

LAELOM Laboratory for Advanced Electron and Light Optical Methods

North Carolina State University College of Veterinary Medicine

1060 William Moore Drive, Raleigh, North Carolina 27607

STANDARD OPERATING PROCEDURE

Subject: Operation of the Nikon 2000S Inverted Photomicroscope		
SOP No:	Authorized By: _____	Effective Date: April 25, 2008
Version No: 2.0	Signature _____ Date _____	Page: Page 1 of 7

Instructions for the Nikon 2000-S Inverted Photomicroscope

1. Turn on the Nikon **Halogen Light Power Supply** and Nikon **Digital Sight Power Supply**. If intending to do epifluorescence work, turn on the Nikon **Intensilight C-HGFI unit** (the Intensilight unit takes about 10-15 minutes to totally stabilize, though it can be used for viewing immediately).
2. Turn on the **Computer** and **Monitor**.
3. Log in as **laelom** for Novell.
4. Click on **NIS-Elements F 2.30** icon to start camera program. It is set up for the **DS-U2 Camera** specifications. Once the program is started, a live image should automatically come up. If it doesn't, click on **Live** icon at the lower right of the **Camera** menu on the lower right of the screen.
5. Put vessel or microscope slide on stage (the latter with cover slip side down, in most cases).
6. Push in the white button labeled **Lamp** at the lower left side of the microscope. Adjust **Halogen** light source illumination level for your viewing comfort through the oculars with the knob on lower left side of microscope stand.
7. Choose appropriate **Objective** for your needs (**all of these objectives are dry objectives; do not use immersion oil**).

Objectives Installed and Their Uses

(All of the Objectives are Dry Objectives. Please do not Oil Them)

Magnif.	N.A.	Lens Type	W.D. (mm)	Uses	Mount Thickness (in mm)	Possible Condenser Settings
10 X	0.30	Plan Fluor	16	BF, oblique, epifluor	1.5	BF or 20 (for oblique)
20X	0.45	Plan Fluor, ELWD	7.4	BF, Hoffman Contrast, epifluor	0-2	BF or 20 for Hoffman
40X	0.6	Plan Fluor, ELWD	2.7-3.7	BF, Hoffman Contrast, epifluor	0-2	BF or 40 for Hoffman
60X	0.7	Plan Fluor, ELWD	1.5-2.1	BF, Hoffman Contrast, epifluor	0.5-1.5	BF or 60 for Hoffman

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8. When working with **Hoffman Contrast**, select the appropriate **Condenser** setting indicated on the chart above and rotate the **Polarizing Lens** at the top of the **Condenser Assembly** to achieve the amount of contrast desired.
9. For the **Hoffman Contrast Condenser**, the height of the bottom of the **Condenser Assembly** should be set at 40 mm from the **Stage** (all the way to the top of the track) to assure even illumination. This is very different from normal Kohler illumination adjustments. To center the **Condenser Assembly**, choose the **BF** condenser setting. Then stop down the **Field Diaphragm** (lever on the right side of the **Light Tube Housing**) until the edge of the diaphragm can be seen. It will not be in sharp focus. Center the **Diaphragm** image with the 2 knurled **Silver Knobs** at the front of the **Condenser Assembly**. Open the **Field Diaphragm** until it is just outside of the field of view.
10. The **Exposure Control** menu at the right side of the screen has a place called **AE Compensation** that can be changed to change the brightness of the live image (which will change the brightness of the captured image as well).
11. Turn the **Port Knob** on the lower right of the microscope stand to **Eye** for 100% transmittance of light to the **Oculars** or to **Side** for a 50:50 split of the light between the **Oculars** and the **Camera Port**.
12. Focus the **Specimen Image** on the **Live Screen**, then adjust the interpupillary distance of the **Oculars** and focus each one independently on the **Specimen** so that you can switch between ocular viewing and working on the **Live Screen** easily.

