

## Measuring Area Using Thresholds

### Threshold Introduction

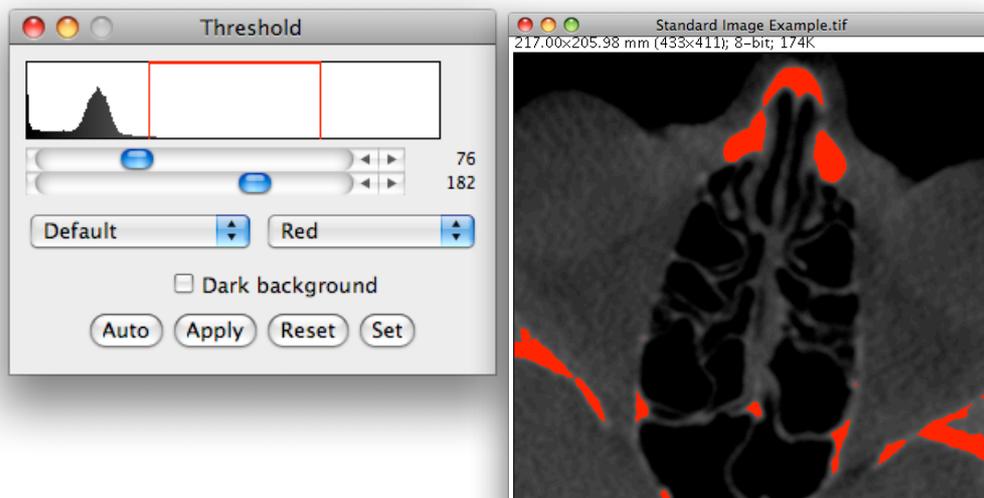
In 8 bit greyscale images (and colour images, but more of that later) there are 256 ( $2^8$ ) intensity graduations which can be assigned to a pixel. A pixel with an intensity of 0 is black, a pixel with a value of 255 is white, everything in between is a shade of grey. Images with 16 bits have 65,536 ( $2^{16}$ ) intensity graduations, with 0 still representing black but 65,536 representing white.

Thresholding works by separating pixels which fall within a desired range of intensity values from those which do not, (also known as 'segmentation'). In the ideal world the pixel intensity values of the feature(s) you're interested in will be a unique subset of the image, and thresholding them from the other features in the image is easy. In practice it seldom is.

Thresholding can be a very effective method of measuring complex or disjointed features in an image. A few keystrokes can measure what would have been a near-impossible task by hand. But there is a catch - potentially quite a big one. When we look at an image we are extremely good at separating out what we are interested in, irrespective of how complex the image may be. Image analysis software has no intuitive moves up its sleeve, it will take all the information in an image and treat it literally. If a stray pixel falls within a threshold range it will be counted, even if to us it's obviously a false positive.

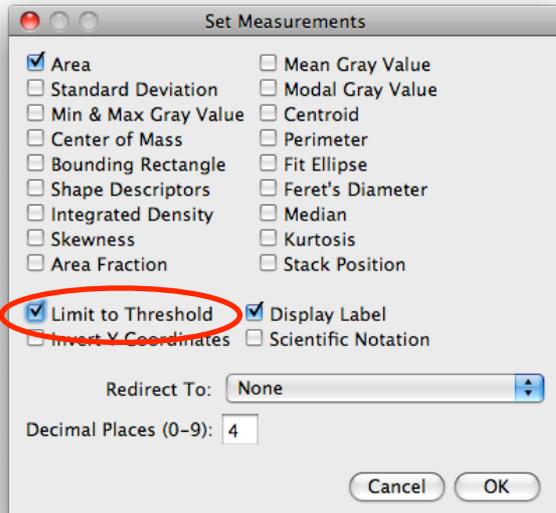
### Example

In this example an image has been chosen that 'behaves' quite well, in many cases additional steps will be required to produce reliable thresholding results.



Activate thresholding (Image / Adjust /Threshold ... ) and the window above appears. The histogram represents the distribution of pixel intensities in the image, black towards the left (0) and white at the right (255). Dragging the sliders selects different regions within the greyscale, pixels in the image which fall in the selected range are highlighted red. In this case, all the pixels between 76 (dark grey) and 182 (mid grey). Discontinuous regions can be selected and those with complex boundaries delineated from the background.

The selected area can be measured using the usual measurement option (Analyze / Measure menu option or apple / m keystrokes) but first measurements must be limited to only the selected pixels. Open the Analyze / Set Measurements menu option and tick the limit to Threshold box. Press Ok to save the changes and then make your measurement.



Label	Area
1 Standard Image Example.tif	44696.9669

Without the Limit to Threshold option ticked the entire area of the image (44,697 mm<sup>2</sup>) is measured.

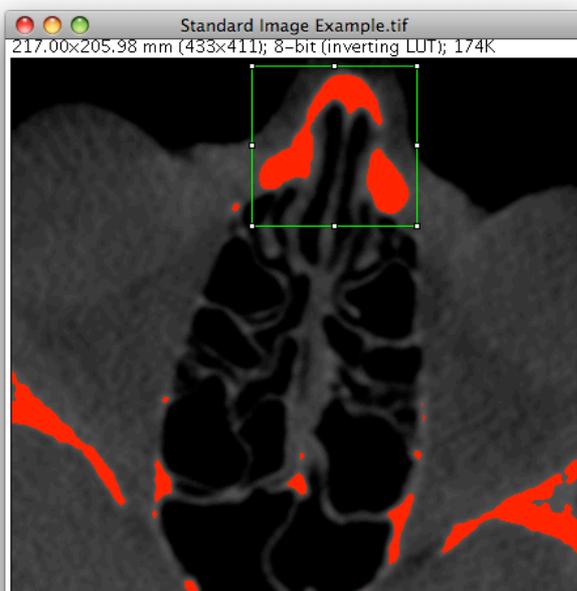
Label	Area
1 Standard Image Example.tif	1595.3605

Ticking the Limit to Threshold option measures only the selected thresholded areas of of the image (1,595 mm<sup>2</sup>).s

The Results window will appear and can be saved as usual for future use.

