INTRODUCTION TO IMAGEJ

The open source software imageJ is a great tool for image manipulation and analysis and is commonly used by microscopists worldwide. Not only is it very powerful and versatile, allowing the user to create their own macros and plugins, it is also completely free.

ImageJ can be downloaded from this address:

http://rsbweb.nih.gov/ij/download.html

In this short tutorial you will learn to do two basic things, both very important for this course. There are several other functions in imageJ you might be interested in depending on your project later in the course.

This tutorial was written using ImageJ 1.44p on a 64bit windows 7 computer.

MAKING COLOURED PICTURES FROM BLACK AND WHITE IMAGES

This guide describes how to apply artificial colours to gray scale images and assemble these into a single image. This is necessary since most high performance fluorescent microscopes use black and white cameras but many fluorescent samples are multicoloured.

1. Open your images in imageJ
2. Select the first image and open the contrast adjuster (*Image > Adjust > Brightness/Contrast* or ctrl+shift+C)
3. Adjust the contrast so the image looks nice (choose an interval that best fits the investigated sample)
4. Go to *Image > Lookup tables* and choose a colour table that looks nice (preferably one that fits the dye)
5. Go to *Image > Type* and pick *RGB colour*.
6. Do steps 2-5 to the other image(s) as well
7. Make sure you only have the correct images open
8. Choose *Image > Stacks > Convert Images to Stack*
9. Choose *Image > Stacks > Z project*
10. Choose an interval that includes all slices and use average or max intensity
11. Click OK
12. Save the image (the colours can be adjusted in *Image > adjust > colour balance)*
13. Don’t forget a scale bar!

SCALE BARS

Without a scale bar a microscopy image does not say much. There are several ways to add a scale bar in imageJ but all of them rely on you knowing distances in the image. There are many ways to know distances in the image if you have knowledge of your sample, for example:

1. Knowing the pixel size of your camera and the magnification of your optics.
2. Knowing a distance in the sample (e.g. channel width in microfluidics).
3. Calibrating the camera and optics with reference samples.

In the first case, if you know the pixel size of your CCD and the magnification of your optics system you can calculate what distance each pixel in your image corresponds to in reality. A decent approximation of your imaging system's magnification is to simply use the magnification of your objective. For instance, using the 60x water immersion objective and the iXon camera with its pixel size of 10µm, you get that each pixel in the image corresponds to 10/60µm ≈ 170nm.

In the second case you can measure a feature of known size in your image and then use this measurement as base for your scale bar.

In the third case, you can image a sample with known features and use this to measure features in your image. The software AndorIQ used for two of the microscopes in this course has a feature which allows you to save such measurements and measure on screen during image capture. For this reason there is a special microscope slide with an engraved micrometer ruler in the lab.

Using imageJ is of course not the only way to go. In any software where you can add a rectangle to your image you can make a scale bar. Just make sure it is the right size, even if you change the size of your image.

ADDING SCALE BARS

1. Open your image
2. If you know your pixel size:
    a. Click *analyse=> set scale*
    b. Fill out the form (pixel distance = 1, known distance = your distance, pixel aspect ratio = 1 (i.e. square), unit = your unit)
    c. Go to step 4
3. If you know a distance in your sample (either due to its features or a measurement in AndorIQ):
    a. Select the line tool
    b. Draw a line of known distance
    c. Click *analyse=> set scale*
    d. The length of your line should show up in known distance
    e. Fill out the rest of the form (known distance = your distance, pixel aspect ratio = 1 (i.e. square), unit = your unit)
    f. Go to step 4
4. Now imageJ knows the scale of your image.
5. Click *analyse => tools => scale bar* and fill out the form as you see fit.