Kohler Illumination

1. Prepare microscope for use:
   a. Turn on microscope at *black rocker switch*. Green indicator light will come on.
   b. *Daylight filter* (NCB 11) is pushed in. *Neutral density* (ND) filters may be in or out as required – usually out.
   c. *Light diverter* is in the 'Bino & Photo' position.
   d. *Condenser turret* at 'A' [brightfield setting].
   e. *Filter block selector* at 'DIA-ILL'.
   f. *Condenser top-lens* is in place.
   g. *Polarizer* out of light path.

2. Kohler illumination: [does not apply to 4x lens – see below]
   This looks like a lot of steps, but generally only needs a few seconds after you've done it a few times. This should be done through the eyepiece.
   [Note: readjust *field and iris diaphragms* whenever you change objectives.]
   a. Open *field* (black ring on base) and *iris* (condenser turret slider*) diaphragms*.
   b. Select *objective* to be used (generally it is easier to use a low power first before going on to a higher power).
   c. Focus on specimen.
   d. Adjust lighting to comfortable level with *rheostat* (if lighting does not change it may be in 'photo' mode).
   e. Close *field diaphragm* completely.
   f. Adjust *condenser*, with black knob on left, until edges of *field diaphragm* are sharp and in focus (may have a halo).
   g. Open *field diaphragm* until edges not seen in viewing field. May also need to center diaphragm with two silver knobs on condenser body.
   h. Slowly close the *iris diaphragm* until the image just starts to darken. This gives the best compromise between resolution and contrast.
      [Note: Highest resolution when iris is fully open, but the contrast is low; depth of focus increases with a closed iris].
   i. Adjust light intensity as needed.

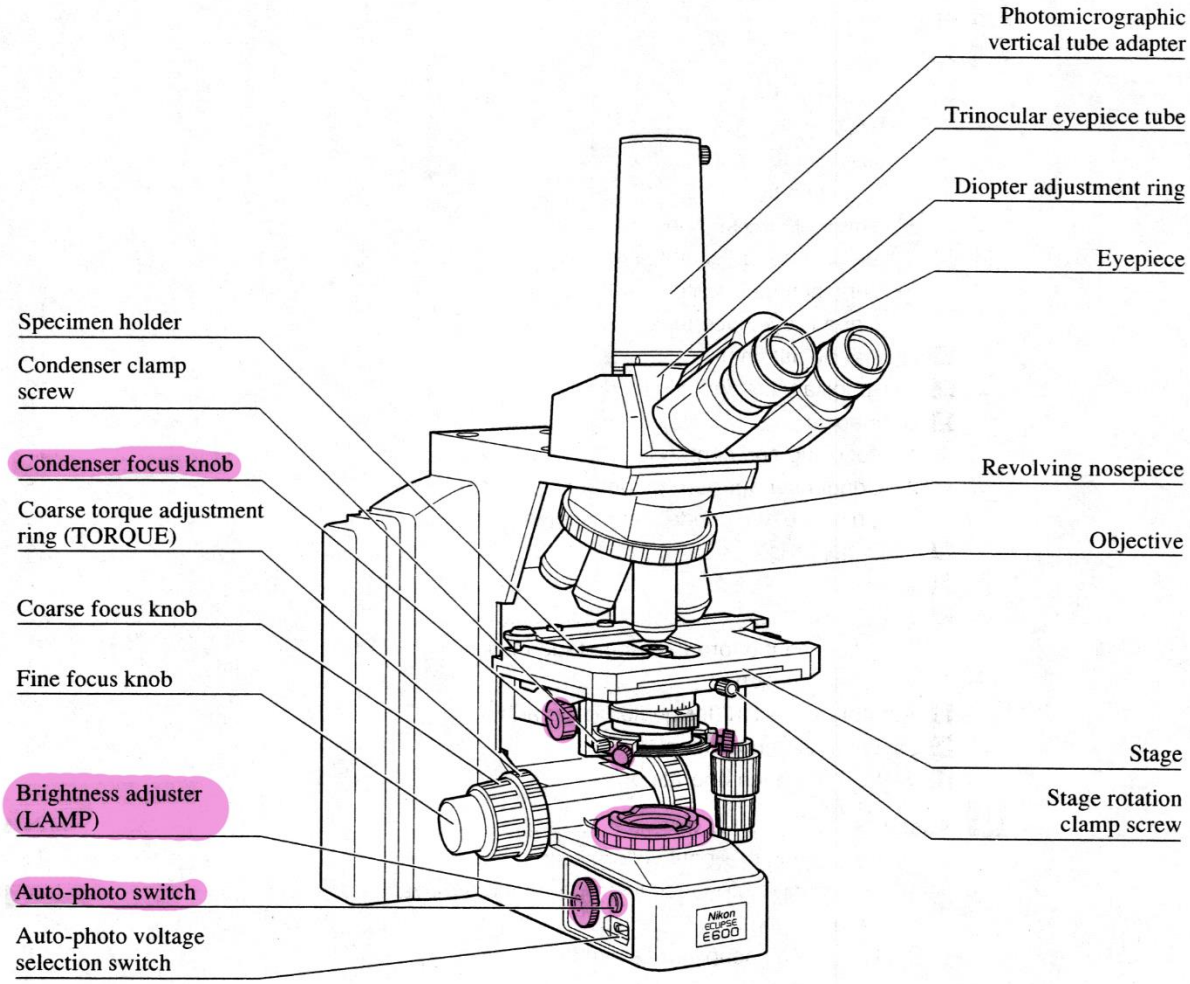
3. Using the 4X lens:
   a. Do Kohler illumination with the *10X lens*.
   b. Fully open the *field and iris diaphragms*.
   c. Move 4X lens into position.
   d. Move the *condenser top-lens* to the side.
   e. Refocus on the specimen.
   f. Close the *field diaphragm* until the image just starts to darken.
      [Note: *With* the condenser top-lens moved to the side the field diaphragm acts like the iris diaphragm in step 2h above].
   g. Adjust lamp intensity as needed.
   h. When finished with the 4X lens put the *condenser top-lens* back in place.

