

## Raster Images

The “ImageJ” program is a tool 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 (“Graphics Image Format”): the default for raster images created from vector graphic source data (like a PowerPoint 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, while GIF images can contain any RGB color, they can only contain 256 unique colors at one time.
- c. Bmp (“Bitmap”): Similar to a jpg, but usually without compression or loss. They take more disc space to store.
- d. HEIC: The new default for Apple devices. It exists specifically to impede compatibility with other platforms, so avoid it if it’s adjustable!

## 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. Strangely, PowerPoint, to create animations, first converts the screen image into a raster image before animating.

When creating diagrams, NEVER use a raster image when it is possible to use a vector graphics image instead. In other words, don’t draw diagrams in “paint”, GIMP, etc.

Also, when sharing images, modern phone cameras, by default, create very high resolution images. “Resolution” means the number of pixels. In most cases, the majority of those pixels are extraneous garbage. If it is in any way possible, you should generally *crop* (delete portions of the image on each of the 4 edges) and also *rescale* (replace every group of 4 (etc.) pixels with one pixel having the average value of the original group) every image *before* using it or sharing it. It’s never OK to email

4 Megabyte images... 1 MB is about the reasonable limit, and smaller is usually better. Powerpoint, etc. all run better when the embedded images are smaller.


## Scientific Measurements with ImageJ


Imagej is a program published for free by the National Institute of Health (NIH). It is designed to allow scientists to convert images into qualitative data so that you can perform calculations, etc. based on your photographs. Photographs might be images of the night sky, or images of bacteria seen through a microscope, or images of the earth taken with satellites or other airborne cameras. You might think of it as “tracker” for single images, but with a lot more power.

**File/Open:** Function is obvious. You can also drag an existing image right onto the toolbar. Images open in their own window, and the toolbar remains open in its own window.

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

As you hover your mouse over regions of the screen, you’ll see the position of the mouse in the currently selected units system (the default is pixels), and you’ll see the color of the pixel underneath the mouse cursor (in RGB format).

**Magnifying Glass** : The default is: zoom in one “level” per click. Control-click will zoom out. Shift-click will undo all zooms and restore the image to original. The “original” scale uses one pixel of your monitor for each pixel of the image.


**Straight Line** : Used to choose a linear region of the image for analysis. It draws a yellow line on the image (but the image is unchanged... the line is not part of the image, it’s *on* the image). This is 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:** Of course, for a new image, all distances are measured in pixels. However, if you have a straight line (yellow) on the screen, you can use *Set Scale* to get imageJ to report future measurements in “real” units. For this tool, there are 4 main boxes for data entry. ImageJ fills out the first one (how many pixels long is the current yellow line). You fill out the next three. So, if you’ve already drawn a line connecting the endpoints of a ruler in your image, you would enter:


- The length is 30.48 (but see below...)
- The pixel ratio should probably stay at 1.0. This option is here for older cameras for which each pixel was a rectangle rather than a square (!).
- Unit of length. The default is “unit”, but here, we’ll change it to “cm”, because a ruler is 30.48 cm long.




Your units can be cm, lightyears (or ly... the units are just text added to each number). Now, whenever you draw a new “straight line”, the toolbar will display the length of the line in the appropriate units (until you perform some other action). If your image is a screen shot of a plot of Volts per second, you might draw a line along the vertical plot axis so that future vertical


measurements are in V. This is tricky, because diagonal lines will have units that are some combination of volts and seconds, so avoid diagonals when using scales with plots.


**Segmented Line** : Right-click on “Straight line” to see this option. Used to click on a series of points that are not necessarily linear, and right-click to stop drawing new lines. So, if you have a picture of a curved 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.

**Analyze/measure:** Using the units you already created, immediately tells you info about the stuff you’ve drawn on top of your image (lines, rectangles, etc.)

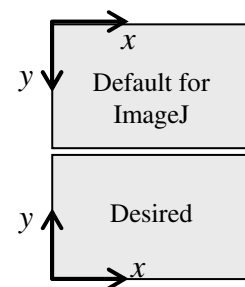
**Freehand** : Similar to a segmented line, but you don’t click to create new points, you just draw. The resulting curve will be a closed curve, not an open-ended curve! If you need an open-ended curve, right-click on “straight line” and choose “freehand line”.

**Rectangle/Oval/Etc.**   : As with lines, etc., the selected region includes handlebars (white squares on the yellow line(s)) so that you can change your selection later if you decide you didn’t get it quite right the first time.

