



Olympus IX70

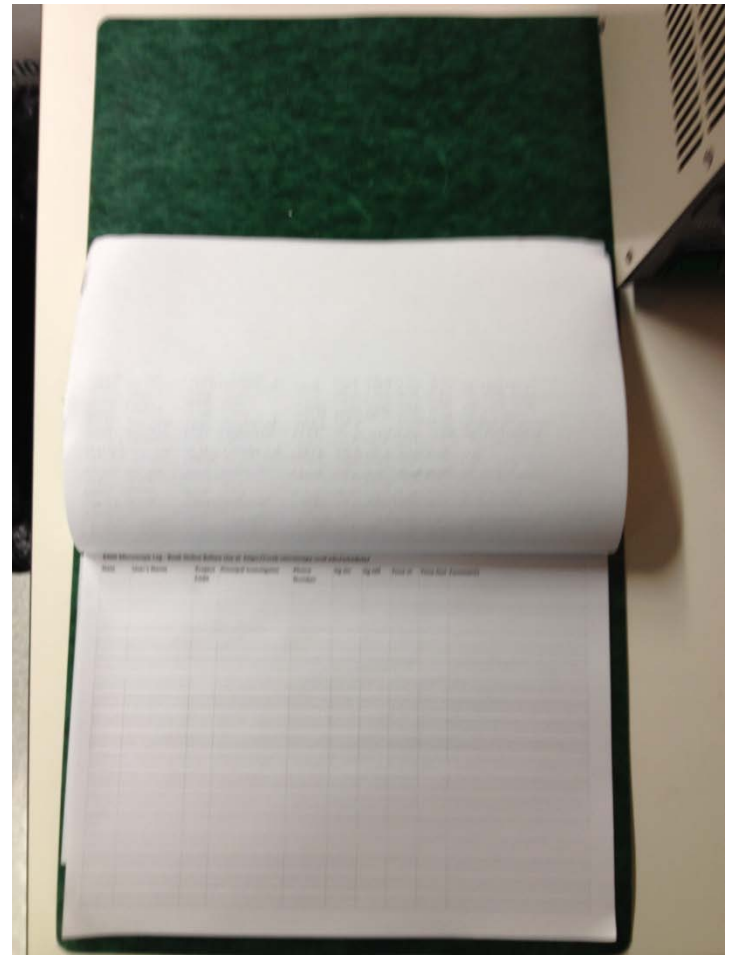
Introduction to the
NRI-MCDB Microscopy Facility
IX70 Inverted Microscope

Contents

- Start-up
- Preparing for Imaging
 - Part I – General
 - Part II – Transmitted
 - Part III – Fluorescence
- Shut-down

