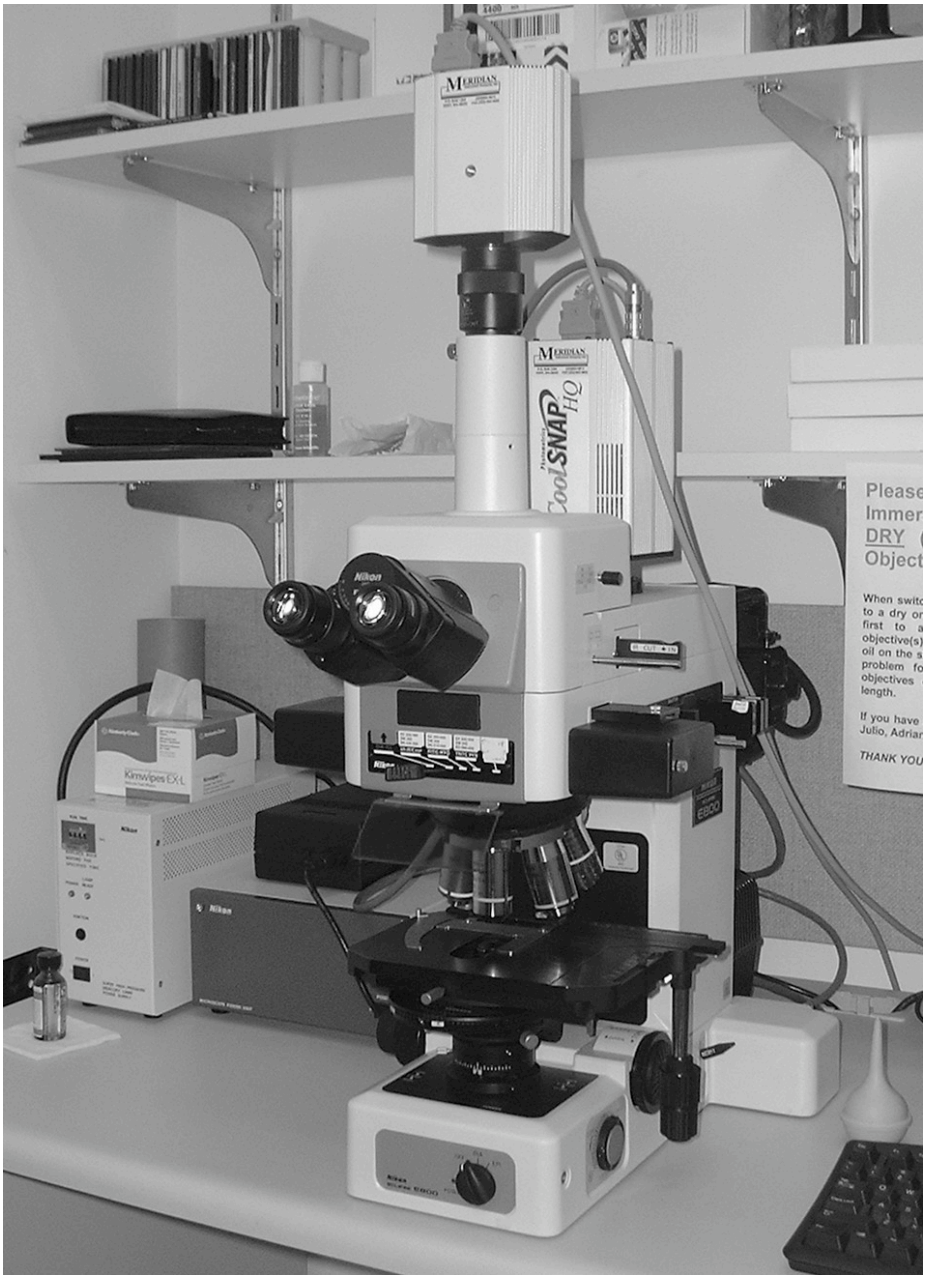
Nikon E800 Operating Instructions.



You can request electronic copies of this manual by contacting imaging@fhcrc.org. Copies are also available on the Scientific Imaging web site. Please send your comments and/or suggestions to imaging@fhcrc.org.

Prepared by Scientific Imaging. 11/11/2003

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Nikon E800 Operating Instructions.

