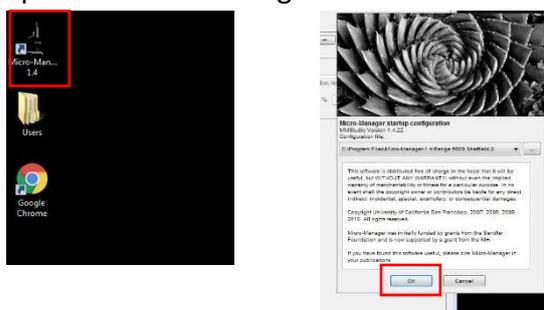


Olympus Epifluorescent Microscope – Walk Through

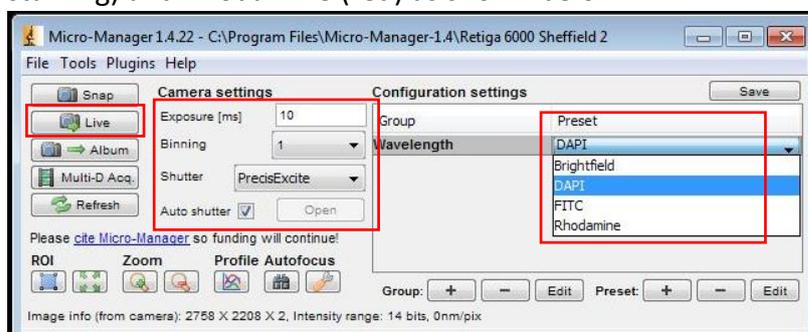
A. Turning on the equipment

1. Turn on the PC and monitor
 2. Turn on the Retiga 6000 camera, located on the left hand side of the microscope, by pushing in the small button on the camera marked on/off
 3. Turn on the brightfield LED light by pressing the blue button on the box on the right hand side of the microscope
- N.B. You only need to do this if you are going to require brightfield images (i.e. “normal” microscope images). It is not necessary to do this if you only want fluorescent images.
4. Open the micromanager software on the PC desktop, and click OK



B. Viewing your sample via the eyepiece

5. Select the following options in micromanager
Binning – this won't have any effect on your eye so doesn't matter what you set here
Exposure – this also won't have any effect on your eye
Shutter – PrecisExcite
Auto shutter - select
Preset – select an appropriate preset channel for your sample in the configuration settings from the dropdown list.
You can choose between brightfield, DAPI (for blue stained samples), FITC for (green staining) and Rhodamine (red) as shown below.



6. Slide the knob on the right hand side of the microscope fully forward to the  position (light will go to the eyepiece rather than the camera).
7. Click “Live” on the micromanager window and look down the eyepiece. Move your sample on the stage as required to find the appropriate area and focus using the microscope focussing knob.
8. If you wish to check a second colour in this way, change the preset to the new channel and repeat the process.

C. Viewing your sample with the camera

9. Select the following options in micromanager

Binning – start with this at 1 but you can increase it later if your signal is weak

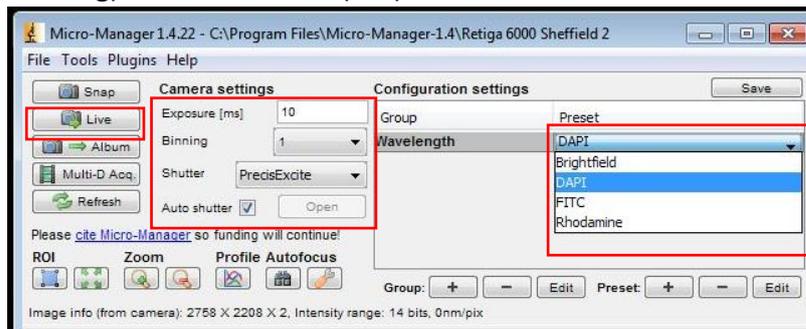
Exposure – start at 10ms for DAPI and 150ms for FITC or Rhodamine channels, but you will probably need to change this later depending on the intensity of the signal

Shutter – PrecisExcite

Auto shutter - select

Preset – select an appropriate preset channel for your sample in the configuration settings from the dropdown list.

You can choose between brightfield, DAPI (for blue stained samples), FITC for (green staining) and Rhodamine (red) as shown below.



10. Slide the knob on the right hand side of the microscope fully backward to  the position (light will go to the camera rather than the eyepiece).
11. Click “Live” on the micromanager window and watch the screen. Move your sample on the stage as required to find the appropriate area and focus using the microscope focussing knob.
12. If you wish to check a second colour in this way, change the preset to the new channel and repeat the process.