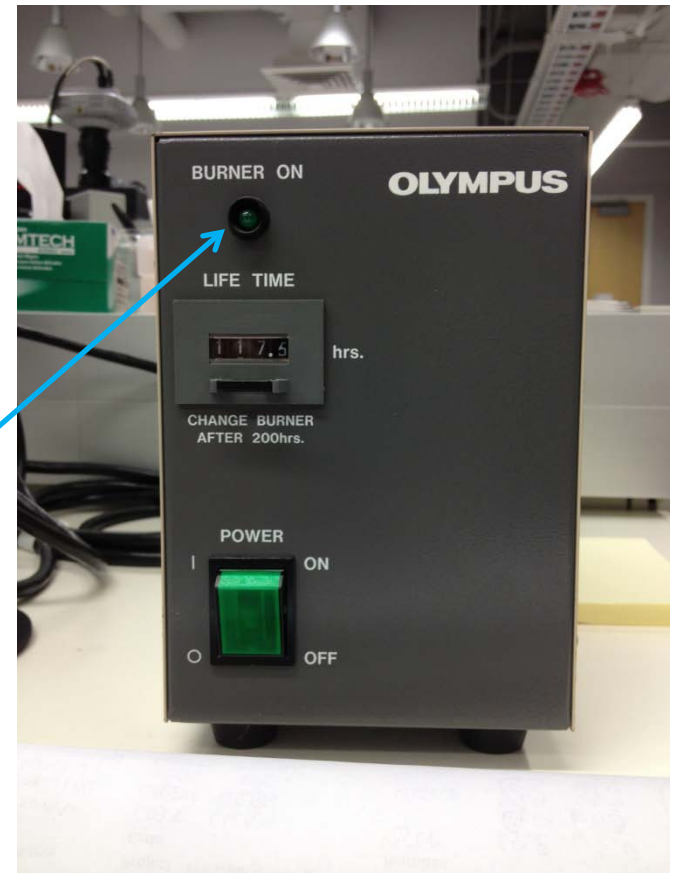
Step 1: Sign-in

- Record the following:
 - Date:
 - Your name:
 - Your Project Code (i.e. Index Code):
 - Your Principal Investigator (PI):
 - Extension (Optional):
 - Hg On (hour listed on the Mercury Burner):
 - Hg Off – (fill-in when you finish):
 - Time-in (the time you arrived):
 - Time-off (the time you left):
 - Comments (any notes on system condition)



Step 2: Mercury Burner

- Turn-on the burner if you are viewing or imaging fluorescent samples.
- Confirm that the burner ignites (i.e. you see a glowing BURNER ON indicator light).
- Leave the burner on for at least 30 minutes before shutting it down.



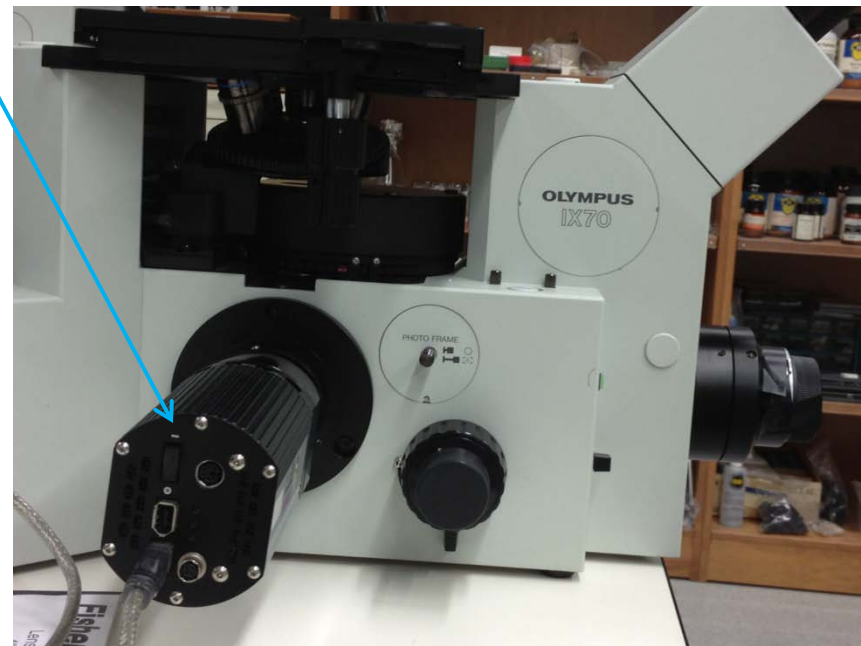
Step 3: Turn on the Microscope

Turn on the microscope using the switch in the back. This supplies power to the tungsten-halogen light used for transmitted light imaging. You need to turn it on if you will be doing transmitted imaging (e.g. Brightfield, Phase)



