Analysis

Task 6. Make a composite image

If you haven't already done it, visit the Molecular Probes and Nikon sites to look at the spectra for the three fluorophores and the properties of the Nikon filter sets.

1. Make a so-called "merge" file from your three fluorescence images. To do this, open all 3 images in ImageJ, then select "Image>Color>RGB merge…". If you put the DAPI image in the blue channel, the fluorescein image in the green channel and the rhodamine image in the red channel, your composite merged image will have pseudocolor representations of the three fluorophores that are close to the real colors. If you took the three images without moving the specimen, this image should show you the relative distributions of dsDNA, tubulin and F-actin in the cells. To save the image with a reasonable file name, use File>Save As. Save pictures for data analysis as TIFF; use JPEG for your lab report.
2. Consider the possibility that blue excitation can cause rhodamine to fluoresce. To what extent will this cause what is called "bleed-through" of rhodamine labeled areas into the "fluorescein" image? Can you think of a way to determine if this is something that could cause difficulty in fluorescence quantitation when data on multiply stained cells is collected?

Task 7. Analyze your image

You have several time-lapse data sets. You want to extract fluorescence intensity values from regions of interest for further analysis. You can do that by hand for each image, but that would be very tedious and runs the risk of inconsistent collection from exactly the same regions in each image of the series. ImageJ has a plugin called Multi Measure that will make this task easier.

1. If it is not already on your computer, you need to download an ImageJ plugin that has the ability to collect data from a series of images for a number of different regions of interest. The plugin is called "Multi Measure" and can be gotten at http://www.optinav.com/imagej.html. Download the Multi_Measure.jar application and put it into the plugins folder under Micro-Manager or ImageJ. Restart Micro-Manager or ImageJ. Now you should find Multi Measure under the ImageJ "Plugins" menu item. Multi Measure is preloaded on the Bioimaging workstation computers.

   To use Multi Measure, follow the instructions that are outlined at the McMaster University site on ImageJ add-ons:

   http://www.macbiophotonics.ca/imagej/intensity_vs_time_ana.htm#IntvTime_1ROI.

   These instructions are given below with modifications.

   a. Open your time lapse series using ImageJ's "File>Import>Image Sequence...". Select the first image in the series and click "Open". You want to import all of the images, so the starting image is 1 and the increment is 1. With the Sort Names Numerically turned on, the image sequence will sort from the first to the last image as you want.

   b. Open "Multi Measure" plugin ("Plugins>Multi Measure").

   c. Select your image sequence window ("Window>your file name ").