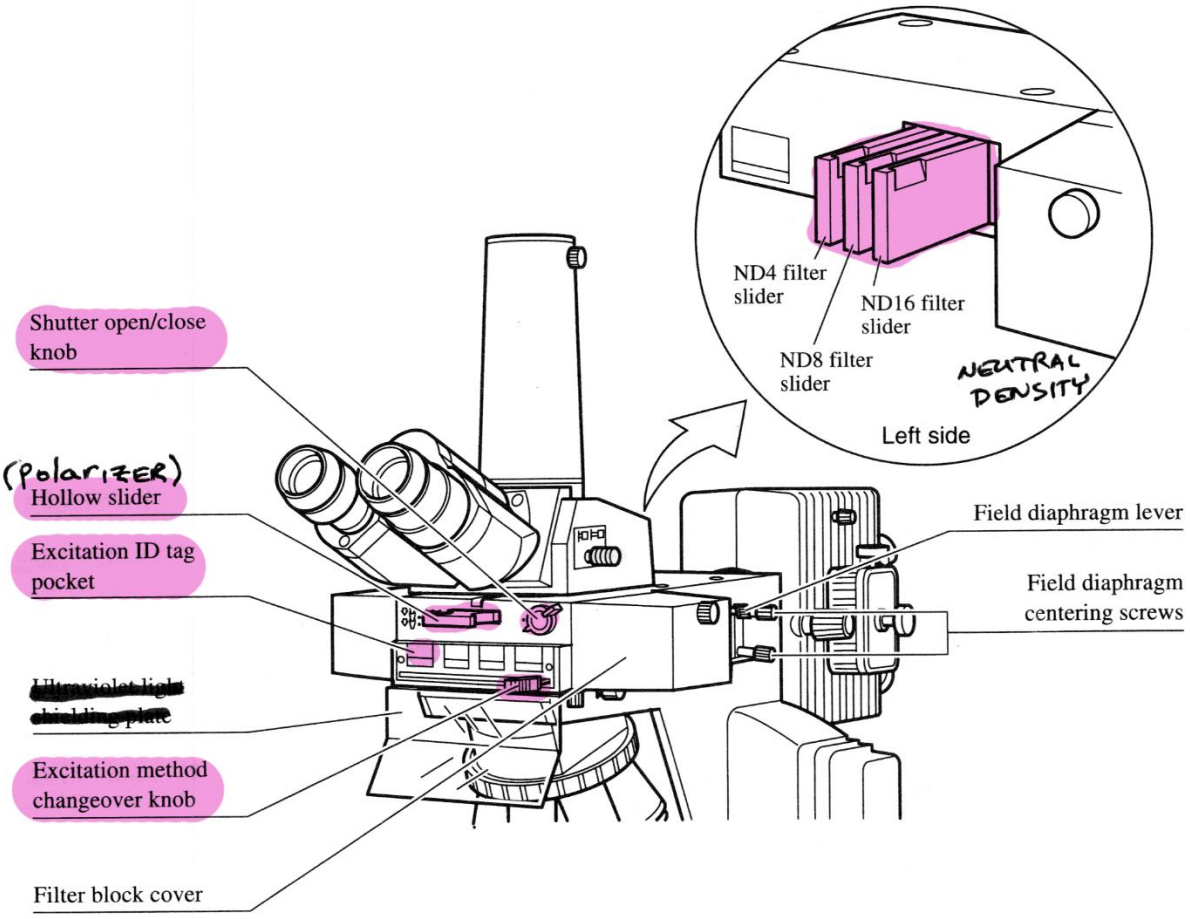
Image collection with software:

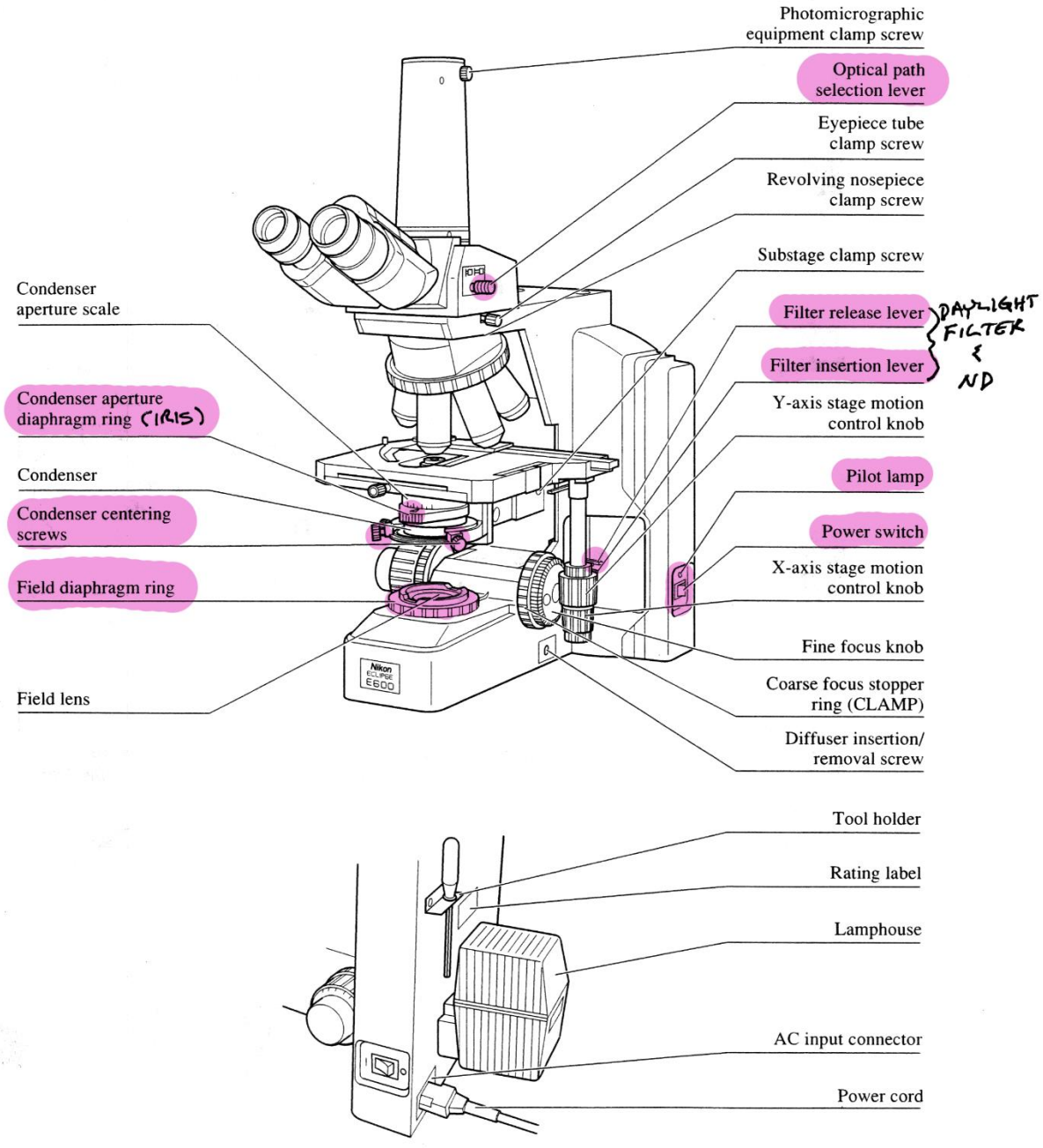
1. Do Kohler.
2. Reset software to defaults.
3. Set exposure time. May need to put light diverter in 'Photo' position for very dim images.
4. Check focus on computer screen.
5. White balance.
6. Fine tune exposure with histogram.
7. Take picture.
8. Save as *.tif file.