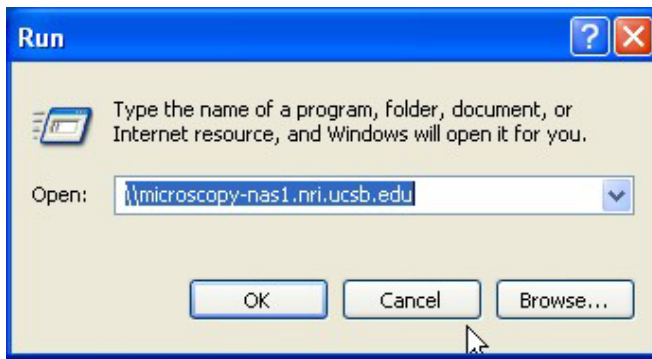
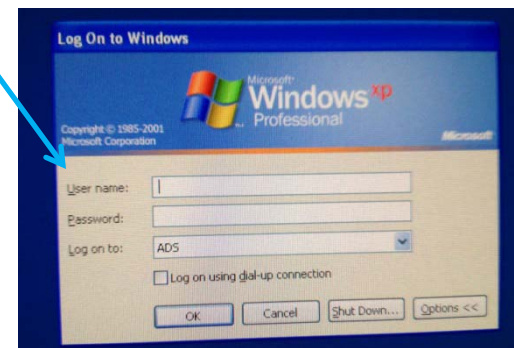
Step 4: Turn Camera Power Supply

- Switch-on the power to the camera if you plan to image your samples.
- The camera is mounted on the left-hand side of the microscope on the side-port.



Step 5: Turn-on/Set-up the computer

- Turn-on the computer, located under the counter below the microscope.
- Log-in using your ADS account name and password.
- From the start menu, select Run and then type
 - [\\microscopy-nas1.nri.ucsb.edu](http://microscopy-nas1.nri.ucsb.edu)
 - Create a shortcut for future

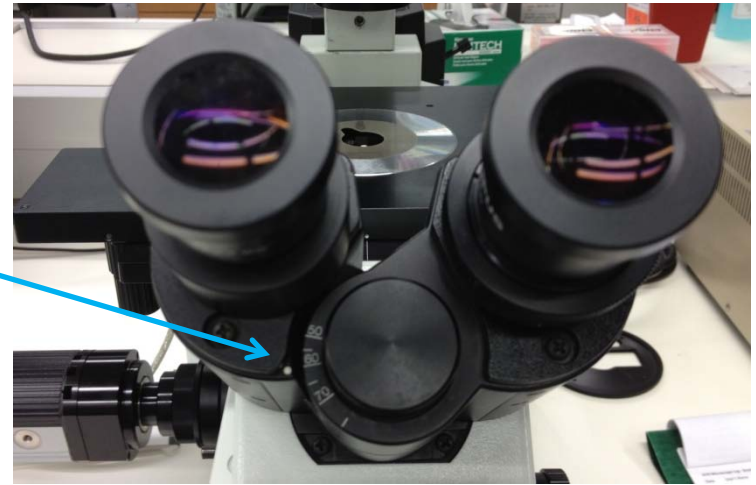


Preparing for Viewing and Imaging

- Part I General Preparation
- Part II – Transmitted Light Applications
- Part III – Fluorescence Applications

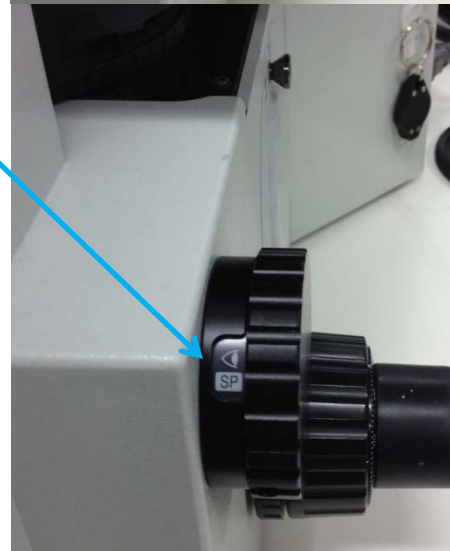
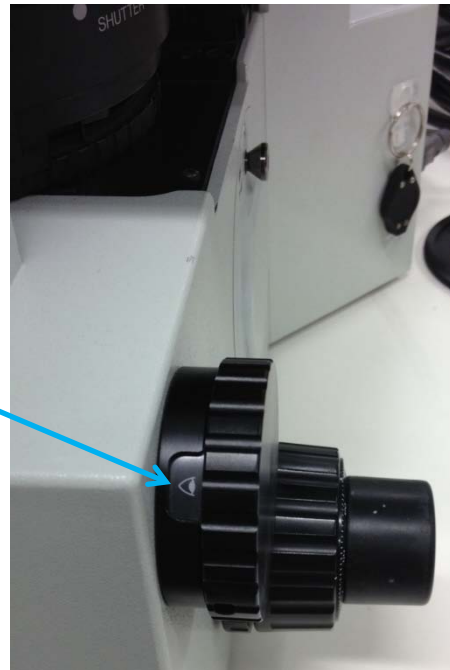
General Prep - Adjust the Eyepieces

