Quick Reference Guide to Using the Nikon Optiphot
(Details begin on following page)

Start-up

1. Epi-fluorescence.

1.1. Turn on the xenon lamp before anything else
  1.1.1. Power supply on shelf above microscope
  1.1.2. Press black rocker switch
  1.1.3. Press the orange button momentarily, and release
1.2. Turn on the microscope lamp
  1.2.1. Green push button at left, front base of microscope
1.3. Select your objective lens;
1.4. Select desired epi-fluorescent filter with slider over objective turret
1.5. Open fluorescent shutter
1.6. Set the trinocular beamsplitter to direct the image to the eyepieces;

2. Brightfield.

2.1. Turn on the microscope lamp
  2.1.1. black push button at left, front base of microscope
2.2. Select your objective lens;
2.3. Set the trinocular beamsplitter to direct the image to the eyepieces;
2.4. Select "DIA" on filter slider;
2.5. Adjust lamp intensity with slider across base of microscope
2.6. Focus on the specimen
2.7. Set Koehler illumination
2.8. See following pages for use of condenser for darkfield and phase contrast.

Shut down

3. Turn off the equipment.

3.1. Switch off the xenon lamp
  3.1.1. Lamp must be on for at least 30 minutes
3.2. Turn off the transmitted lamp
3.3. Remove slide
3.4. Clean any immersion oil or mounting medium from lens and stage

Did you record your usage on the Nikon reservation calendar?

Details on Getting Started With The Microscope
See the Nikon Optiphot manual for additional information about the microscope.

Start-up

1. Epi-fluorescence.

1.1. When using fluorescence, turn on the mercury lamp before anything else;
1.2. The xenon arc lamp power supply sits on the shelf over the microscope;
1.3. Press the rocker switch labeled "POWER" towards "I", the green light will come on;
1.4. Press the button labeled "IGNITION" for about 3-4 seconds, never more than 10 seconds;
1.5. The amber "LAMP READY" flashes, then is steady in 1-2 minutes, after lamp stabilizes;
1.6. If the amber indicator doesn't come on in 3 minutes, turn off the power, wait 5 minutes then proceed from step 1.2;
1.7. If the bulb fails to ignite, it may be too hot from prior use, the bulb may be faulty or may need to be reseated. Contact facility staff.

2. Transmitted Illumination with halogen lamp (Figure 1).

2.1. Power switch turned on by the black switch, left front corner of the microscope base;
 2.1.1. This lamp is used for brightfield, phase contrast, and darkfield microscopy
2.2. Lamp intensity is controlled by 3 means:
 2.2.1. Lamp voltage controlled by slider at front of the microscope base
 2.2.2. Neutral density filters mounted below the condenser (Figure 1, Table 2)
  2.2.2.1. Avoids need to change lamp voltage, which alters white balance
 2.2.3. Photo Button – sets lamp to 9V, which is much too bright for the digital camera

3. Beam Splitter (Figure 3, Table 1)

3.1. 3 options for dividing the light between the eyepieces and camera port

Position	Image path
In	100% to eyepieces
Middle	14% to eyepieces/86% to camera
Fully out	100% to camera

Table 1. Beamsplitter positions.

