Operating Instructions for CCD2

Turn on system

- Turn on fluorescent arc amp (flick rocker then hold down red button), turn on digital camera, then turn on main green rocker on lower-RHS of microscope.
- Log into network (as yourself) then PC (as PGUEST)and open Metamorph (This microscope **must** be completely turned on **before** you open Metamorph). Open the Acquire window from the main menu bar, and set the Acquire window 'illumination' drop-down menu to 'current shutter'.
- Click the Display tab in the Acquire window and tick the 'autoscale' box.
- Place your slide on the stage and select an appropriate objective from the drop-down menu in the main menu bar of Metamorph.

View your image with binoculars:

- Click eyepiece.jnl
- Set 'illum' drop-down menu on main menu bar to desired illumination; brightfield, phase or specific type of fluorescence.
- Set 'current shutter state' button (found on task bar on RHS of Metamorph window) so that it looks green/grey = active, then look down binoculars.

View/capture your image in Metamorph:

- Click camera.jnl
- Set 'illum' drop-down menu on main menu bar to desired illumination; brightfield, phase, DIC or specific type of fluorescence.
- If doing brightfield, phase or DIC, then press the colour temperature button (3200K) on the RHS of microscope.
- Click the 'autoexpose' button to establish effective exposure time. This will capture an image (that may not be in focus) and the resulting exposure time will be automatically entered in the exposure box.
- Click 'show live' button in the Acquire window. Adjust focus using the 'show live' window. (If the shutter speed is too slow then increase the live binning value and/or reduce the exposure time).
- Click the same button or F2 on keyboard to close live window.
- Click the Acquire button in Acquire window to capture the image.
- To adjust contrast/brightness, click on the display tab in the Acquire window, uncheck the auto-scale box and use the sliders to adjust grey levels. This will NOT change the actual image, only the way it is displayed. You should standardise grey levels for controls/experiments in order to make accurate comparisons.
- Save the acquired image to your personal folder on the appropriate server (permanent), or to SCRATCH disc (deleted every Monday).

Capture Multiple Wavelengths:

Open the Acquire Multiple Wavelengths box from the acquire section of the main menu bar in Metamorph. Choose how many channels and select which type of illumination you want from the drop-down menu on each channel..Initially turn on the autoexpose for each wavelength. Focus using the live window before capturing the stack. Save the resulting stack (*stk file).

Combining Images

Colour Combine: Open all the images you wish to combine. Open the Colour Combine dialogue box from the display section of the main menu bar. Choose the source (individual images or images in a stack). Pick the image appropriate to each colour (RED, GREEN, BLUE) or leave as 'none'. Combine the images and save the result (as a *TIF). Open the Scale Image dialogue from the display section of the main menu bar and adjust the scale for each channel. Even when saved, this will NOT change the image itself, only the way it is displayed.

Overlay: Open all the images you wish to combine. Open the Overlay dialogue box from the display section of the main menu bar. Choose the source (individual images or images in a stack). Overlay the images and save the result (as a *TIF). Open the Adjust digital contrast dialogue from the display section of the main menu bar and adjust the contrast for each channel. Adjustments are permanent once the image has been saved.

After you've finished

- Check CCD2 bookings on BRIAN; if microscope is booked during next couple of hours then leave everything turned on, just log out of the computer. If no-one has booked after you for two hours or more, then log out of computer and turn off microscope, camera and arc lamp, and start the stopwatch.
- Clean objectives with **lens tissue**, clean stage and bench with normal tissue. Throw away tissues/lens tissue in green bins; slides and coverslips in orange glass bins.
- Please put blue plastic cover over microscope optics, but please avoid contact of the plastic cover and the hot arc lamp!