- Adjust the spacing to accommodate your interpupillary distance.
- For those with vision corrected to normal, position the diopter to 0. To correct the system for your eyes adjust the diopter.

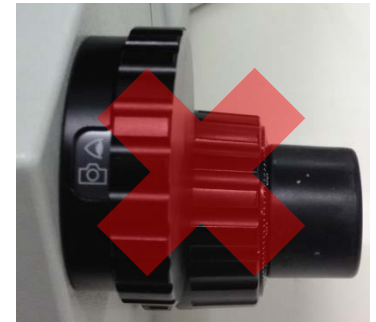


General Prep – Set the beamsplitter

- For viewing with the eyepieces rotate the beam to the eye.
- For imaging rotate the beam to the eye/SP (side-port) icon.

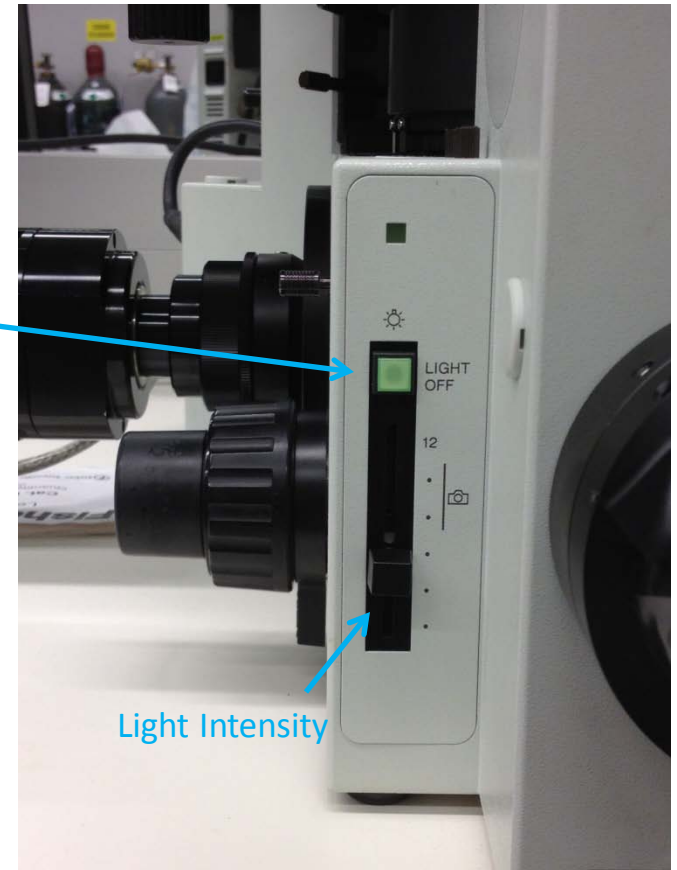


The film camera port is inactive



Part II: Preparing the microscope for transmitted imaging

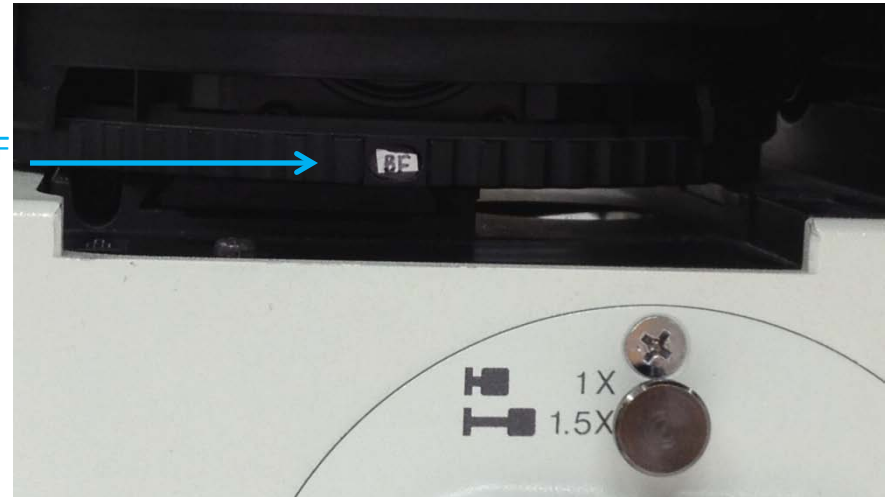
- Adjust the light intensity.