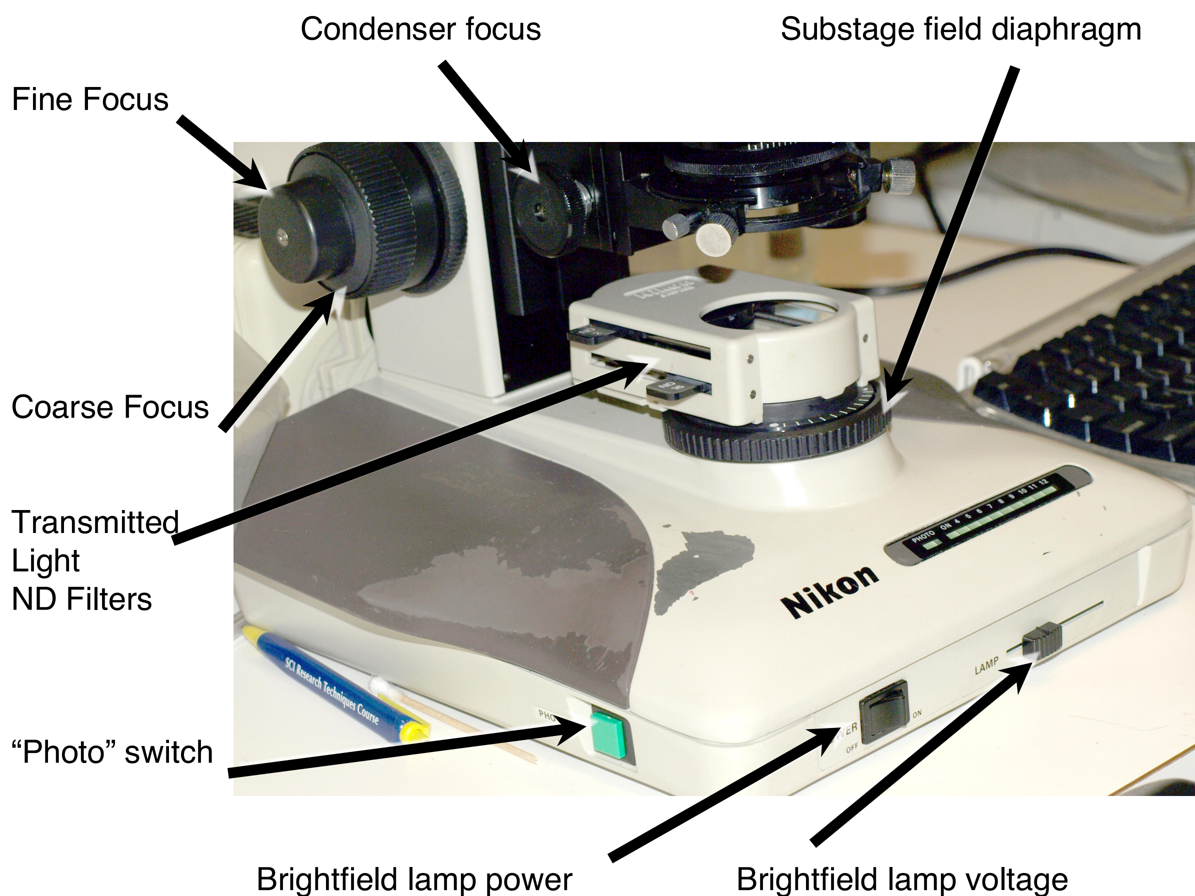

Figure 1. Sub-stage Controls

Filter	Light Reduction	% Transmission
ND2	1/2	50%
ND4	1/4	25%
ND16	1/16	6.25%
ND2+ND4	1/8	12.5%
ND2+ND16	1/32	3.125%
ND4+ND16	1/64	1.562%
ND2+ND4+ND16	1/128	0.781%

Table 2. Neutral density filters and combinations for transmitted light.

4. Select your objective lens

4.1. Choose the objective lens compatible to your specimen and needs

4.1.1. low NA will provide low resolution and weak fluorescence, but large working distance

4.1.2. low magnification providse large field of view but may not magnify sufficiently for full resolution at the camera