1. The Nikon E800 Microscope.

The Nikon E800 is a fixed-stage upright microscope configured to image in transmitted light, DIC, and epifluorescence modes. The Nikon is equipped with the following objectives:

10x/0.45	PlanApo	DIC L	∞/0.17	WD 4.0	
20x/0.75	PlanApo	DIC M	∞/0.17	WD 1.0	
40x/0.95	PlanApo	DIC M	∞/0.17	WD 0.14	
40x/1.3	PlanFluor	DIC H	∞/0.17	WD 0.2	Oil
60x/1.4	PlanApo	DIC H	∞/0.17	WD 0.21	Oil
100x/1.3	PlanApo	DIC H	∞/0.17	WD 0.2	Oil

The last three objectives (shaded) are Oil immersion objectives.

The condenser is: C-CU with 0.9 N.A. dry lens (swing-out)

And contains the following inserts: A(bright field) /DIC L/ DIC M/ DIC H/ø/ø/ø

DIC lens prisms are inserted above each individual objective lens.

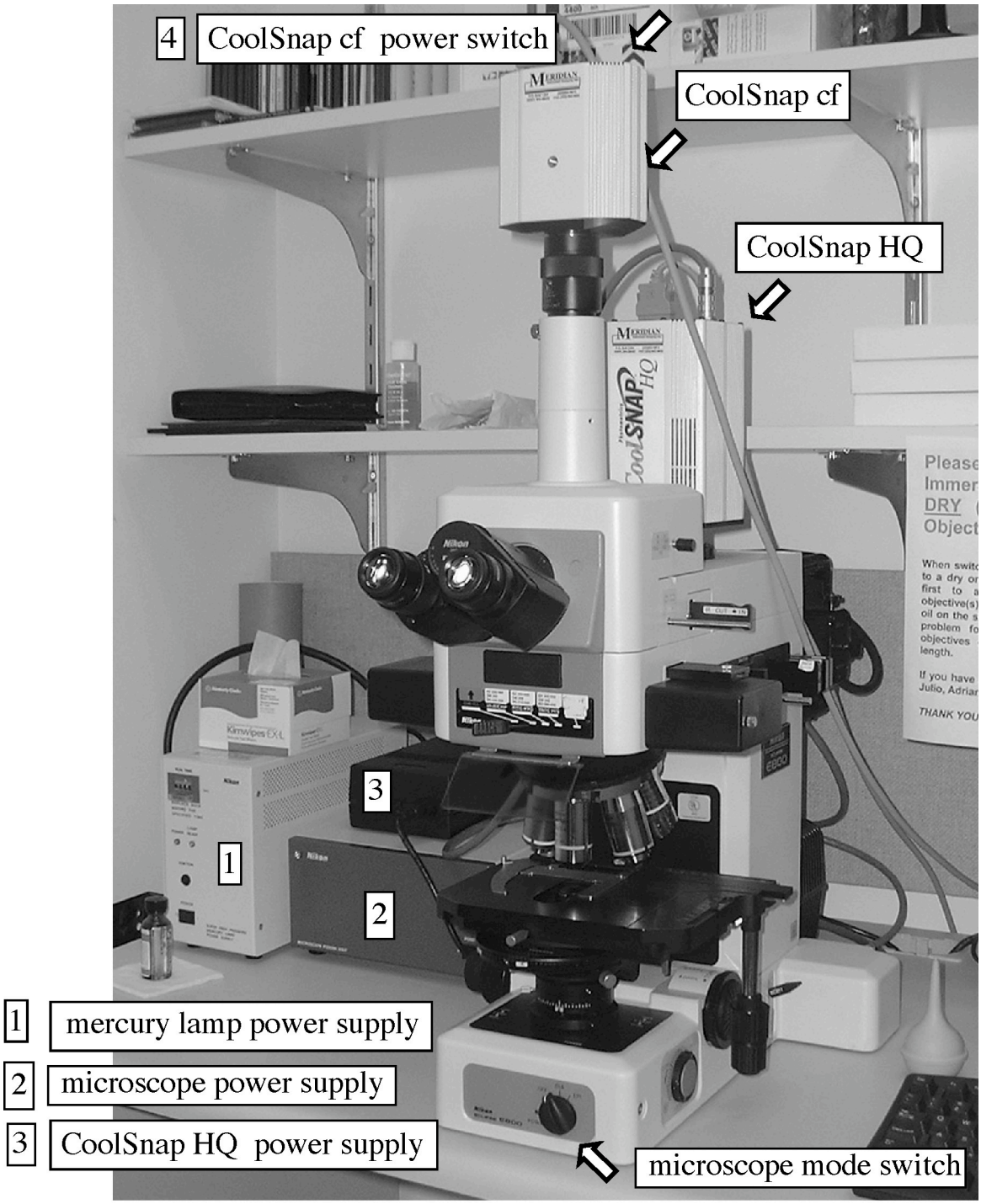
The Nikon also has a filter cube slider with the following filter cubes:

1. empty (VIS)
2. UV2E/C DAPI 330-380/400/435-485
3. FITC, HYQ 466-500/505/510-560
4. TRITC, HTQ 530-550/565/590-650
5. TripleBP(DAPI/F/R) #83100

In addition, we have the two following cubes:

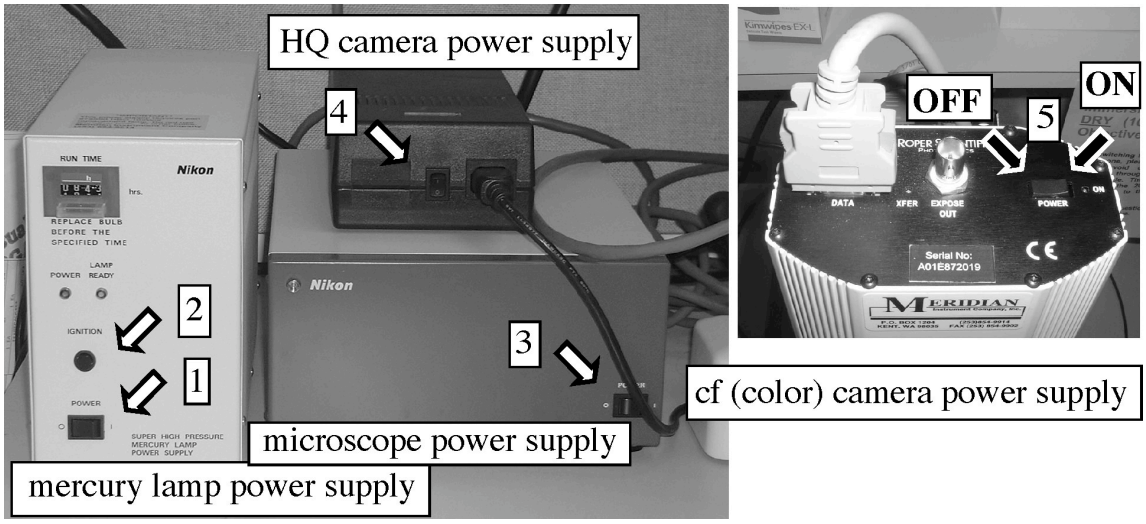
Cy5, HYQ # C38705
TexRed, HQ # C24693 (540-580/595/600-660)

These cubes can be interchanged on slider position #5 (ask the staff)


4 CoolSnap cf power switch
CoolSnap cf
CoolSnap HQ
3
1
2
1 mercury lamp power supply
2 microscope power supply
3 CoolSnap HQ power supply
microscope mode switch

Nikon E800

2a. Turning ON the system.



2.1 Power ON Mercury (Hg) arc lamp (if using fluorescence).

2.2 Power ON microscope.

2.3 Power ON CoolSnap HQ high-resolution monochrome camera (if necessary)

2.4. Power ON CoolSnap cf color camera (if necessary)

2b. Turning OFF the system.

