Stereo2/3 Quick Start Guide

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

SAMPLE CLEANING AND PREPARATION

- Make sure your sample container (e.g. your Petri dish) is *scrupulously* clean and dry before placing it on the microscope stage.
- Use a sample container sized appropriately to your sample; a 1cm or smaller embryo can be contained in a 35mm Petri dish. This will help to minimize spills.
- Do not fill sample containers to overflowing, as the microscope must be kept dry.

STARTUP

- Check the microscope and table to be sure they are clean before doing anything else.
- Set the zoom wheel to the lowest setting (0.7x).
- Check the eyepieces to be sure that they are a comfortable distance apart.
- If you will be doing reflected light or epifluorescence imaging, make sure that the plastic stage insert is in place and that the black or white side is facing up (as per your needs).
- If you will be using transmitted or oblique illumination, ensure that the glass stage insert is in place and that it is scrupulously clean.
- Ensure that the clear plastic cover is in place over the stage (yes, you **must** use this).
- Push the bino/camera slider to *bino* to send the light to the eyepieces.
- Log in to the computer. Log in to *AD* rather than *IMBPC* or *LAB-STEREO1*.
- Follow the instructions that pop up in the *Windows Script Host* dialog box, and the click *OK*, thus closing the dialog box.
- Place your sample on the stage and centre it below the objective.

LIGHT PATH

- Turn on one of the lights.
    - Both Stereo2 and Stereo3 allow for epifluorescence imaging. This lamp is turned on through the *Lamp On* icon on the computer desktop.
        - To switch between filter cubes, use the slider on the left side of the microscope body (not the base). Basic red, green, and blue filter cubes are available on both Stereo2 and Stereo3.
        - The epifluorescence shutter is located in the tube that enters the left side of the microscope body. Be sure to close this shutter when not actively observing or imaging your specimen.
    - Stereo3 has a dual-head fibre optic light which produces directional lighting that can be used for reflected-light imaging and to accentuate the shape of your sample.
    - To perform brightfield, oblique, or darkfield transillumination, turn on the lamp in the microscope base.
    - To set up brightfield imaging
        - Ensure that the LBD filter rod on the right-hand side of the microscope is pushed in and that the brightness control knob at the rear of the right-hand side is set to produce a neutral light.

        - Adjust the brightness of the light using the ND (neutral density) filters on the right-hand side of the microscope.
        - Turn the switch at the front of the right-hand side of the microscope to the rear position.
        - Push the rod on the front of the microscope all the way in and turn it fully clockwise.
    - If you wish to use oblique illumination, simply adjust the angle of illumination using the knob on the front of the right-hand side of the microscope base so that the background is dark but your specimen is bright. You may need to zoom in to exclude regions of your sample that aren't lit and regions of the background that are.
- Carefully bring your sample into focus, and adjust the magnifying power of the microscope using the zoom wheel.

IMAGE CAPTURE IN *NIS ELEMENTS F*

- 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*.
- 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.

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*. The *Live Quality* button 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.

WHEN YOU ARE DONE

- Clean up **all** spills in the working area. This is an OH&S issue with which all personnel **must** comply.
- Turn off all lamps whether you used them or not.
- Transfer your files off the microscope computer and onto your network folder.
- Log out of the computer.

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 can 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?