

# Olympus BX51 on Aura

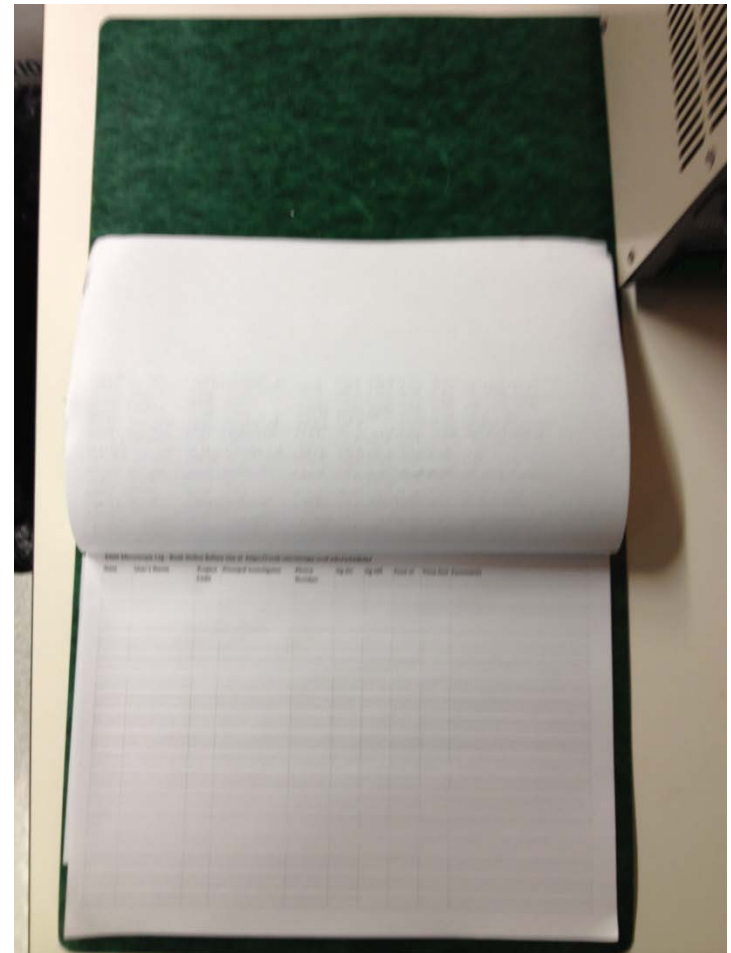
Introduction to the  
NRI-MCDB Microscopy Facility  
BX51 Upright Microscope

# Contents

- Start-up
- Preparing for Imaging
  - Part I – General
  - Part II – Transmitted – Brightfield
  - Part III – Transmitted DIC (Differential Interference Contrast)
  - Part IV – 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 side. This supplies power to the tungsten-halogen light used for transmitted light imaging. It is needed for transmitted imaging (e.g. Brightfield, DIC or Darkfield)



# Step 4: Turn Camera Power Supply

- Switch-on the power to the camera if you plan to image your samples.
- The power switch is located on the top of the camera.



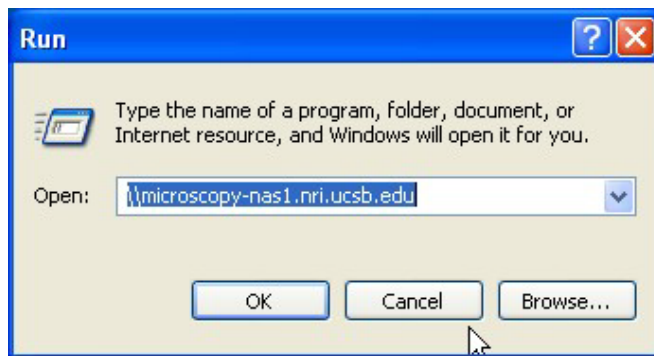
# Check camera color setting

- Use Mono for grayscale imaging and fluorescence
- Use RGB for color imaging.



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

- Turn-on the computer, located under the counter to the far left of 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 use.