IMPORTANT: IF YOU ARE THE LAST USER, ESPECIALLY AFTER HOURS OR DURING WEEK-ENDS, ALWAYS REMEMBER TO POWER SYSTEM OFF WHEN DONE:

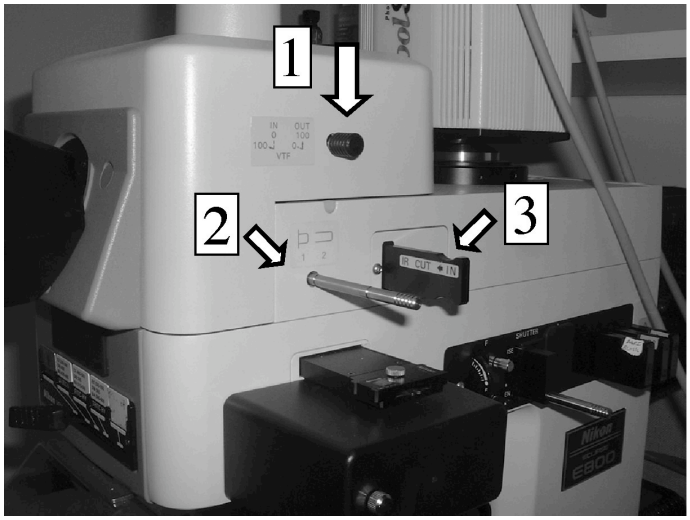
1. POWER OFF ALL SWITCHES (EXCEPT COMPUTER)

2. CLEAN UP IMMERSION OIL FROM OBJECTIVES USING LENS TISSUE (FOLD ONE OR TWO SHEETS OF TISSUE IN TWO ALONG THEIR LENGTH, HOLD BY THE EDGES, AND GENTLY WIPE OBJECTIVE; REPEAT WITH NEW TISSUE IF NECESSARY).

3. MAKE SURE ALL LAMPS AND CAMERAS ARE OFF BEFORE YOU COVER MICROSCOPE.

3. Selecting the light path.

Two sliders on the right side of the eyepiece select the light path.



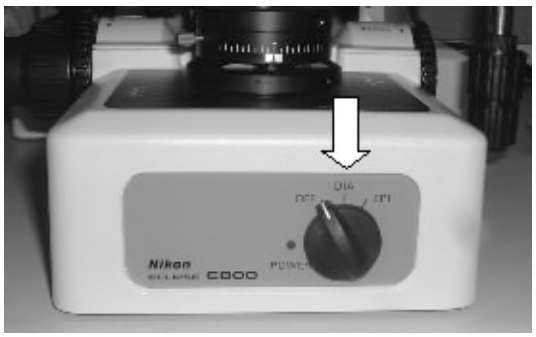
Lower Slider (2) IN: Coolsnap HQ High Resolution Monochrome CCD Camera
Lower Slider (2) OUT: Coolsnap cf Color CCD Camera/Binocular viewing (eyepiece)

Upper Slider (1) IN: Eyepiece
Upper Slider (1) OUT: Coolsnap cf color CCD

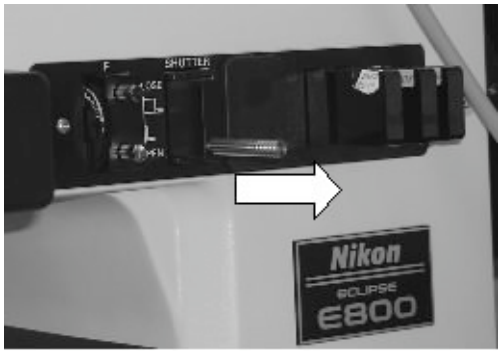
Slider 3: Infra-Red protection for CCD camera (leave in the engaged (IN) position).

4. Operating in transmitted light mode.

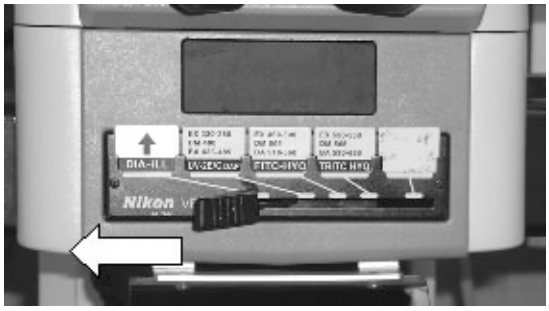
3.1 Use switch on the front base of microscope to select transmitted light (DIA).