- If the LIGHT-OFF button is illuminated – the transmitted light is off.
- Note, if the **green filter** is in, the transmitted light appears green.



Part II: Confirm that no extra optical components are in place

- Remove any fluorescent filter cubes from the light path
- Remove all Phase Rings

Rotate to BF
(no cube)



Condenser is set for BF
(Brightfield)



Part II: Establish Kohler Illumination

- Place a slide on the stage
- Rotate the 10x objective into position using the nosepiece.



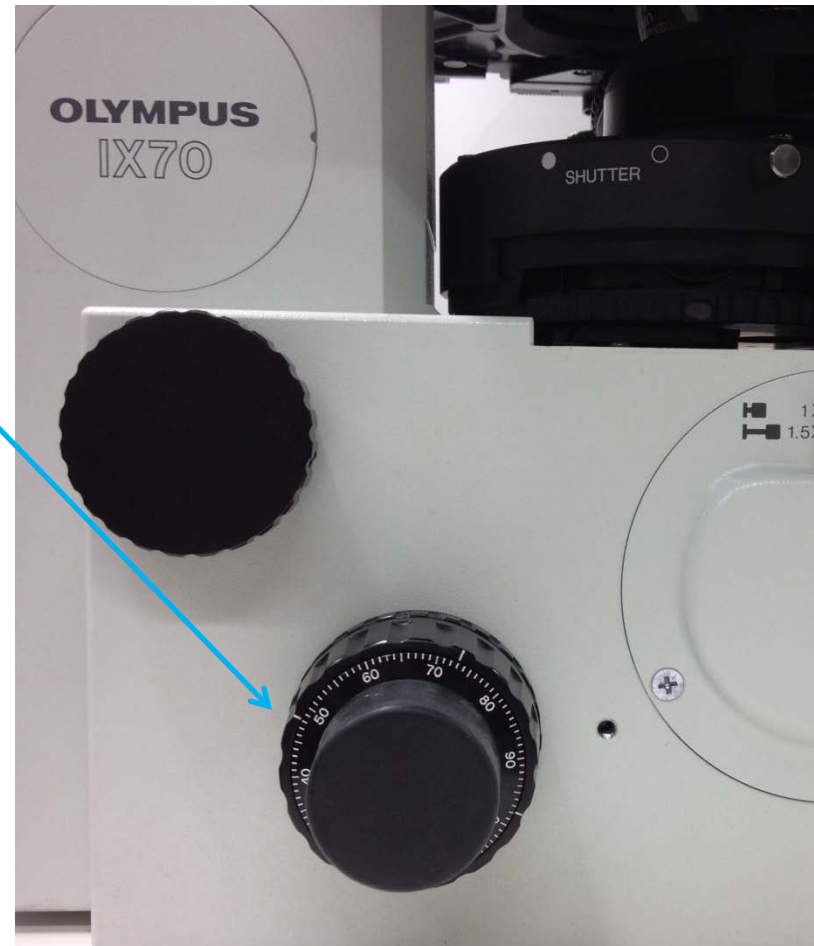
Part II: Establish Köhler Illumination

Use the condenser focus knob to position the top of the condenser rack near the mark.



