CCD1: Fluorescence Photography

Microscope

- Push slider on LHS of actual camera IN (this pushes colour filters OUT of light path)
- Check main green rocker on microscope is OFF
- Turn on fluorescent lamp power unit
- Turn on UniBlitz (=shutter between arc lamp and microscope)
- Make sure switcher box is set to MM (not SPOT)
- Push the slider on top-RHS of microscope IN to see down binocular eyepiece
- Place your slide on the stage
- Use the filter wheel to select appropriate filter cube
- Flick switch on UniBlitz (N.C - N.O) to open fluorescent shutter; you should now see fluorescent light beam
- Focus and select field of view

Computer

- Pull the slider on top-RHS of microscope OUT to send image to camera/monitor.
- Flick switch on UniBlitz (N.O - N.C) to close fluorescent shutter
- Turn on digital camera
- Log in to computer, open **SPOT**
- Select preset from drop-down list in bottom right corner of SPOT interface
    - 12 B (12 bit blue fluorescence)
    - 12 G (12 bit green fluorescence)
    - 12 R (12 bit red fluorescence)
    - 12 FR (12 bit far red fluorescence)
- Use **live focus preview** to adjust focus of image on screen
- Hit **F10** to Compute Exposure Time.
- Hit **F9** to capture image
- **Save image** (TIFF) to a folder on your server
- If necessary, do Flatfield correction (follow instructions on screen). This time use presets with FLAT suffix from drop-down list in bottom right corner of SPOT interface
    - 12 (flat) B blue (12 bit blue fluorescence)
    - 12 (flat) G green (12 bit green fluorescence)
    - 12 (flat) R red (12 bit red fluorescence)
    - 12 (flat) FR far red (12 bit far red fluorescence)

After you've finished

- Check CCD1 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 is booked after you for 2hrs or more, then log out of computer, turn off microscope, UniBlitz shutter, 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 avoid contact of the plastic cover and the hot arc lamp!

Troubleshooting

- Choose appropriate neutral density setting on slider on back/top RHS of microscope (bright enough to see specimen, but not too bright or camera will not be able to compute exposure time and/or may cause bleaching)