3.2 If Hg lamp is ON, engage Hg lamp block (pull slider OUT) to block Hg lamp illumination



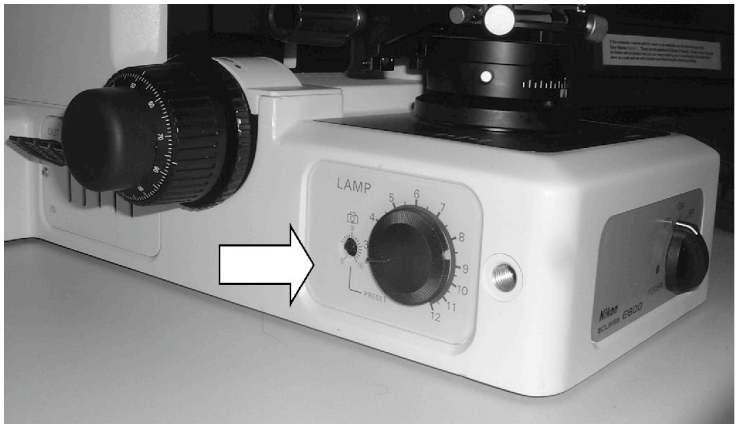
3.3 Select blank filter cube (DIA-ILL)



3.4. Choose objective. Apply a SMALL drop of immersion oil to sample if using immersion lens. Inspect objective markings and chart on the wall to make sure you do not apply immersion oil to a dry lens.

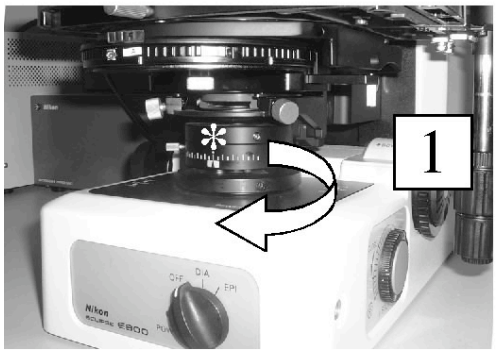
3.5. Focus on sample.

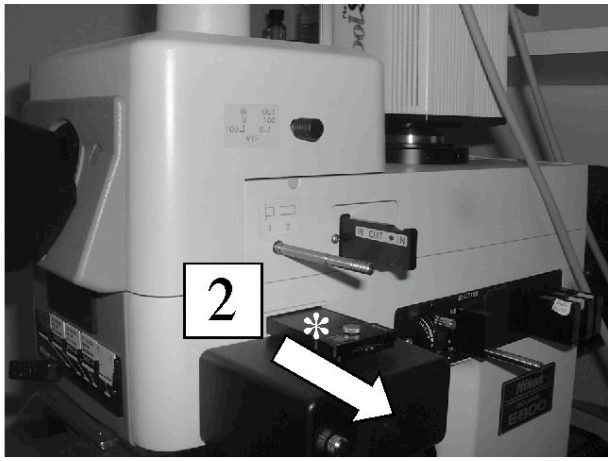
3.6. Adjust illumination intensity with power dial on left side of microscope



3.7. Engage filters for transmitted illumination if required (e.g. neutral density to attenuate light). Push lever down to engage filter.

3.8 Remove polarizer and analyzer from the light path. (swing polarizer (1) out; pull analyzer (2) out).





3.9. Set Condenser turret to position A (for normal transmitted light)

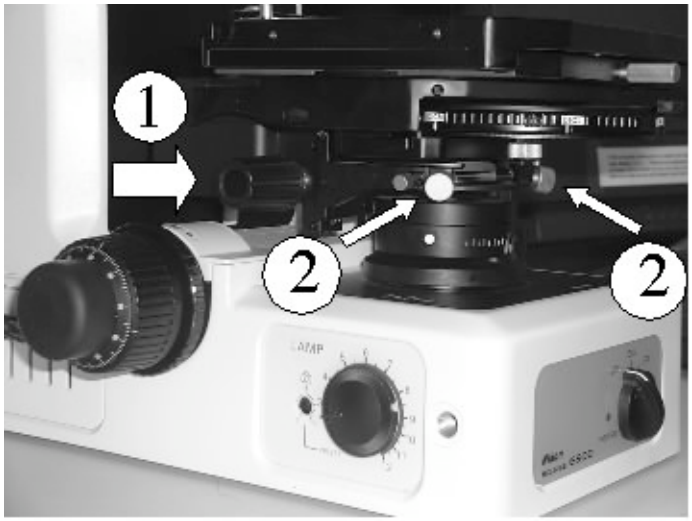
5. Adjusting the microscope for Koehler illumination.