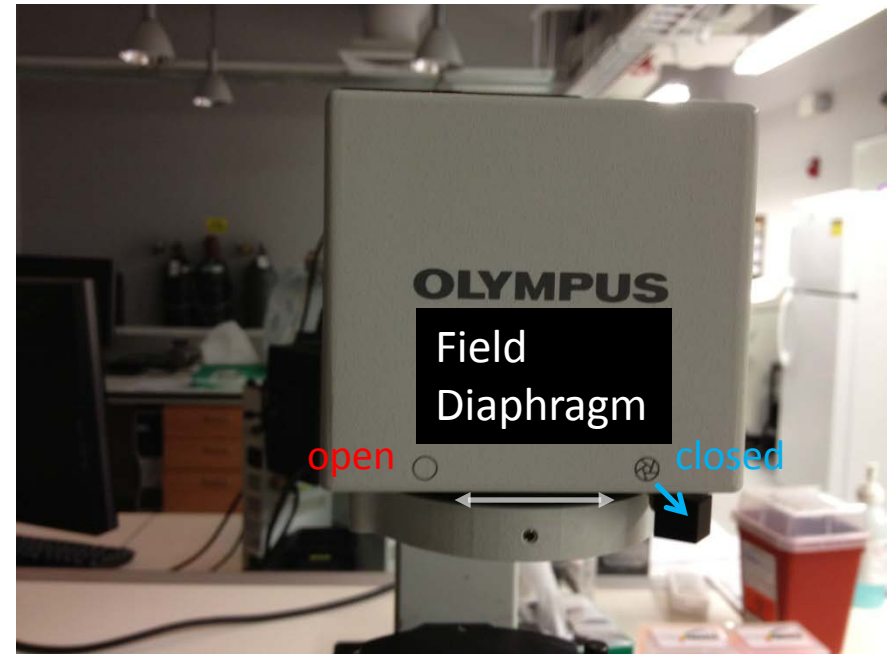
Part II: Establish Köhler Illumination continued

Use the microscope focus knob to bring the sample into crisp focus.



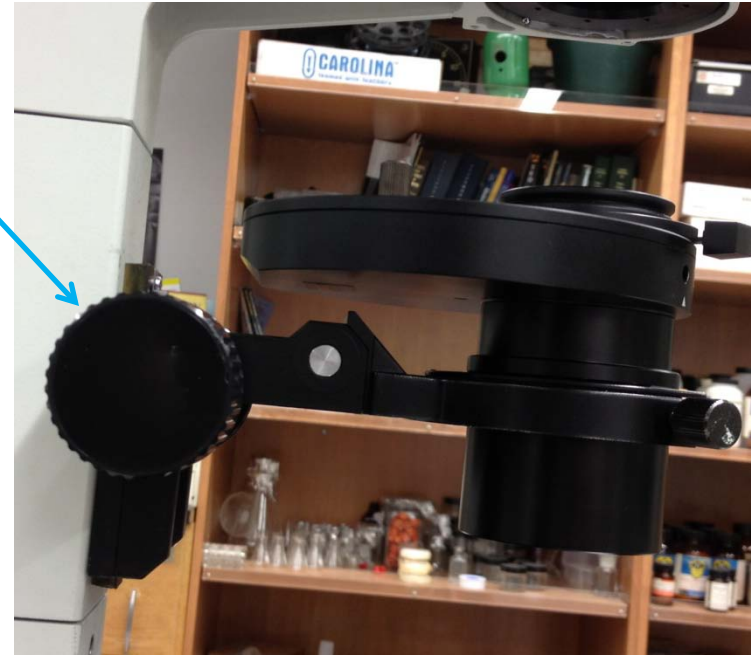
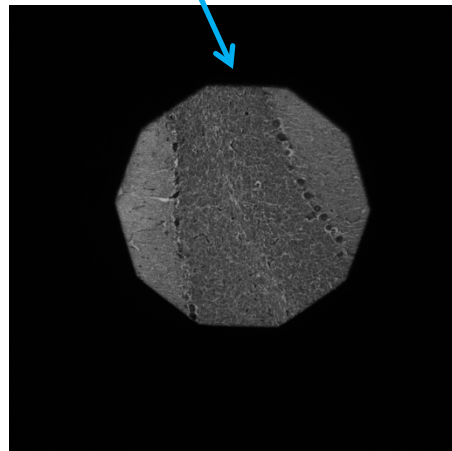
Part II: Establish Köhler Illumination continued