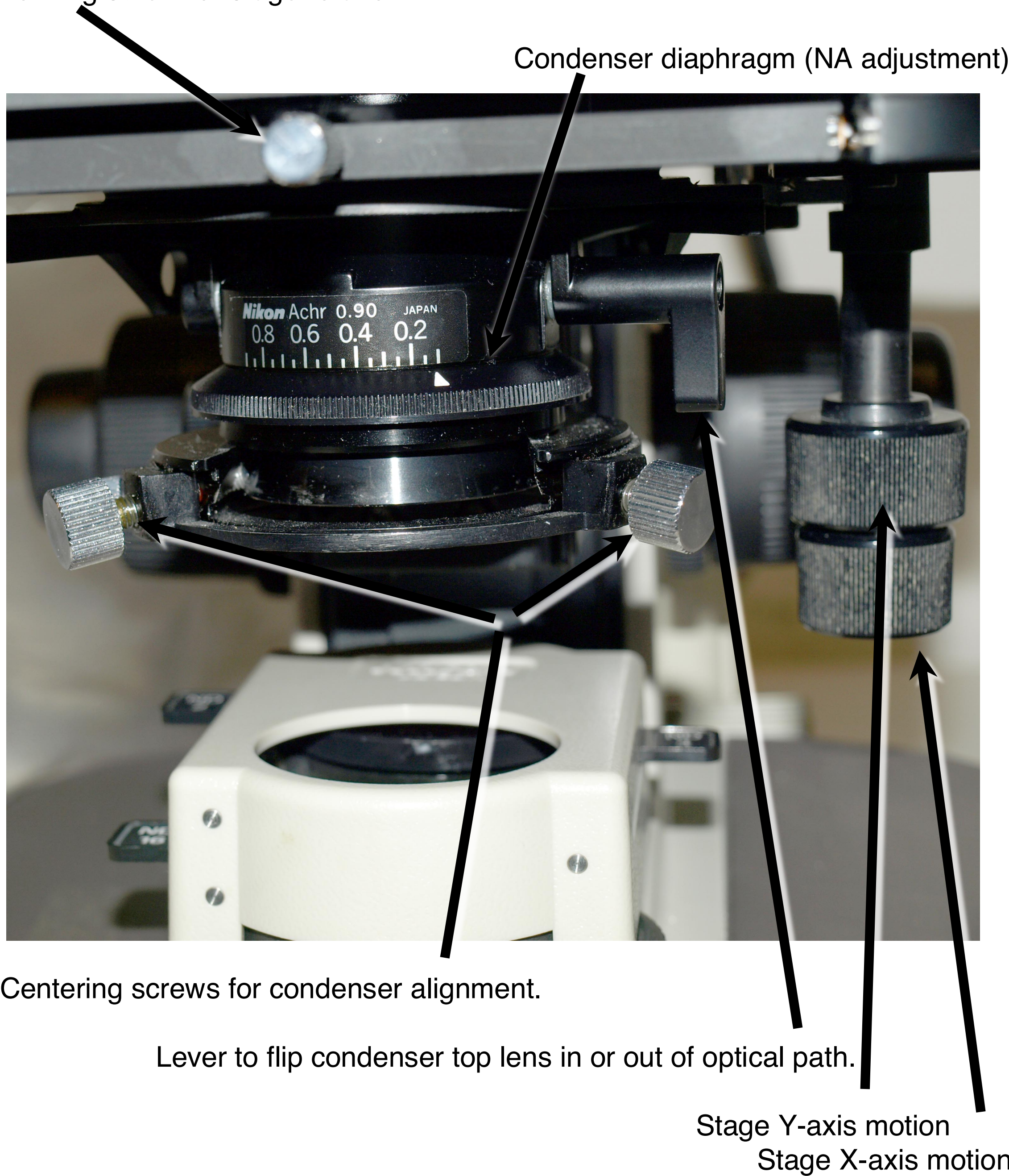

Figure 2. Sub-stage Condenser

A. Performing Brightfield transmitted light microscopy

1. Focus on specimen

1.1. Place slide on the stage and hold in place with the stage clip

1.1.1. Begin with low magnification for greater ease in surveying your specimen to identify regions of interest

2. Adjust the focusing eyepiece

2.1. Find a small object with sharp boundaries

2.2. Use the fine focus using observing with one eye

2.3. rotate the upper portion of the other eye piece until the object is in similar focus with both eyes

3. Set Koehler Illumination for objectives 10X and above (Figure 1, Figure 2)

3.1. Note: this should be performed every time the objective is changed

3.2. Focuses the condenser to the same plane as the objective, set the incoming light column and condenser NA

3.2.1. This is essential for best contrast, resolution and even illumination

3.3. Open the condenser diaphragm for maximum brightness

3.4. Make certain the condenser top lens is flipped up

3.5. Close the field stop diaphragm to its minimum diameter

3.6. Use the condensor focus knob to bring the leaves of the field stop sharply into focus while observing through the eyepieces

3.7. Roughly center the spot of light with the centering screws

3.8. Open the field stop until it just reaches the edge of the field of view

3.9. Adjust the centering until it is concentric with the field of view

3.10. Close the condenser diaphragm to match the NA of the objective

3.10.1. If the objective NA is higher than .85, set condenser to maximum

3.11. Double check the eyepieces – if the image is blurry, close the field stop, re-focus condenser then re-open the field stop

4. Set Koehler Illumination for objectives below 10X

4.1. Follow the steps above, except that after step 3.7, flip the condenser top lens out of position

5. Setting contrast filters

5.1. A color filter inserted into the light path, as paddles at the lamp housing or placed on the condenser, can increase contrast.

