

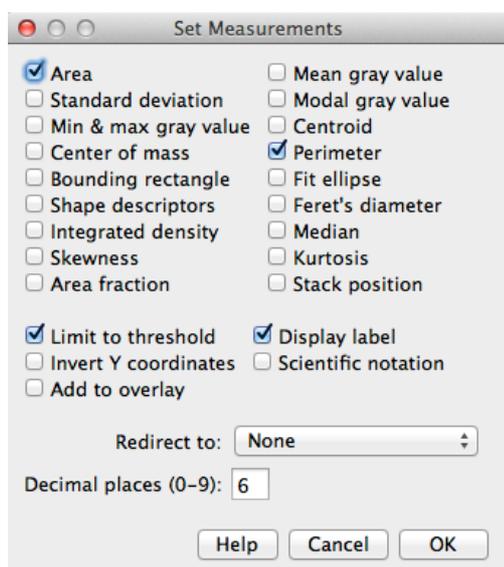
## Spatial Calibration of an Image - ImageJ / Fiji

The first step is to ensure the original images are as sharp and clear as possible. It's often assumed images from a microscope can be 'cleaned up' later. Often they can't be, so try and get it right from the beginning if possible.

### Image Format

Unless there's a need for the colour information in the image, it's often easier to convert it to greyscale when thresholding images, ie. selecting pixels with values which fall within a defined range. To change to 8 bit greyscale go to Image / Type / 8 bit. This produces a grey image with 256 shades of grey.

### Set Measurements

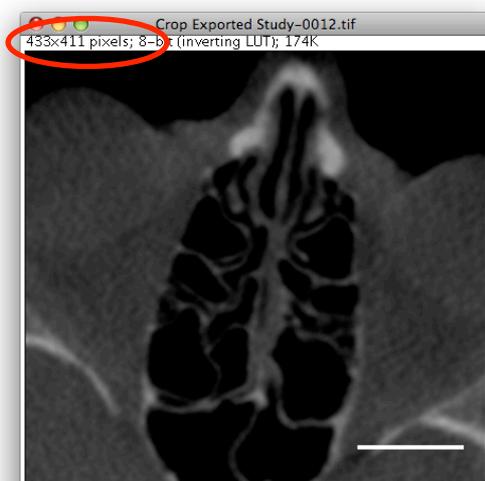


Decide what you want to measure by opening the 'Set Measurements' window under Analyse / Set Measurements.

Tick all the options you want to measure, but in this case also tick 'Limit to Threshold' and always tick 'Display Label'. The former means only pixels included in the threshold range will be measured, the latter means the columns of data produced will be headed 'Area', 'Perimeter', etc. Don't think you will remember what a number of anonymous columns are a week later - you won't!

Click 'Ok' and proceed.