- Use the field diaphragm slider to the closed position.



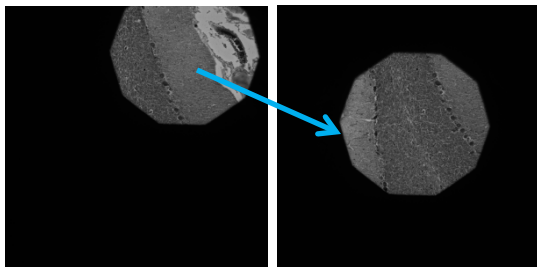
Part II: Establish Köhler Illumination continued

Use the condenser focus knob to further adjust the condenser height so that the outline of the field diaphragm appears crisply focused when viewed through the microscope.



Part II: Establish Köhler Illumination continued

- Use the centering knobs located on the left and right sides of the condenser to center the view of the field diaphragm.

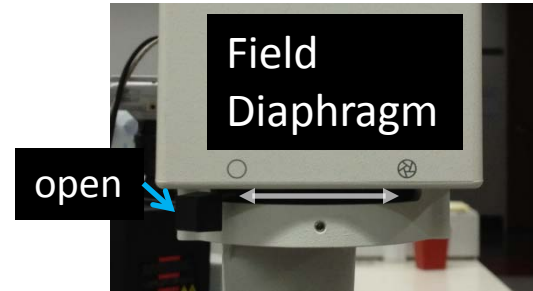


Centering Knob

Centering Knob

Part II: Establish Köhler Illumination continued

- Slide the field diaphragm toward the open position.
- As the view of the field diaphragm approaches the edge of the field of view, use the centering knobs on the condenser to fine tune the position of the light.
- Once the light is optimally centered, open the field diaphragm just beyond the field of view – no more.



Centering Knob

Centering Knob

Part II: Establish Köhler Illumination continued

- For brightfield images adjust the condenser diaphragm to optimize contrast
 - $\frac{3}{4}$ of the entire light disc visible in the eye tube after removal of the eyepiece for stained specimens
 - $\frac{1}{2}$ or less for transparent specimens
 - Or to taste but the more closed the diaphragm the lower the NA of the system



Phase Contrast

- The condenser diaphragm should be completely open.
- Rotate the condenser turret so that the appropriate Phase Ring is in view through the condenser port
 - 4x – no phase
 - 10X, 20X Phase 1
 - 40X Phase 2



Part II: Reestablish Köhler Illumination

- Köhler illumination is objective specific
- Reestablish Köhler Illumination for each objective

Part III: Preparing the microscope for fluorescence imaging

- Press the LIGHT-OFF button if you are alternating between transmitted and fluorescence imaging. (The button light is on when the transmitted light is off.)
- Otherwise, turn-off or leave off the transmitted light using the microscope power switch on the back of the microscope.