D. Obtaining one colour images from samples

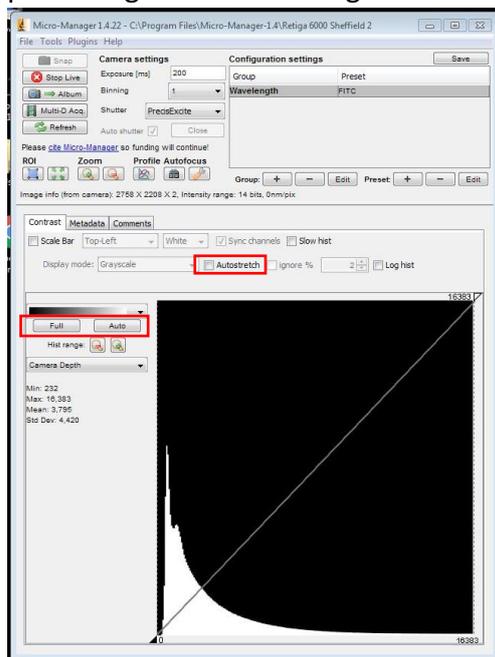
13. Use the “Live” procedure using a camera, described above, to obtain an image for the single colour required.

N.B. All images generated using the Live procedure will be greyscale rather than coloured images but this can be change later in ImageJ (see step 18 for how to do this).

14. Optimise the image, by increasing the exposure time for less brightly stained samples and decreasing the exposure time for more bright samples. If the signal is low you can also increase the binning (shown below), this will improve the signal to noise ratio, but take care with the highest binning values as these can cause your image to start to look pixelated.

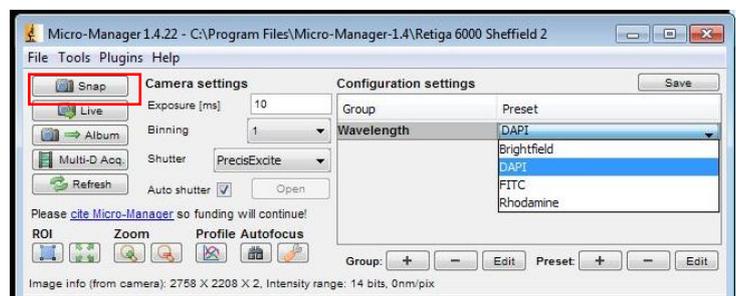


15. You can also modify the appearance of the image by changing the histogram display options, as highlighted below. This allows you to set lower and upper clipping levels - pixels values lower than the black triangle will appear as black, pixel values higher than the white triangle will appear as white. The steeper this line, the higher the contrast: moving the line to the right will darken the image, while moving it to the left will brighten the image. The "Full" button sets the lower and upper clipping levels to the camera's full intensity range. The "Auto" button will adjust the levels automatically, based on the extreme pixel values in the image. For "Live" imaging you can perform this adjustment continuously by checking the "Auto-stretch" box (highlighted below); once checked you can then set a percentage of outliers to ignore.

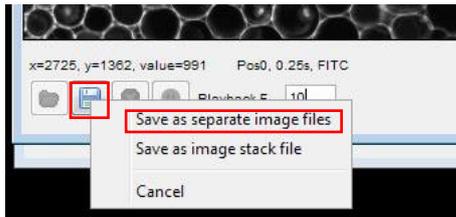


Note however that changing the histogram settings this does not change the raw data, only the way that the image is presented on the screen.

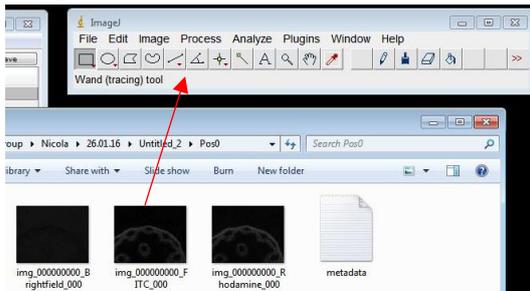
16. To save an image either click "Album" on the image (can keep doing this to save a number of images), or "Snap" on micromanager window (this takes a new image)



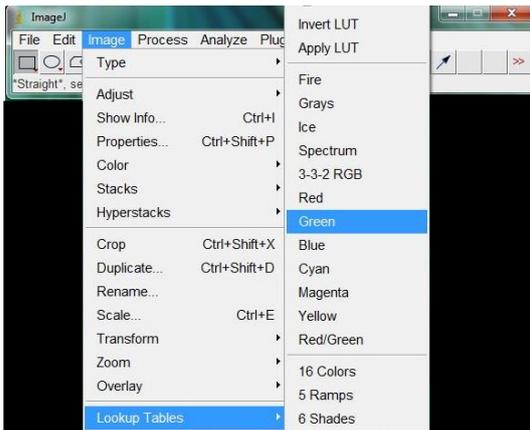
17. Then click the floppy disc on each image generated and select “Save as separate image files”, into your own folder. **Please create a new folder for each imaging session, with the date in the title for the folder.**



18. To add colour to a saved image, go to the “users” folder on the desktop and find your image. **DO NOT** double click to open the image, instead drag and drop the file onto the ImageJ bar.