Typically, the microscope will be properly adjusted. The only adjustments you need to do is to open the field diaphragm to just enclose the field of view for your objective, and adjust the condenser aperture. The full procedure is described below.

5.1 Stop down (close) field diaphragm by rotating dial on left side of microscope base

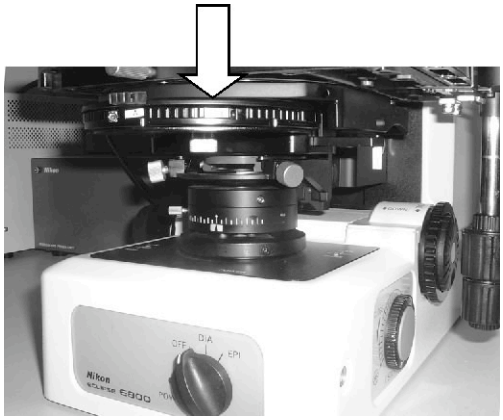
5.2 Focus field diaphragm by moving condenser UP or DOWN (1)

5.3 Center field diaphragm using condenser centering screws (2)



5.4. Open field diaphragm barely wider than the field of view (see Fig 4.1)

5.5 Open condenser aperture diaphragm to about 75% of the objective back aperture. You can examine the objective back aperture by removing one eyepiece and looking down the tube.



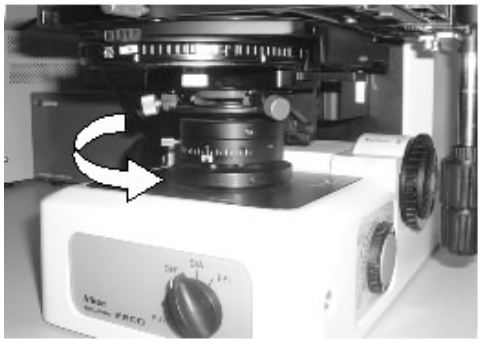
As an approximation, the condenser aperture should be set to minimum for 10x objective and to max for 100x objective:

10x: setting	~2	
20x	~5	<open-close>
40x	~7	O | | | | | | | | | o
60x	~9	10 0
100x	~9	

6. Adjusting the microscope for DIC.

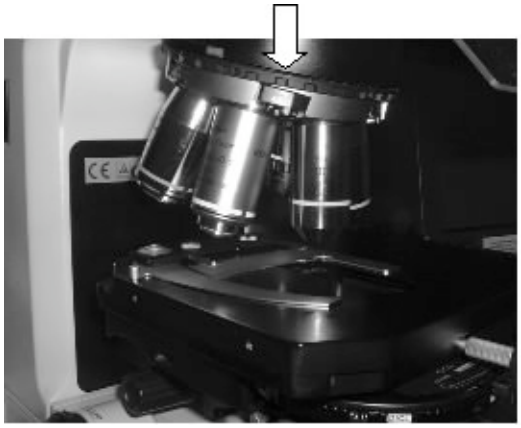
Typically, the microscope will be properly adjusted. Normally, you only need to select the condenser prism matching your objective, and engage the polarizer and analyzer. The whole procedure is described below.

6.1 Engage Polarizer (swing over light path; left) and Analyzer (slide IN; right)



6.2 Select position A on condenser turret (no DIC prism engaged)

6.3 Disengage objective prism by pulling prism slider OUT (without removing completely; the DIC objective prisms are the little sliders located just above each objective). Note: make sure to disengage the prism directly above the objective in use (in the image below, the prism shown belongs to an objective that is NOT in use).



6.4 Verify that field is dark. Rotate polarizer for minimum transmission (normally when set to –o-)

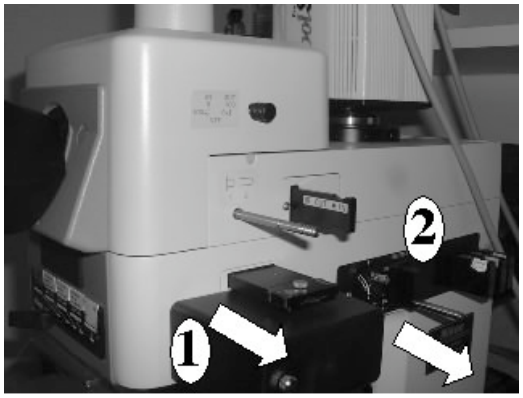
6.5 engage objective prism (push IN)

