Calibrating Images

ImageJ Skill

Calibrating an image allows you to measure distance, area, and pixel value in meaningful units.

Spatial calibration

Spatial calibration defines horizontal and vertical dimensions of an image in real-world units such as millimeters, centimeters, miles, etc.

When there is a known distance in the image

When the image contains a grid, ruler, or object of known size:

1. Use the straight line selection tool to select a known distance on the image.
2. Choose Analyze > Set Scale….
3. Enter the **Known Distance**.
4. Enter the **Unit of Length**.
5. Click **OK**.

When you know the scale (e.g., 1 pixel = 0.025 mm)

1. Choose Analyze > Set Scale….
2. Enter the **Distance in Pixels** (1).
3. Enter the **Known Distance** (0.025).
4. Enter the **Unit of Length** (mm)
5. Click **OK**.

Uncalibrating an image (removing the scale)

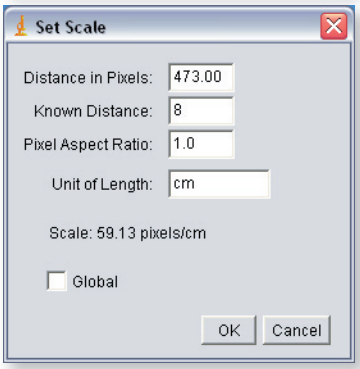
1. Choose Analyze > Set Scale…
2. Enter 0 for the **Known Distance,** or delete the **Unit of Length**.
3. Click **OK**.

Density calibration

Pixel values often represent measurements—temperature, elevation, brightness, etc.—recorded at regular intervals in a grid pattern. Sometimes the pixel values are the actual measurements, but often they are only proportional to the actual measured values. Density calibration is the process of scaling the pixel values to approximate the original measured quantities.

The information needed to density calibrate an image can come from either of two sources—measurements made on the image itself (such as from a calibration bar), or from image metadata (information about the image, often provided in a separate text file, printed manual, or Web page).

Setting scale



What is the Pixel Aspect Ratio?

ImageJ always displays pixels as squares, but some imaging devices use *rectangular* pixels. The Pixel Aspect Ratio setting tells ImageJ how to adjust for non-square pixels, so that length and area measurements are accurate.

You can determine the pixel aspect ratio of a camera or scanner by creating an image of a circular object and carefully measuring the height and width of the object in the image. Divide the measured width by the height (both in pixels) to get the pixel aspect ratio.

For example, the width of this scanned nickel was 255 px and the height was 250 px, a pixel aspect ratio of 1.02. Unless otherwise noted, leave the pixel aspect ratio set to 1 (square pixels).

Density calibration from image measurements

If an image includes a density scale or regions of known value, you can calibrate it by taking measurements directly from the image. In this example, the image represents sea surface temperature near the East coast of the United States. The calibration bar relates pixel brightness to the temperature in degrees Celsius.

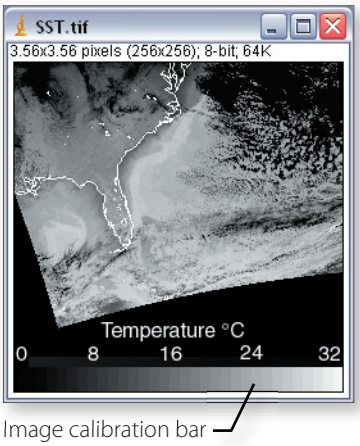
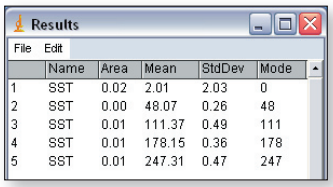


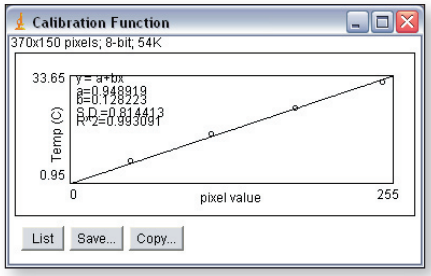
Image calibration bar

1. Choose Analyze > Clear Results to delete any previous measurements.
2. Use a selection tool to select a small portion of the calibration bar on the image, and press **M** to measure the average pixel value of the selection. Repeat this for one or more other regions of known temperature.