### Combining Thresholding and ROI

Although thresholding is very useful, as can be seen in this example if you were trying to measure the area shown in the previous example you would be including areas outside your ROI. Eliminating these from measurements can be relatively easy, or next to impossible. With image analysis there is a great diversity in images and what people want from them, consequently there are often no standard answers. Imagination and perseverance are often your best options.



To remove the unwanted areas at the bottom of the image, use a selection tool to mark your ROI, either before or after you threshold it.

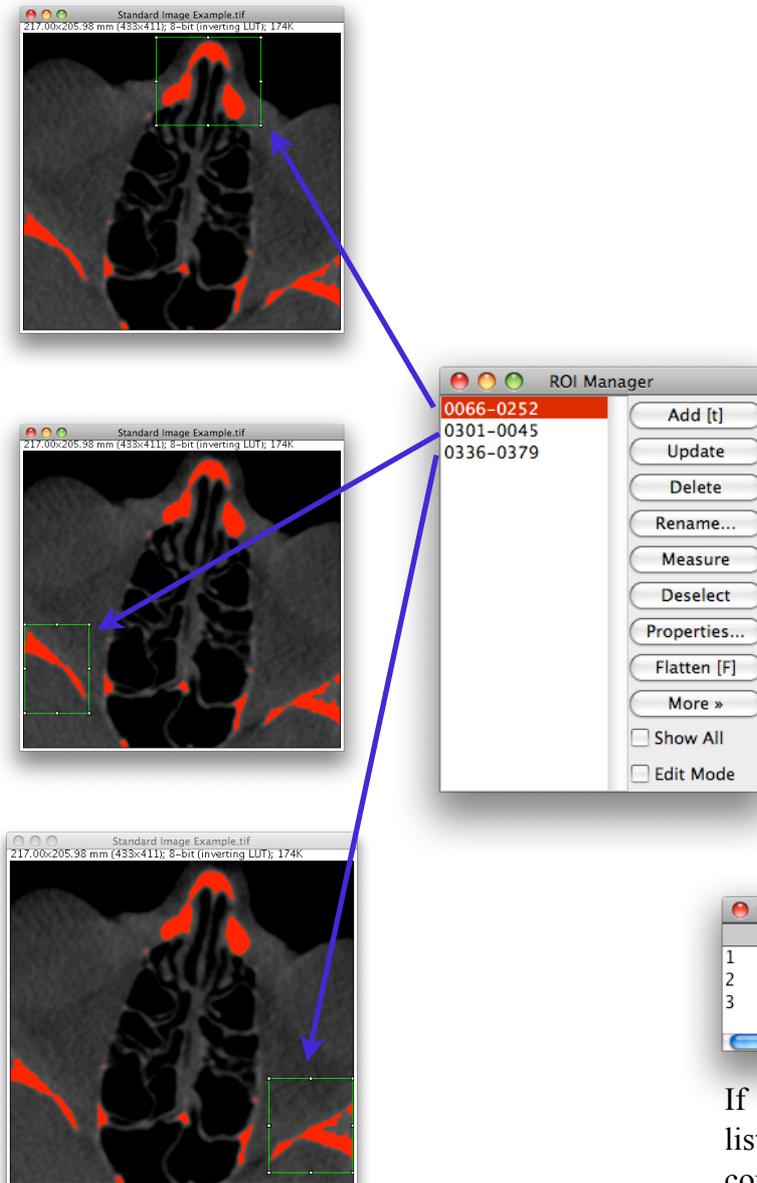
Measurements will now be limited to pixels which (1) fall within the selected area **and** (2) are within the selected threshold intensity range.

Both selection area and threshold ranges can be adjusted 'live' and new measurements taken by pressing apple / m. Each new measurement is added to the Results window.

## Combining Thresholding and Multiple ROIs

If you want to measure multiple ROIs then ImageJ's ROI Manager is a powerful tool for marking and measuring many separate regions within an image, or a stack of images.

Activate the ROI Manager via the Analyse / Tools / ROI Manager ... menu path.



Select a ROI as described earlier and add it to the ROI Manager's window by pressing 't', or clicking the button in the window.

Select a new ROI (with any of the selection tools) and add them to the ROI Manager's window. Repeat as required.

Using the ROI Manager's option buttons ROIs can be renamed, their boundary colours changed and so forth.

A ROI will only be visible in the image if it is highlighted on the ROI Manager's list.

	Label	Area
1	Standard Image Example.tif:0066-0252	771.5597
2	Standard Image Example.tif:0301-0045	335.5481
3	Standard Image Example.tif:0336-0379	450.0765

If multiple ROIs are marked on the list (Command key to make non-contiguous selections and Shift for contiguous selections.)

As with any part of ImageJ, (or other image analysis programs), explore the menus of the ROI Manager as it contains other features which may be of use to you. One of the reasons instructional documentation can be sparse is it's impossible to anticipate all the possible uses it will be put to. Your images and information you wish to extract from them may be unique so a customised method may be the only option.