

Making a Look Up Table (LUT) Using Fiji

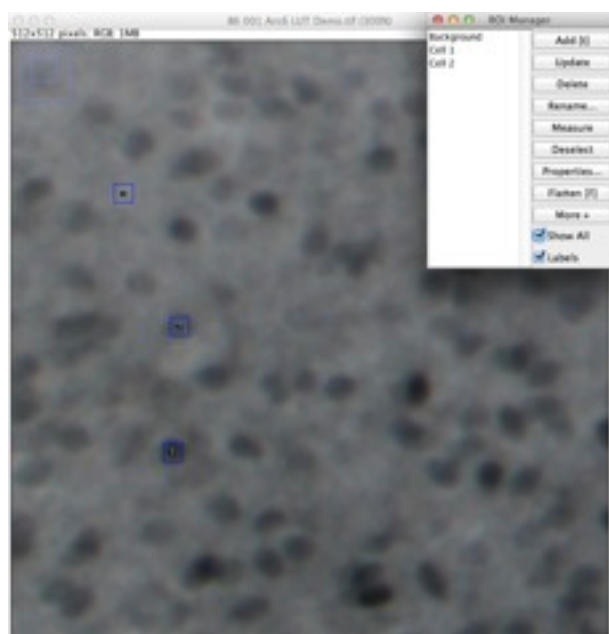
Download the open source software Fiji from <http://fiji.sc/wiki/index.php/Fiji> It's essentially the same as ImageJ, but comes with many pre-installed plugins and can update itself automatically.

Introduction

Look up tables (LUTs) can be very effective at highlighting features of interest in greyscale images. We're poor at distinguishing differences in shades of grey, so it can become very subjective as to whether a zone of grey is 'in' or 'out' of what we want to analyse. On the other hand, we're very good at distinguishing colour and relate to it much easier than we do to grey. The trick therefore is to colour the features we're interested in - but as objectively as possible.

Determining Greyscale Characteristics

Once an image has been turned into an 8 bit greyscale Image / Type / 8 Bit, the next step is to characterise the greyscale intensity of the feature(s) we're interested in. It is not recommended this is done 'by eye', but by selecting the area(s) and measuring the statistics with regards mean intensity and possibly standard deviation and maximum / minimum.



In this example, the image contains background, light grey cells (Cell 1) which are not wanted for analysis and dark grey cells which are (Cell 2).

Each area has a small region of interest (ROI) marked on it and added to the ROI Manager (Analyse / Tools / ROI Manager).

The Analyse / Set Measurements options are set to measure mean greyscale value. Choosing 'Measure' in the ROI Manager will produce a results' window showing the mean greyscale value for each ROI.

In this case the background has a mean pixel intensity of 128 and ranges from 121 to 133. Cell 1 has a mean of 85 and ranges from 76 to 95. Cell 2, the one of interest, has a mean intensity of 59 and ranges from 48 to 77.

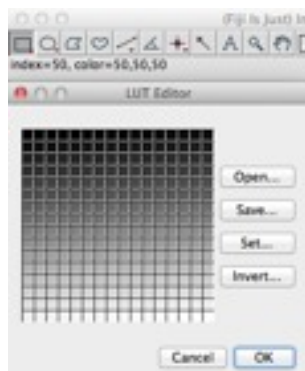
Results				
Label		Mean	Min	Max
1	B6 001 Arc6 LUT Demo.tif:Background	128	121	133
2	B6 001 Arc6 LUT Demo.tif:Cell 1	85	76	95
3	B6 001 Arc6 LUT Demo.tif:Cell 2	59	48	77

This indicates that a selection range from 50 to 70 would be a good starting point to highlight the cells of interest and avoid both the background and the unwanted cells.

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Making a Customised LUT

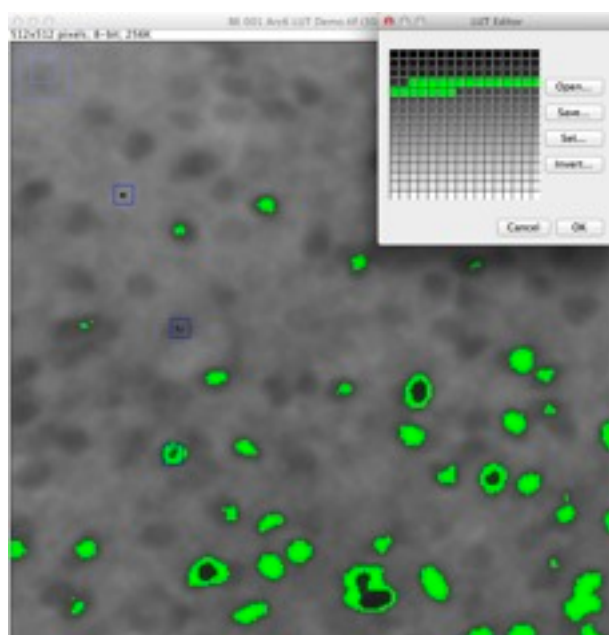
Using the example above, a customised LUT is made by using the Image / Colour / Edit LUT option. To select the intensity range required, in this case 50 - 70, move the cursor over the table and note the 'colour= x,x,x' values in the main menu. These represent the RGB values, but in a greyscale image will all have the same value.



When the appropriate starting value is reached, click on the square and drag the cursor until the upper intensity value is displayed, 70 in this example. This opens the colour editor, which determines the colour(s) assigned to this intensity range.



Choose a starting colour for the beginning of the intensity range (50) and press 'Ok', which brings up the colour to assign to the final intensity (70). If you choose the same colour the whole range from 50-70 will be the same. Choosing a different final colour produces a colour range between from 50-70. Your call.



In this example some of the marked cells have intensity areas lower than the assigned LUT. It can be edited to suit, or the holes filled later in a binary image, using Process / Binary / Fill Holes.

If the lighter grey cells were to be filled, the LUT can be re-edited and another colour range added as suits.

Try to avoid using red as a LUT colour as it's often the threshold marker and having red on red makes for a frustrating exercise.

A custom LUT can be saved and re-applied to other images.

Note: Using a LUT does not alter the underlying pixel values, it simply applies a false colour to make it easier to distinguish. The pixel value (ie. 1-255 in an 8 bit image) determines the intensity and is unchanged. This means quantification can still be done when a LUT is used.