6.6 select matching condenser prism (H, L or M; read DIC label on objective or use Table in section 1)

6.7 for fine adjustments, rotate analyzer dial (make sure to unlock by rotating screw on top)

7. Setting the Microscope for Fluorescence.

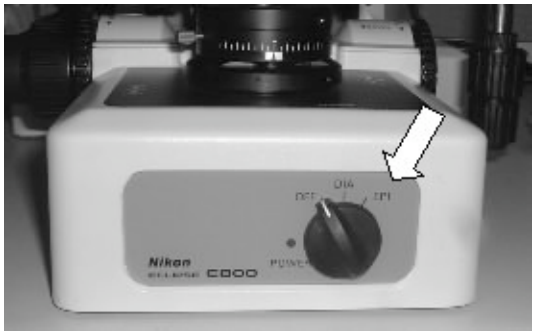
7.1 Disengage Analyzer (1; pull OUT)



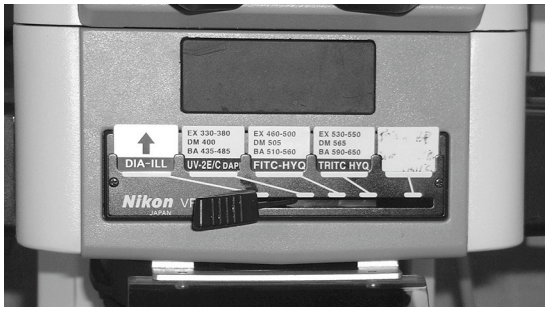
Note: the polarizer is not in the epi-illumination light path, and therefore does not affect its quality.

7.2 Pull epi-illumination shutter OUT to block light (2).

7.3 select EPI setting on front base dial (will work on OFF too)



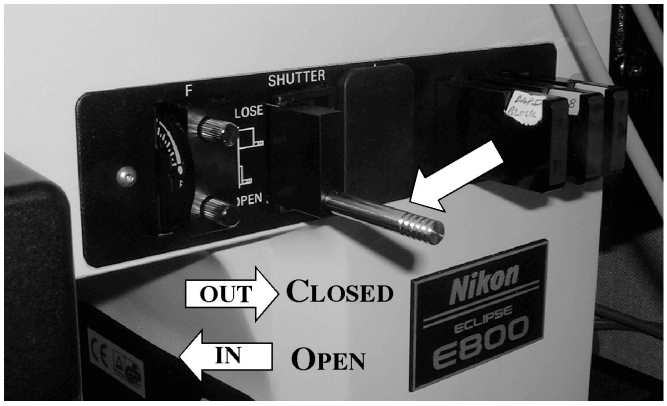
7.4 select fluorescence filter cube by moving slider



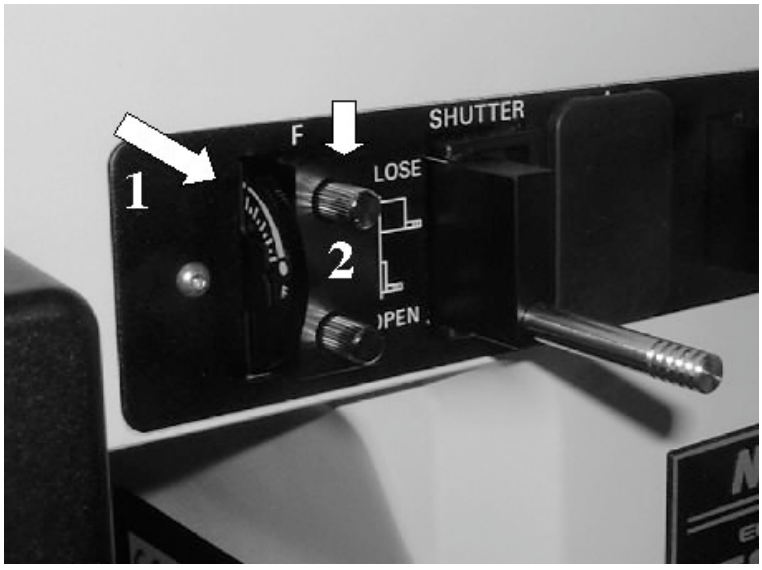
Filter positions (from left to right). [Note: Positions 1-4 are generally fixed. Position 5 may be occupied by a different filter (e.g. Texas Red or Cy5)]:

Position 1: blank (transmitted light)
Position 2: DAPI
Position 3: FITC
Position 4: TRITC (Rhodamine)
Position 5: Triple DAPI/FITC/TRITC

7.5. Open fluorescence illumination shutter by pushing IN



7.6. Select the smallest convenient illumination field aperture using the epifluorescence field diaphragm (1). Center if necessary using the two centering screws (2).



<u>7.7 Always pull shutter OUT when not imaging or viewing to minimize photobleaching of your sample.</u>

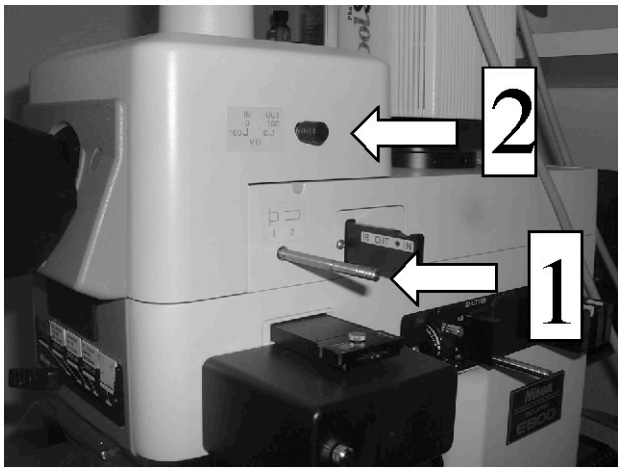
8. Starting Imaging Software (Metamorph).

8.1 Verify that the desired camera is ON (typically, HQ for fluorescence, cf for transmitted light); See section 2.

8.2 double-click on the camera icon on desktop. This will launch Metamorph acquisition software.

8.3 If one camera is not ON, will give error message: “camera not found, do you want to try again?” Answer NO

8.4 Adjust light path for the appropriate camera:



Lower Slider (1) IN: Coolsnap HQ High Resolution Monochrome CCD Camera
Lower Slider (1) OUT: Coolsnap cf Color CCD Camera/Binocular viewing (eyepiece)

Upper Slider (2) IN: Eyepiece
Upper Slider (2) OUT: Coolsnap cf color CCD

8.5 Click on “Acquire Image”. Will open “acquire” dialog window.

On “acquire” window:

•use “show live” to show live image for centering and focusing your sample

• use acquire to acquire single exposure.

Adjust exposure and collect Image.

Use “Save as” to save the image to your local folder. Move later to your fred account.

Use control buttons on left of window to display image histogram, select display colors, and select magnification.

Notes:

To collect multiple color planes (e.g. in fluorescence) with Metamorph, you need to acquire a greyscale image for each channel, by manually selecting th eappropriate fluorescence filters. Once you have the desired images, you can combine them into an RGB color image with Metamorph, or other imaging software.

Metamorph can collect time-lapse images into a multi-layered image file (stack). The stack can be saved as an AVI or QT movie.

Metamorph does not record the type of objective used. For distance measurements on your image, you need to record that information (e.g. by adding it as a file info). Metamorph can measure distances if you have the proper calibration file for the objective you used.

9. Turning OFF the system.

IMPORTANT: IF YOU ARE THE LAST USER, ESPECIALLY AFTER HOURS OR DURING WEEK-ENDS, ALWAYS REMEMBER TO POWER SYSTEM OFF WHEN DONE:

9.1. POWER OFF ALL SWITCHES (EXCEPT COMPUTER)

9.2. CLEAN UP IMMERSION OIL FROM OBJECTIVES USING LENS TISSUE (FOLD ONE OR TWO SHEETS OF TISSUE IN TWO ALONG THEIR LENGTH, HOLD BY THE EDGES, AND GENTLY WIPE OBJECTIVE; REPEAT WITH NEW TISSUE IF NECESSARY).

9.3. MAKE SURE ALL LAMPS AND CAMERAS ARE OFF BEFORE YOU COVER MICROSCOPE.