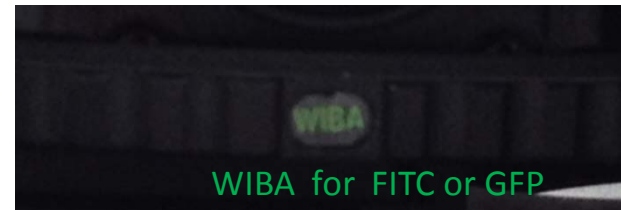


Part III: Filter Turret

- Position the desired filter cube in the light path.
 - NUA – DAPI and other blue appearing probes excited by near UV.
 - WIBA – FITC, Cy2, Alexa488 and other green appearing probes excited by blue light.
 - TR - TRITC, Cy3 and other red appearing probes excited by green light

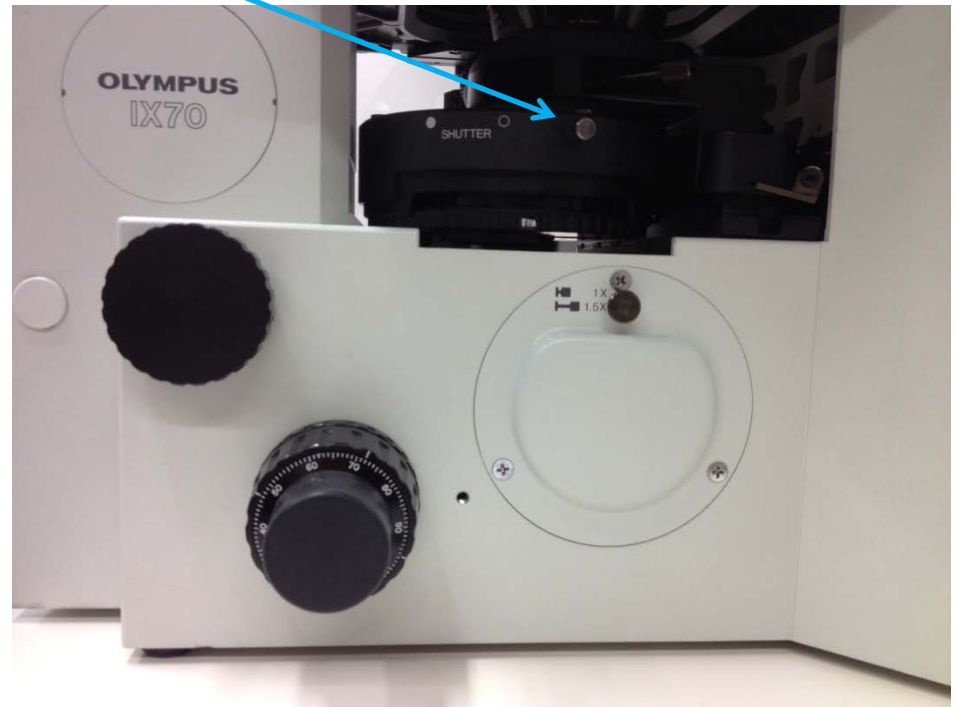


NUA is the DAPI cube



Part III: Open the Shutter

- Rotate the Shutter to open the light path.
- Close the shutter whenever you are not viewing or imaging.



Shut-Down Procedure

- Check the online schedule
 - Shut-down if nobody is scheduled within the next hour
 - Leave the system on if somebody is using the system in the next hour but do the following.
 - Log-off the computer
 - Close the fluorescent shutter
 - Clean-up
 - Return to the 10x objective
 - Sign-off in the log.
- Adjust your online reservation end-time if you finished early

Shut-Down Procedure Continued

- To shut down
 - Shut off the computer
 - Turn off the camera power supply
 - Turn off the microscope power supply
 - Shut down the mercury burner (make certain it has been on for 30 minutes before shutting down)
 - Complete the paper log by filling-in
 - Hg Off (the hours on the bulb)
 - Time you finished
 - Any comments