5.1.1. Viewing blue or brown staining often benefits from using a green or blue filter

6. Controlling epi-fluorescence

6.1. Select filters for specific wavelength bands (“colors”) by the black slider located on the front of the microscope betwee the eyepieces and objective lens turret;

6.2. There is only room for 3 filters cubes

6.2.1. A 4th cube requires manually removing filters, usually the Cy5 and DAPI cubes are swapped, see DMC staff for instruction on changing the 4th filter

6.3. Xenon lamp intensity is controlled by pushing ND filters in or out of the light path

6.3.1. located behind the trinocular head (Figure 3);

6.4. Xenon excitation is controlled by a slider located between the ND filter and the xenon lamphousing (Figure 3)

6.5. The excitation light is also blocked when the filter slider is in the “DIA” position for transmitted light (diascopic illumination)

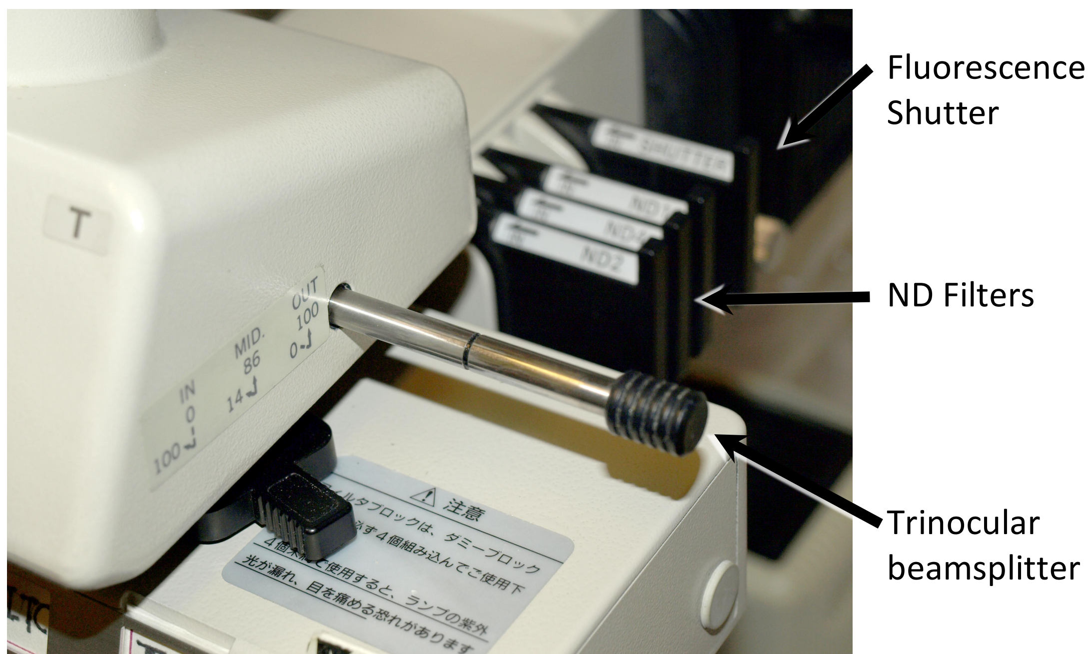

Figure 3. Trinocular Head and Fluorescence ND Filters

Filter Label	Cube Label	"Color"	Part	Nikon
FITC	HQ:FITC	green	96170	C01174
TxR	HQ:TXRD	red	96172	C00178
UV	UV-2E/C	blue	blue	
Cy5	Cy5	far red	31023	C81736

Table 3. Epi-fluorescence filter choices

Lens	μm/pixel	pixel/μm	1 mm reticle field
1/.04 Plan 160/-	8.40	0.12	
2/.05 Plan 160/-	4.24	0.24	
4/.1 EPlan 160/-	2.12	0.47	
10/.40 EPlan 160/-	0.87	1.15	820 μm^2
20/.40 EPlan 160/-	0.43	2.31	412 μm^2
40/.65 EPlan 160/.17	0.21	4.78	200 μm^2
40/.85 Fluor 160/.17*	0.21	4.78	200 μm^2
100/1.30 Oil Fluor 160/.17**	0.09	11.55	

*UV capable, coverslip correction collar
**Variable NA 0.8-1.3

Table 4. Spatial calibration for each objective lens, with the Spot IIe camera.

Objective lens notes.
Other objective lenses are available upon request, such as 60/1.4 oil, 100/1.25 oil.
Lenses designated as "160/-" do not require a coverslip.
Lenses designated as "160/.17" require a coverslip of 170 μm thickness.