**Point** : Used to select just a single point of the image. By default, the position is (0, 0) in the upper left corner. That’s not a usual physics coordinate system!

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

**Analyze/Set Measurements:** Before you make a measurement of anything, you have to pre-specify the kinds of data you want. 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 cm<sup>2</sup> if you’ve 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). Note that one of the options is “invert y coordinates”. By default, the  $+y$  axis is DOWN in images, which isn’t generally how scientists think. To make the  $+y$  axis UP, check this “invert” box. The default  $x$  axis is to the right, as usual.



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. When outputting data for points, the measurement generally returns the  $(x, y)$  positions of *the pixel* you've indicated, in the active units. However, if you request the center of mass of a pixel, you'll get slightly different numbers for  $(x_{cm}, y_{cm})$ . That's because "position" is based on the pixel number, but the center of mass is based on the position of the *center of the entire pixel*. For a single pixel, these results differ from each other by 0.5 pixels, of course.

## Making Measurements

We've already seen that you can determine lengths and colors by just hovering your mouse. If you have drawn a straight line tool, you'll also be told the angle of the line in degrees.

Once you've used the "Analyze/Set Measurements" to determine what kind of data you want, press CTRL-m ("Measure!"). For example, if I've selected mean gray value, and centroid, and then drawn a straight line, CTRL-m shows me this. I seem to get the angle and length for free when using the straight line to select my data.

Results					
File	Edit	Font	Results		
	Mean	X	Y	Angle	Length
1	8.550	2.941	3.020	-36.870	3.866

If, instead, choose Area, mean gray value, and perimeter, and then I draw a rectangle, I might see this. Here, "mean" means average brightness within the selected rectangle.

Results			
File	Edit	Font	Results
	Area	Mean	Perim.
1	0.867	11.934	3.728

**Determining Brightness:** In the "set measurements" tool, "mean gray value" is brightness.

**Determining Color:** Other than seeing the RGB values as you hover, it's a nuisance to get valid exportable RGB values for an entire set of points. There is a "plug-in" that can do this, but the results are a little more chaotic. Go to "Plugins/Analyze/RGB Measure", and this will automatically open a measurement report for all the selected data. Unfortunately, this report is chaotically presented. Other measurement reports have one point → one line of data. This report will have each point represented on 5 separate lines (I've even truncated this report here). On this report, I'm asking about 3 points. For example, one of them is located at (84, 8). For this one point, the RGB values are (180, 79, 199).

Results				
File	Edit	Font	Results	
	Label		Mean	X Y
1			102	16 9
2			180	84 8
3	Red		238	138 7
4			35	16 9
5			79	84 8
6	Green		219	138 7
7			115	16 9
8			199	84 8
9	Blue		241	138 7
10			84	16 9
11			153	84 8

**Exporting and Saving Results:** Once you have the measurements window open, you can "File/Save As" your data. 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 from the report into another 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. To clear all stuff drawn so far, type "shift a".

If you choose new options to save (using the Set Measurements tool), you'll need to run measure again to get the new 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. If you don't first select a region, the entire image is reported on. Clicking "List" will change the output style from a plot to a list or table.

**Analyze/Plot Profile:** Before using this tool, select a Straight line. Then, this tells you brightness as a function of position along the line (in scaled units, or just pixels by default). You'll use this on some of your homework questions.

**Image/Color/Split Channels:** This creates 3 new sub-images from a color image, each of which shows only the R, G, or B portion of the image on its own. 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", which means stars in a night sky, or pollen on a microscope slide. 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 first modify the image a little using...

**Image/Adjust/Threshold:** A "thresholded" image is one that is black-and-white (rather than various shades of gray). In order to count particles, we first 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. Using this tool changes the image as it is stored in ImageJ. When you check the "dark background" option, the resulting image will be *black with white spots*. When it's unchecked, the resulting image will be white with dark spots. 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, this threshold is valid even if you don't "apply" this setting. If you "set" your choices, then the analysis tools will use these values. If you "apply" your choices, ImageJ will change the image "permanently" (but not the source file) so that **all** future calculations use the thresholded version. Usually, I just "set" my levels.

**Analyze/Analyze Particles (Revisited):** This will report using the settings you've already chosen with "Set Measurements". You'll also have to answer some questions, like:

- a. Count & process all the particles, or reject some that are too small or too big?
- b. Examine lopsided ellipses, or just perfect circles? The "circularity" is the (minor axis)/(major axis), and can only range from 0.0 to 1.0.
- c. The third box asks ImageJ to create a new image from the chosen particles.
- d. You of course want to check "Display Results".