*** Similar to a segmented line, but you don’t click to create new points, you just draw.

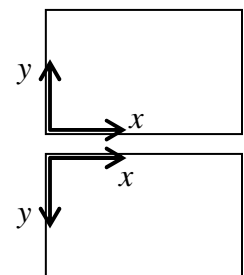
**Select: *Rectangle/Oval/Etc.*** Use to choose an area rather than a linear part of an image.

**Point:** Used to select just a single point of the image.

**Multi-point (right click on point):** Used to select a series of discontinuous points. It’s like “Segmented line”, but there is no line joining the selected points.

**Analyze/Set Measurements:** This lets us choose ahead of time the kinds of things we want to measure. Some of the more useful ones are area, centroid, and mean gray value. Area is in pixels<sup>2</sup> by default, but might be in m<sup>2</sup> if you set the scale already. Centroid generates two values (x and y), either in pixels or scaled units. For a color image, “mean gray value” is the average of the three component colors (R, G, and B).

Also, note that in this menu you can choose to “invert” the y-coordinates. In most scientific graphics, coordinates are as shown in the top picture here (zero at the bottom left corner, positive values as you go up). But, coordinates for images are always as shown in the second picture (zero at the top left corner, positive y values as you go down). By clicking “invert y axis”, you are asking ImageJ to use standard science conventions rather than standard image processing conventions.



The kind of measurements that I do come in several flavors:

1. Measure lengths or positions on a drawing.
2. Determine the color or brightness at a position.
3. Create a profile (color or brightness as a function of position).
4. Create a histogram (a list of pixel counts as a function of color or brightness).
5. Count the number of dots on an image, and determine properties of them (size, brightness, etc.)

There exist almost an infinity of other options, too.

**Measuring Length:** First, select a straight line. The length of this line in scaled units appear at the bottom of the main ImageJ toolbar. Also there are the  $(x, y)$  positions (in scaled units) of the cursor, and the angle of the line in degrees.

**Determining Color/Brightness:** First, select a line, or a point, or a collection of points. Then press CTRL-M (“Measure!”). This brings up a little window that tells you all of the properties that you chose earlier (using the Set Measurements tool).

**Saving Results:** Once you have the measurements window open, you can save this data as a list. By default, it appends “.xls” to the file, so Windows will think it is an Excel file. But really, it is just a tab-delimited ASCII file. So, you can change the extension to “.txt” and open it in notepad, or almost any other program. Or, you can just highlight all the data, and use Copy-and-paste directly into the other numerical analysis program of your choice. If there is more data than you can see on one screen, I find the “Edit/Select All” (CTRL-A) option useful. I also find it convenient to clear the data often, instead of just appending new measurements into this list.

If you choose new options to save (using the Set Measurements tool), you’ll need to re-measure to get those results. Old results already in the window won’t be updated automatically.

**Analyze/Histogram:** Do you want to find out which colors appear most often in the image? Use the histogram. Again, press CTRL-M to get the results. If you don’t first select a region, the entire image is reported on.

**Analyze/Plot Profile:** This tells you color as a function of position (in scaled units, or just pixels by default). You should choose a straight line first.

**Image/Color/Split Channels:** Will create 3 new sub-images from a color image. Look at the names in the title bar of each to see which is which! Each will appear as a grayscale image, showing just the Red Value from 0 to 255 (or the Blue, or the Green). This allows you to easily examine “redness”, separate from overall color. An image that is originally gray will be separated into 3 identical components (i.e., if the grayness of a pixel is 212, then Red = Blue = Green = 212, too).

**Analyze/Analyze Particles:** Use this to count and categorize particles. This is useful for astronomy, geology, metallurgy, and fluid mechanics (images of particles were the main source of data for my PhD thesis, using the technique called “PIV”). Sadly, we can’t even get started with this categorization until we modify the image a little...

**Image/Adjust/Threshold:** A “thresholded” image is one that is black-and-white (rather than gray). In order to count particles, we need to specify a range of colors that identifies “what is a particle” from “what is not a particle”. So, this tool has two sliders. The first slider says “discover every color (gray level) that is brighter than me, and consider it to be part of the body of a particle (like a star)”. The second slider says “select every color that is darker than me”. So, with the two,

you can specify a range of colors to be counted as your particles. Checking the “dark background” option changes whether you want to include or discard this range from your selection. While you are adjusting the levels, you will see a red layer appear directly on the image itself. The red pixels are the pixels you are choosing to include in your particle count. The non-red portions will be ignored. Also, DO NOT “apply” this setting. If you “apply” the setting, then the image is permanently changed into a thresholded image; we don’t want to change it, we just want to set the thresholding properties temporarily.

**Analyze/Analyze Particles (Revisited):** You’ll have to answer some questions, like:

- a. Keep all the particles, or reject some that are too small or too big?
- b. Keep ellipses, or just perfect circles? The “circularity” is the(major axis)/( minor axis), and can only range from 0.0 to 1.0.
- c. You of course want to show the results. Essentially, checking this box makes imageJ automatically type CTRL-M for you after it calculates the results.