Fluoro4 Quick Reference Sheet

ACRF Cancer Biology Imaging Facility
Institute for Molecular Bioscience
University of Queensland
14 August 2012

First and foremost: be mindful of what you are doing at all times. All microscopes are delicate and must be treated as such.

SLIDE CLEANING AND PREPARATION

- Make sure your slide is *scrupulously* clean. All dust, old oil and mounting medium should be removed with a little 70% EtOH.
- If you can use a mounting medium that sets hard, please do so.
- Be sure to give your slides and sealants at least eight hours to set. **Do not bring wet slides to the microscope.**

STARTUP

- Sign in to the computer using your IMB LDAP ID and password.
- Set the eyepieces for your comfort.
- If you wish to do fluorescence imaging, double click on the **Lamp On** icon on the desktop.
- I you only wish to do brightfield or phase contrast imaging, double click on the **Scope On** icon.
- Light sources are located under the microscope table and can only be activated after logging in to the computer.
    - The brightfield lamp will come on as soon as you double click either the Lamp On or the Scope On icons.
        - The switch for this light source is on the left side of the microscope along with the brightness control.
        - The brightness control should be set to 12V and left there. If the image is too bright, use the neutral density filter.
    - The fluorescence lamp, as stated above, will only come on after you click on the Lamp On icon.
        - The orange light on the front of the lamp housing should stop flashing after about 30 seconds. If it does not, turn off the power, *wait 5 minutes*, and restart the lamp.
        - This lamp has an internal shutter and neutral density (ND) filters within the lamp housing. These are controlled *via* the switches on the front of the lamp housing.
        - After ninety (90) minutes, the control box to the left of the microscope will start to flash and beep. This signals that the lamps are about to be turned off.
            - If you wish to continue working, double-click the *Lamp On* icon on the desktop.
            - Otherwise, the lamps will be shut down and the control box will enter cooling mode and the lamps will not be able to be restarted for five minutes.

LIGHT PATH

- There are two switches on the right side of the microscope.
    - The bottom switch should always be set to *Eye*.
    - The top switch should be set to *Eye* for observation, and to *Side* for imaging.
- Objectives – always inspect and clean objectives both **before** and **after** a session
    - Dry (these **never** get oil): 4x; 10x; 20x; 40x
    - Oil-immersion (double check these to be *sure* that *Oil* is written on the side of the objective): 100x (highest resolution).
    - The 20x and 40x objectives also have correction collars to correct for differences in coverslip/plate bottom thickness. *If you are using slides with glass coverslips ensure that the correction collar is set to 0.17.*
- Condenser Filters
    - Diffuser/IR pair – usually pushed in for DIFF.
    - Green filter (for phase contrast) – usually pulled out.

- LBD filter –corrects the colour temperature of the light source; keep pushed in.
- Neutral Density (ND) filter – push in if the image is too bright.

- The Filter Wheel (below the objectives) has six positions:
  - Blank – for brightfield and phase contrast imaging
  - UV – for blue fluorophores (e.g. DAPI)
  - C – for cyan fluorophores (e.g. ECFP, mTFP-1)
  - G – for green fluorophores (e.g. EGFP, Alexa Fluor 488, FITC)
  - Y – for yellow fluorophores (e.g. EYFP, mCitrine)
  - R – for red fluorophores (e.g. mCherry, TRITC).
- Establish **Köhler Illumination** for the best compromise between contrast and detail
  - Select the 10x objective.
  - Select an appropriate filter cube.
  - Bring the sample into focus.
  - Brightfield
    - Set the condenser turret to *A* (for Abbe).
    - Close the field stop and bring the image of the field stop into focus using the condenser focus wheel.
    - Centre the image of the field stop in the field of view using the condenser centreing screws.
    - Open the field stop until it is just beyond the field of view.
    - Set the aperture stop to a value of three-quarters of the numerical aperture of the objective ($0.75 \times A_N$, for the maths minded).
  - Fluorescence
    - Push the field stop rod in until the field stop just shows up in the field of view; then pull the field stop rod out until the image of the field stop just leaves the field of view.
    - Adjust the aperture stop until the image just becomes dimmer.

IMAGE CAPTURE IN *NIS ELEMENTS F*

- Start the software and choose the a camera at the prompt:
  - for colour images choose the DS-Fi2-U3
  - for monochrome choose the DS-QiMc-U3
- The software starts in *Live View* meaning that the software starts with the camera running and the live image displayed onscreen.
- In the Docked Tools (on the right side of the window)
  - Set the camera *Mode* to *Normal*;
  - Set the resolution for *Focus* and *Capture* to the highest settings;
  - Select your exposure *Mode*:
    - if the *Auto Exposure* button is highlighted, auto exposure is active;
    - otherwise you may select your exposure manually
  - If you are using manual exposure, set the *Exposure* time from the drop-down menu or enter a value in the exposure time field;
  - Set the *Gain* as low as possible while still giving an exposure time of < 1s.
  - Under the *Color* heading, use the *Auto White Balance* to correct the colour balance of the camera – find a region in your sample in brightfield that should be clear and then click this button. This is definitely a good idea, even if you're doing fluorescence.
  - To capture an image click the *Capture* button. Once the image has been captured, save your image using *Ctrl + S* or *Menu > File > Save*. *Auto Save* is also available.
- Toolbar Tools
  - *AE Area* – by clicking this button you can display and modify the region being used to determine auto exposure settings.
  - *Annotations* – clicking this button allows you to make annotations directly on your image.
  - *Report* – clicking this button allows you to export your images as PDF's.
- *Zoom* controls can be found in the upper right corner of the image frame.
- Red, green and blue channels can be combined into a single image via *Menu > Edit > File Merge*.

IMAGE CAPTURE IN *NIS ELEMENTS BR*

- Much of this version is the same as *NIS Elements F*.
- The camera buttons are found in the toolbar, but otherwise behave as the camera buttons in *NIS Elements F*. There is an additional *Live Quality* button that uses the actual capture settings to acquire the live image.
- This version of the software is calibrated, meaning that scale bars show actual distances. *However*, for the scale bar to be accurate you must select the objective you are using from the toolbar.
- This software has many other tools and capabilities including background subtraction, smoothing, sharpening, noise reduction and more. *NIS Elements BR* is highly customizable. If you'd like to become a power-user, have a chat to one of the microscopists.

WHEN YOU ARE DONE

- Transfer your files off the microscope computer and onto your network folder.
- Log out of the computer. This will shut the lights down automatically.
- Carefully remove oil from the objective using lens tissues provided and wipe up any spills you may have made.

A NOTE ON DATA STORAGE

The tagged image file (TIF) format is the standard when it comes to acquiring data through the microscope. TIF's are portable and lossless, while other file formats are either not portable (JPEG2000) or are lossy (JPEG). Thus we recommend that you save all your images as TIF files. Also, since your data is precious, we advise that you save your data locally to *C:\\User Data\<yourName>*. This will ensure that should something go wrong with the network your data will not be lost. Also, please do save to *C:\\User Data\<yourName>* rather than to *My Pictures* so we cand clean the hard drive when we need to.

Finally, please remember that the optics on these microscopes are **very** delicate. Please treat the microscopes gently and with respect. Think of them not as pieces of lab equipment, but as **very, very expensive** lenses for very special cameras. A really nice lens for up-close work for your camera can run about $2,000 and you wouldn't grind pieces of glass into that, would you? Microscope lenses can cost much more than that, so why would you grind slides into them or leave oil on them?