Helpful Hints:

1. Microscopists generally collect low magnifications first before proceeding to a higher magnification. This will be especially important when doing fluorescence imaging.
2. Imaging is easier if all the sections are cut and stained uniformly. You won't have to make as many hardware and software changes to compensate for the variations.
3. Collect the best images you can. Bad images will generally remain bad even after extensive post-processing [GIGO], and will most probably contain a lot of digital artifacts.
4. Check your images for composition, color, contrast and focus.
5. Archive your original images. Many times while post-processing images you will make a mistake that cannot be undone.


Photomicrographic vertical tube adapter
Trinocular eyepiece tube
Diopter adjustment ring
Eyepiece
Specimen holder
Condenser clamp screw
Condenser focus knob
Coarse torque adjustment ring (TORQUE)
Coarse focus knob
Fine focus knob
Brightness adjuster (LAMP)
Auto-photo switch
Auto-photo voltage selection switch
Revolving nosepiece
Objective
Stage
Stage rotation clamp screw
Nikon ECLIPSE E600


ND4 filter slider
ND16 filter slider
ND8 filter slider
NEUTRAL DENSITY
Left side
Shutter open/close knob
(Polarizer)
Hollow slider
Excitation ID tag pocket
Ultraviolet light shielding plate
Excitation method changeover knob
Filter block cover
Field diaphragm lever
Field diaphragm centering screws


Photomicrographic equipment clamp screw
Optical path selection lever
Eyepiece tube clamp screw
Revolving nosepiece clamp screw
Substage clamp screw
Condenser aperture scale
Filter release lever
Filter insertion lever
DAYLIGHT FILTER & ND
Condenser aperture diaphragm ring (IRIS)
Y-axis stage motion control knob
Condenser
Pilot lamp
Condenser centering screws
Power switch
X-axis stage motion control knob
Field diaphragm ring
Fine focus knob
Coarse focus stopper ring (CLAMP)
Field lens
Diffuser insertion/ removal screw
Tool holder
Rating label
Lamphouse
AC input connector
Power cord

MicroPublisher 5.0

Magnification	1 x 1 Bin	2 x 2 Bin	3 x 3 Bin	4 x 4 Bin
4x	746 p/mm	373 p/mm	249 p/mm	186 p/mm
10x	1.86 p/um	932 p/mm	621 p/mm	466 p/mm
20x	3.73 p/um	1.86 p/um	1.24 p/um	932 p/mm
40x	7.46 p/um	3.73 p/um	2.49 p/um	1.86 p/um
60x	11.18 p/um	5.59 p/um	3.73 p/um	2.8 p/um
100x	18.66 p/um	9.33 p/um	6.22 p/um	4.66 p/um
Box Size	2560 x 1920	1280 x 960	852 x 640	640 x 480
File Size	14.1 M	3.52 M	1.57 M	900 K

p/mm = pixels / millimeter

p/um = pixels / micrometer