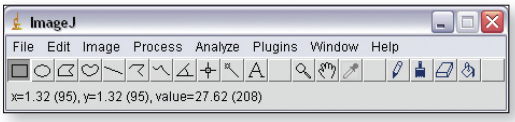


	Name	Area	Mean	StdDev	Mode
1	SST	0.02	2.01	2.03	0
2	SST	0.00	48.07	0.26	48
3	SST	0.01	111.37	0.49	111
4	SST	0.01	178.15	0.36	178
5	SST	0.01	247.31	0.47	247

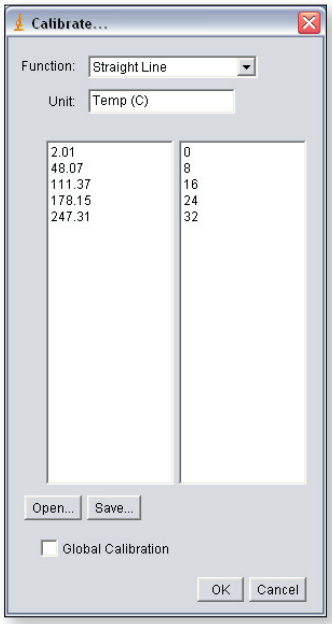
3. Choose Analyze > Calibrate… to open the **Calibrate** window.
4. The average pixel values you measured should be listed in the left-hand column of the **Calibrate** window. Enter the known value (Temperature in this case) in the right-hand column for each measured value.
3. Enter the appropriate units in the **Unit** box.
4. Select **Straight Line** from the **Function** popup menu.
5. Click **OK**. A plot of the calibration function and a list of pixel values and their corresponding calibrated values are displayed. Close these windows.



As you move the cursor around the image, the status bar will now display both the calibrated and uncalibrated pixel values. Pixel measurements (mean, minimum, maximum, etc.) will also be made using calibrated values.



The Calibrate window



Calibration functions

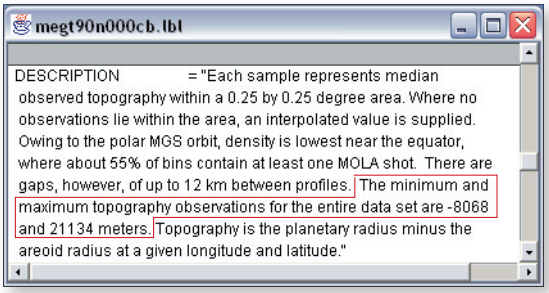
Not all image calibrations use a straight line function. In general, you should choose the simplest function that "fits" the calibration data you entered.

The correlation coefficient, labeled R^2 on the calibration plot, tells how well the function you chose fits the data. The closer this value is to 1, the better the fit. (A correlation coefficient of 1 represents a "perfect fit." However, don't get too excited if your $R^2 = 1$. With only two points, you will *always* get a perfect fit, but that doesn't mean that your calibration is perfect!)

Density calibration from image metadata

In this example, you are working with an image of the surface of Mars. The pixel values represent elevation (dark = low, bright = high).

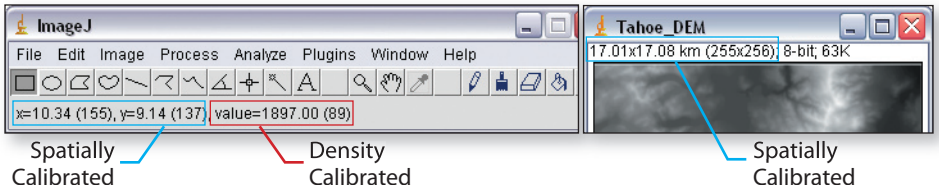
The image is accompanied by a label (.lbl) file containing image *metadata* (information *about* the image data). From this file, you learn that the elevation values in the image range from -8068 meters to 21134 meters. The minimum and maximum values in the image range from 0 to 255.



1. Choose Analyze > Calibrate… to open the **Calibrate** window.
2. Enter the known minimum and maximum pixel values in the left-hand column and the corresponding minimum and maximum elevation values in the right-hand column.
3. Enter the appropriate units in the **Unit** box.
4. Select **Straight Line** from the **Function** popup menu.
5. Click **OK**. A plot of the calibration function and a list of the pixel values and their corresponding calibrated values are displayed. Close these windows. The ImageJ status bar and **Results** window will now display calibrated pixel values.

How to tell if an image is calibrated

Spatially calibrated images show X and Y in both calibrated units and pixels in the ImageJ status bar. The image window status bar also shows the dimensions of the image in calibrated distance units.



Density calibrated images show pixel values in both calibrated units and raw values in the ImageJ status bar.

Removing density calibration

To remove the density calibration from an image:

- Choose Analyze > Calibrate…
- Set the calibration function to None.
- Click **OK.**

Be an image detective

Spatial and density calibration data can come from many sources. It may be found in separate label (.lbl) or header (.hdr) files, or it may be embedded at the beginning of the image file itself or in the image documentation. You may even be able to get the information from the person or agency that provided the images.

The Calibrate window

To enter or change values in the **Calibrate** window, click in a column and type the number. Press the **Return** key to enter the next value.