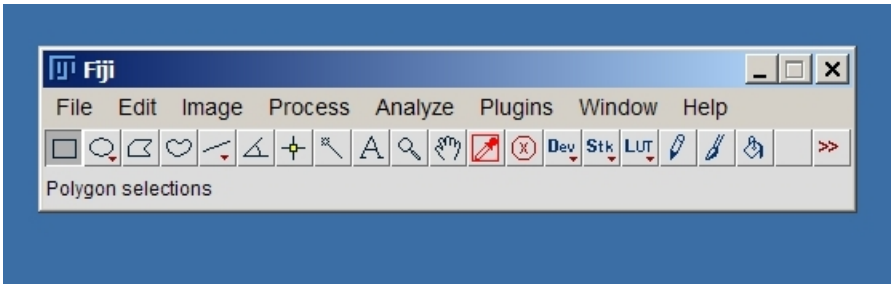
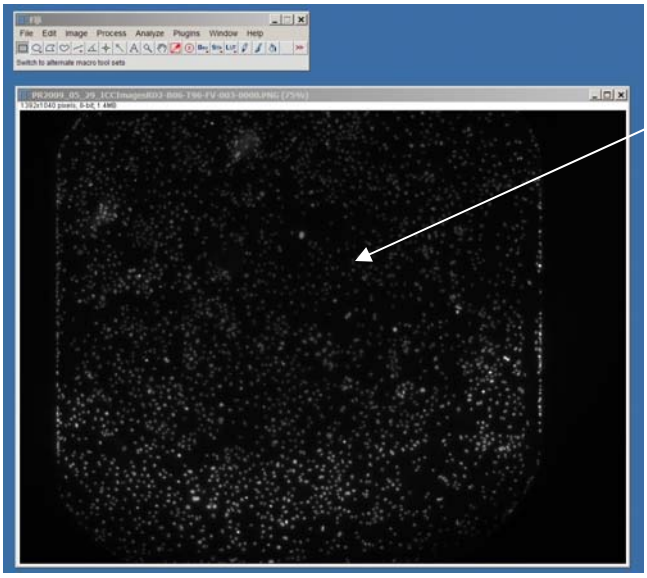
Image Enhancement & Overlay – (Visible and Fluorescent Images)

In many experiments in live cell imaging, a series of images are taken both in visible and any number of fluorescent images. Cells may be counted, or tracked, based on a fluorescent label, or to measure cell death vs cell division. Fiji or ImageJ can display an overlay of the fluorescent images on the visible image. This allows the researcher to see the fluorescent label with respect to all the cells in the image.

Fiji is an image processing package based on ImageJ
http://pacific.mpi-cbg.de/wiki/index.php/Main_Page



First load an image: File > Open



In general, when taking fluorescent images, it's always better to expose the cells to as short an exposure as is necessary, since you may damage the cells. In order to see all the signal, the image may need to be enhanced.
Be careful, you may see something that really isn't there !

For more information, please contact:
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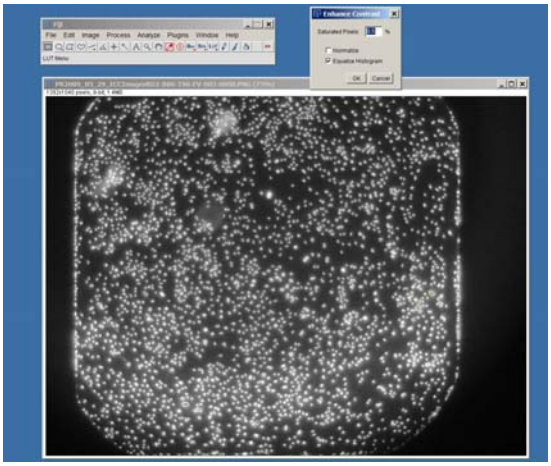
In order to see all the signal in the image, one method is to Equalize the Histogram. That basically means to stretch the signal from 0 to 255.

Process > Enhance Contrast Select Equalize Histogram

Equalize Histogram



Take a look at Wikipedia for a good description of these image enhancement processes.