### Set Scale

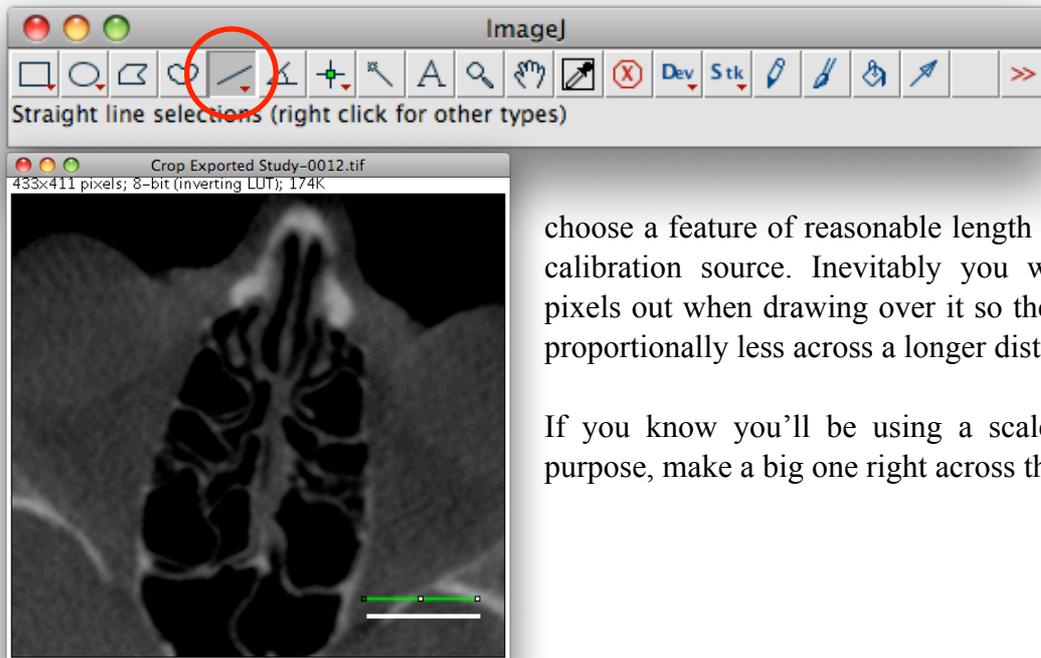


If you want to measure 'real' dimensions, rather than just pixels, you will need to calibrate the image, ie. tell the software 'how many pixels equal a  $\mu\text{m}$ , mm, and so on'.

An uncalibrated image will only show the pixel dimensions of the image, a calibrated image will also show the dimensions in the appropriate units.

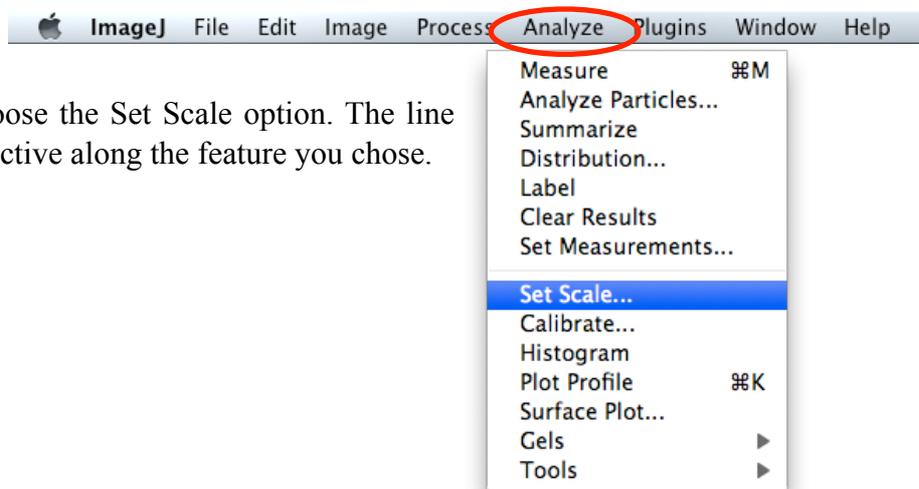
To calibrate an image from a scale bar, or other feature of known length in the image, the line tool is selected from the main control panel and a line drawn along the calibration bar or feature. To force the line to draw horizontally or vertically hold down the shift bar while dragging the line tool.

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Try to choose a feature of reasonable length to use as your calibration source. Inevitably you will be a few pixels out when drawing over it so the error will be proportionally less across a longer distance.

If you know you'll be using a scale bar for this purpose, make a big one right across the image.



From the Analyze menu choose the Set Scale option. The line you selected should still be active along the feature you chose.

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The value to change in the window which appears is the Known Distance, this is the length of the feature you measured. In this example the length of the scale bar was 50mm so 50 is entered in the box. The unit of length is also entered in the appropriate box, although this is just a label and has no effect on the numerical values of measurements.

To apply the same calibration to other open images, or ones you will open, tick the Global option.

The scale at the bottom indicates how many pixels per unit of measurement.

When the calibration values are known they can be noted and entered directly into this window on future occasions, without using the line tool step - if they were recorded under the same conditions.

Once calibrated the image window will show its dimensions in the appropriate units in addition to the pixel dimensions.

All dimensions will now be displayed as 'real' measurements, not just as the number of pixels.

Once you have set the scale of your image, open the Analyse / Set Scale menu to see what the scale has been set to. Record these numbers and enter them as described below - for images taken under the same magnification. It is more accurate than re-drawing a line each time. You may make as small error drawing the line, but it will at least be a consistent error. This is particularly important as square and cubic functions (area and volume) will compound any inconsistent errors.

If you already know the pixel dimensions of your image, go to the Analyse / Set Scale menu and enter the details in the box shown.

If you have many image of the same scale, tick 'Global' to propagate the scale to images you open, or have open already.

Always draw a line immediately after setting a scale and check it is showing the correct dimensions,. It's easy to make an arithmetic error or enter the wrong numbers. The line tool displays a 'live' length in the main menu bar.