N.B. Don't worry if the file looks black or very dark in the folder before you open it, unless you are viewing files as extra large icons you will probably not be able to see any image. In ImageJ select Image, Color, Look up Tables and choose the required colour from the list (Green in the example below), then save the coloured image.

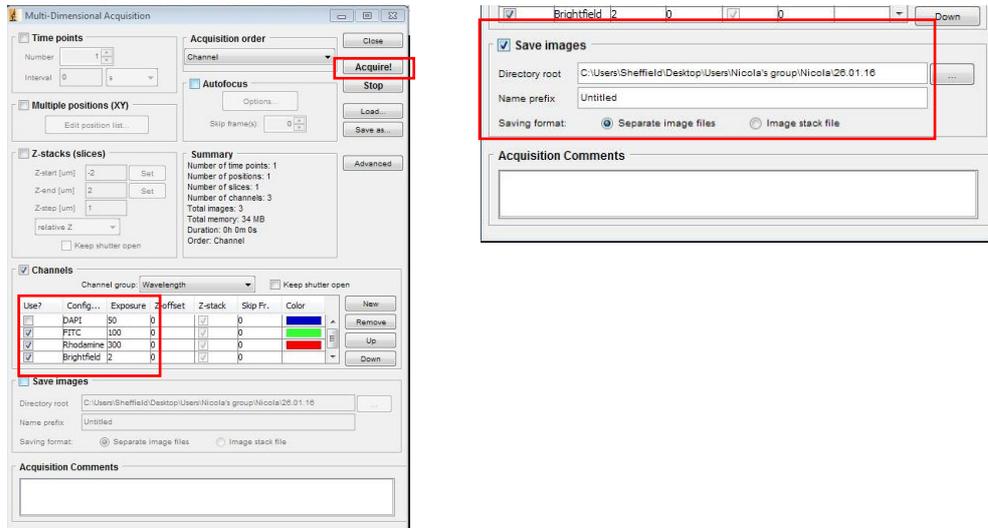


E. Obtaining multi-colour images from samples

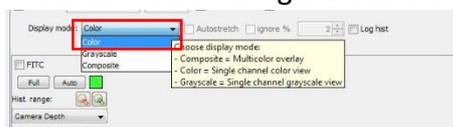
19. It is probably easier to first use the Live single colour method (section D) to determine the exposure time required for each channel separately. Once the exposure times have been determined, select Multi-D Acq to obtain an image with two or more colours, or fluorescent and brightfield together.



20. Select the channels required for your image, set the exposure time in the table for each channel and click “Acquire”. If you wish to autosave all images generated in this way you should also select “save images”, enter the correct location and name prefix if required for these images and ensure that you have chosen “separate image files”

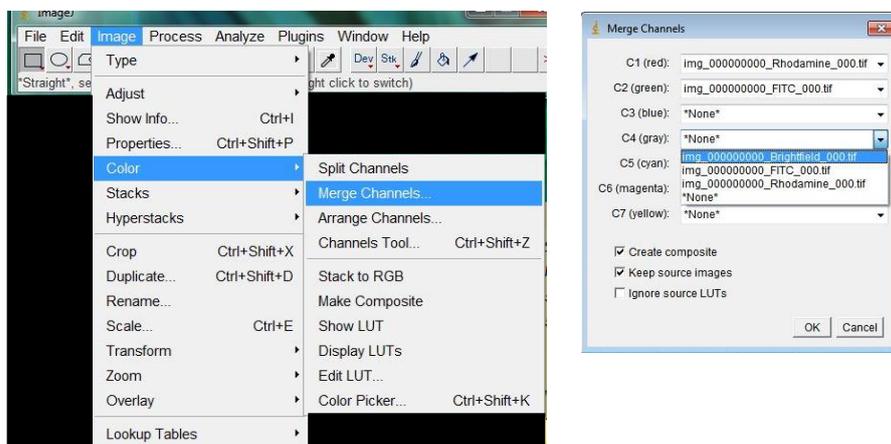


21. Once the image is obtained make sure that you select “Color” in the display mode option on the main micromanager window.



22. Save the image by clicking on the floppy disc icon at the bottom of the image (if you did not choose the autosave option) and choose to save as “separate image files” (see step 17).
 23. To open image and generate a composite from these separate files drag and drop the appropriate files into ImageJ (see step 18).

When you have transferred all the images for each of the channels into ImageJ select Image, Color, Merge Channels. Then assign the correct images to the correct colours from the dropdown list, select “Create composite”, “Keep source images” and “OK”. Remember to save the image that you have generated.



F. Shutting down the equipment

24. When you have finished, make sure you have saved all the images you need, exit the software, turn off the camera and the brightfield LD (if used).
25. Remove your samples, replace the dust cover over the objectives, clean up any spills and tidy the area.
26. Shutdown the PC and monitor.