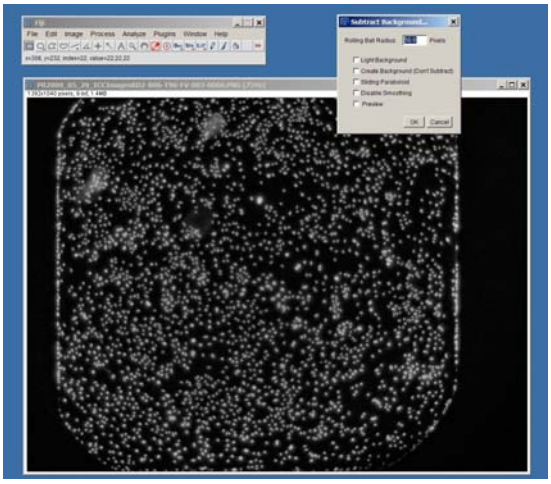
http://en.wikipedia.org/wiki/Histogram_equalization

Or

http://imagejdocu.tudor.lu/doku.php?id=gui:process:enhance_contrast

Process > Subtract Background

Subtract Background



The cells are now separated and are easier to see. It would be much easier to count these cells or possibly track these cells over time, with just a few enhancements.

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Our goal is to overlay this image onto a second image, we can also add color to the image.

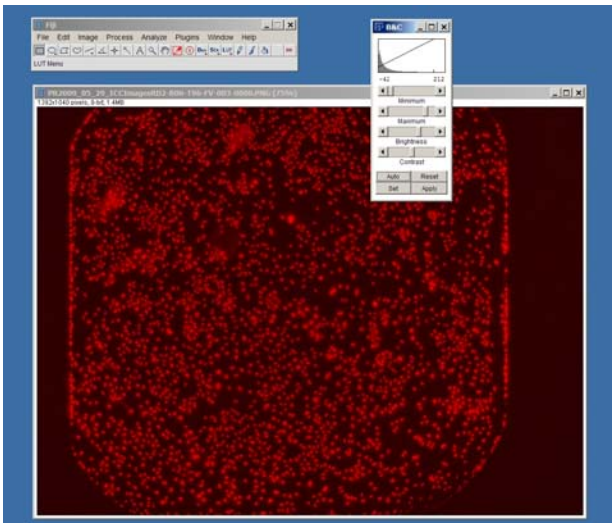
Image > Lookup Tables > Red

This process just adds color to a gray scale image, it's a nice feature to select a color that matches the fluorescent image.

Image > Adjust > Brightness / Contrast

The grayscale image is now colorized, but you may need to increase the brightness, to more easily locate the individual cells.

Colorize and brighten



This process should be repeated for each image that you want to overlay. For example, you may want to overlay a fluorescent Red and a fluorescent Green over a visible image.

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Here is a quick way to overlay a set of images, use Merge Channels

Image > Color > Merge Channels

You can select which image is what color, Red, Green, Blue or Gray

Image > Color > Channels Tool

Image 1 & Image 2

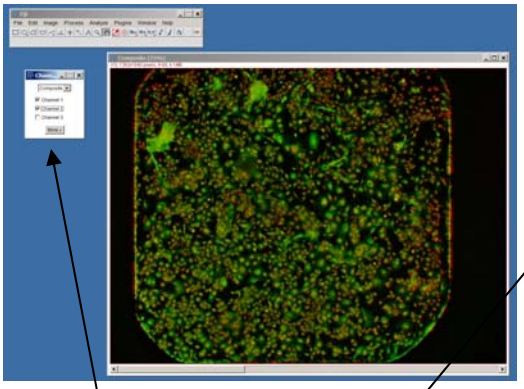
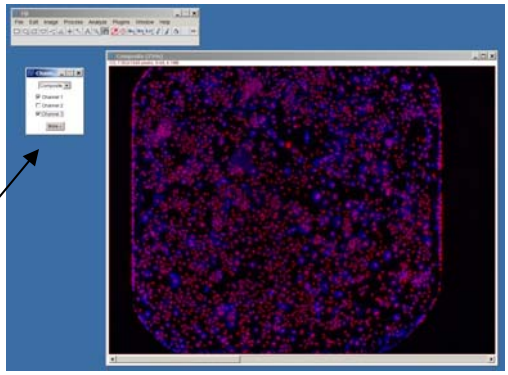
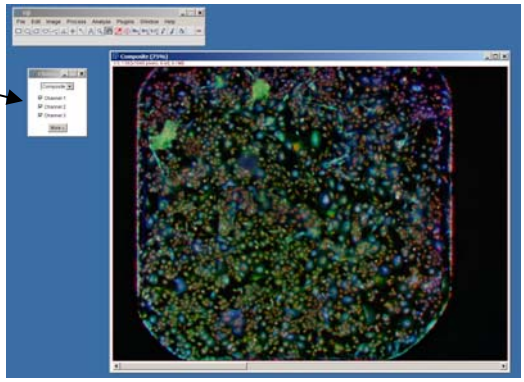


Image 1 & Image 3



To turn on or off specific channels

Image 1, Image 2 & Image 3



By turning on and off the channels, you can observe which cells are fluorescent

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