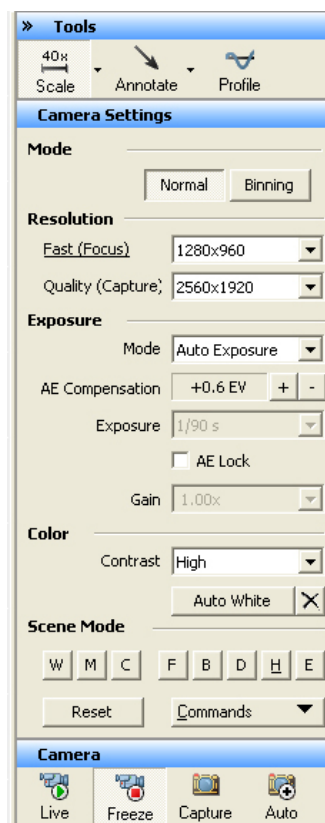
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13. To Capture an Image:

Basic Settings to Start



- Click on the **Live** button.
- Focus and frame subject on **Monitor**.
- The **recommended procedure is**: go to **File, Save As**, and choose file type and location (e.g., a stick you own). Save files as either **JPEG or TIFF files, not JPEG 2000 files, which are not read by all systems**. After saving the file, click on **Live** to get the live screen back.
- A second option (**not recommended**), is to click on **File**, click on **Options**, and then set up a **File Prefix** (preferably your initials), and then all the images you take will be saved

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under your **File Prefix** in the **Client Images** folder, as JPEG files with sequential numbers (**do not save them as JPEG 2000** files because they are not easily read by some programs).

- e. Clicking on **Auto** saves image as JPEG file into **Client Images**, with sequential numbers and **should not be used** as a capture mode.
- f. The **Capture** button only saves images to RAM and then you need to go to **File, Save** and then save your image. If you do not save the images as you go, the RAM will fill up and slow the operation of the program, without having saved the images anywhere. The **Freeze** button just stops live imaging.

Different Ways to Save Files and Resulting File Sizes*

Mode	1280 X 960 Jpeg (lowest)	1280 X 960 TIFF	2560 X 1920 Jpeg (lowest)	2560 X 1920 TIFF
File, Save As	500 + kb	3.5 + mb	500 + kb	3.5 + mb
Freeze, File, Save As	500 + kb	3.5 + mb	500 + kb	3.5 + mb
Capture, File, Save As	500 + kb	3.5 + mb	2 + mb	14 + mb

* For on-screen viewing, emails, or PowerPoint presentations, a 500+ kb Jpeg file is quite adequate. For print purposes, a 3.5 mb TIFF file is usually adequate.

- 14. **To fill the screen with an image**, go to **View** and click on **Full Screen**. To get back to the smaller screen with functional buttons, right click.
- 15. **To save Operational Parameters** (white balance, etc.), click on the **Command** button in the lower right side of the screen, click on **Save Settings**, and save to **your initials**.
- 16. **For Epifluorescence Work:**
 - a. Turn **C-HGFI** unit on. When light quits flashing, the bulb is lit.
 - b. Choose **ND (neutral density)** setting that gives you enough signal (1 is the most illumination, and 32 is the least). Transmittance of more light will quench the fluorochrome faster.
 - c. Make sure that the **C/O Lever** on the right side below the **Nosepiece** is in the **O Position (open)**.

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- d. Use the **Knurled Disk** above the **C/O Lever** to choose the appropriate filter cube (**UV, B or G**). The other positions have no filters and are used for Hoffman Contrast or bright field imaging.

Filter Cubes Installed in the Nikon 2000-S

Designation	Nikon #	Excitation Wavelength	Uses*
UV	UV-2E/C	340-380 nm	DAPI, Alexa Fluor 350 and 405), AMC, BAO 9
B	B-2E/C	465-495 nm	FITC, GFP,BODIPY, DiO
G	G-2E/C	528-553 nm	TRITC, Alexa Fluor (546,555,568), Cy3, DiI, Propidium Iodide

*For a full list of recommended labels for each cube, see Appendix A at the end of these instructions.

- e. The **ND Filters** on the **Light Housing** at the lower right rear of the microscope stand should be pulled out (toward you), since the **ND Filters** on the **C-HGFI unit** are more flexible.
 - f. Open the **Shutter** on the **C-HGFI unit**.
 - g. A low signal will often give false images on the monitor, so make sure that you look through the oculars to see what is actually there.
- 17. To Add Size Bars**, start with **Live Image** (you cannot change the scale on captured images, once you have selected one)
- a. Go to **Tools**
 - b. Open pull-down **Menu**
 - c. Select **Objective Magnification** in use
 - d. Click on **Scale Icon** and **Scale** will appear on image (To remove **Scale**, click on **Scale Icon** again).
 - e. Right click on **Scale** on image, and a **Scale Properties** bar appears. Left click on **Scale Properties** to change font, line, width, horizontal or vertical display of bar, etc.

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- 3. Exit Windows**
- 4. Turn off computer and monitor**