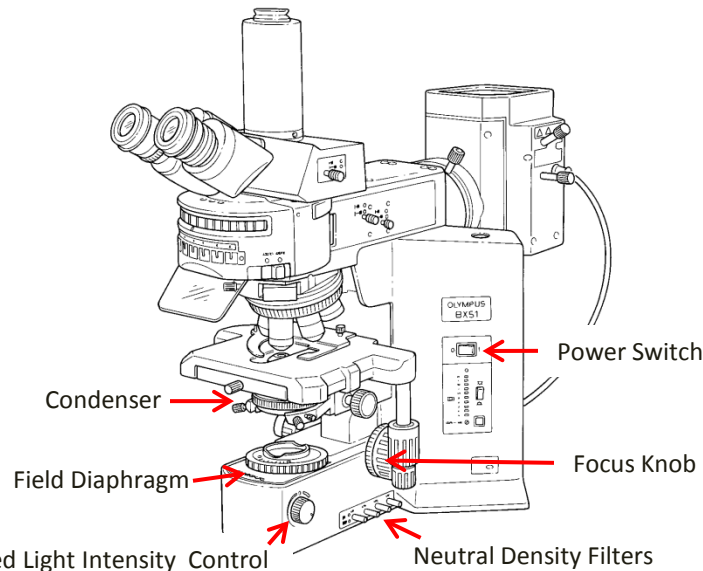




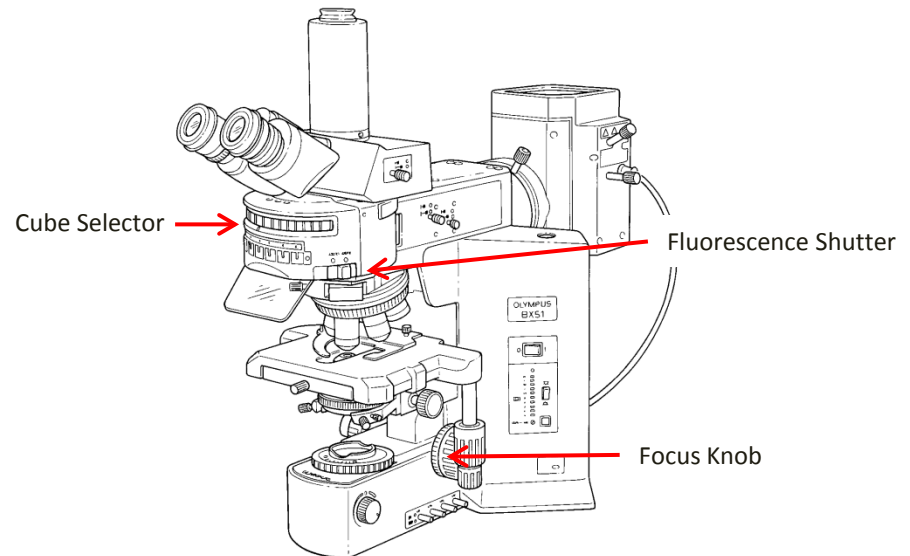
# Preparing for Viewing and Imaging

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

Critical for Transmitted  
Light Applications



Critical for Transmitted  
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.



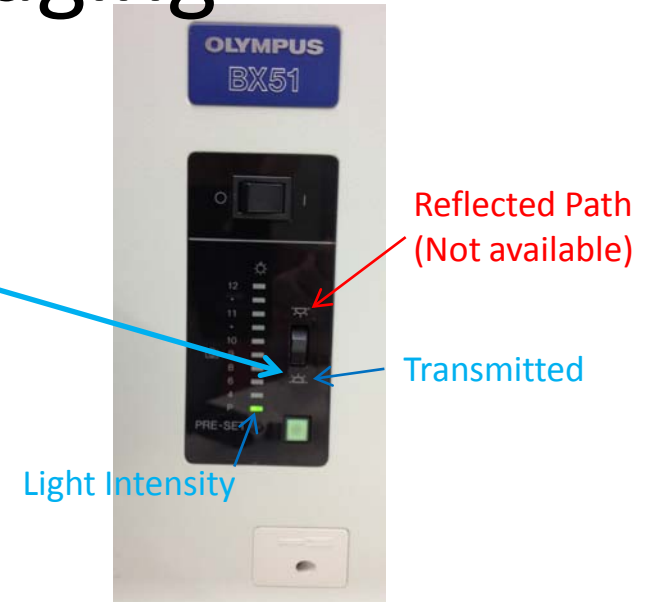
# General Prep – Set the beamsplitter

- For viewing with the eyepieces position the slider all the way in.
- For transmitted light imaging the middle or all to the camera position will work.
- For imaging fluorescence pull the slider all the way out to direct all the light to the camera.

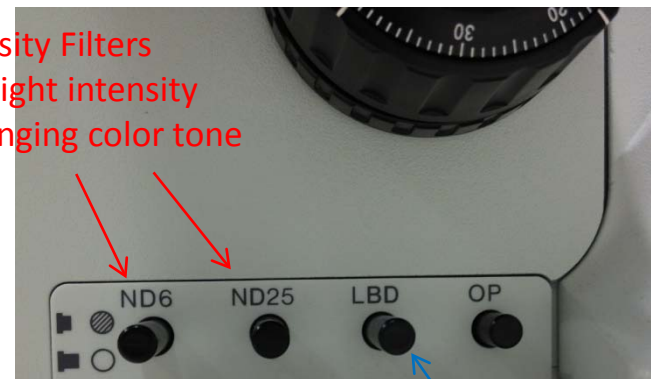


# Part II: Preparing the microscope for transmitted imaging

- Direct light to the transmitted path.
- Adjust the light intensity.
- For transmitted color imaging use the neutral density filters to adjust the light intensity. At low magnification add all the ND filters and remove them as you increase magnification (because you need more light at higher magnification).



Neutral Density Filters change the light intensity without changing color tone of the light

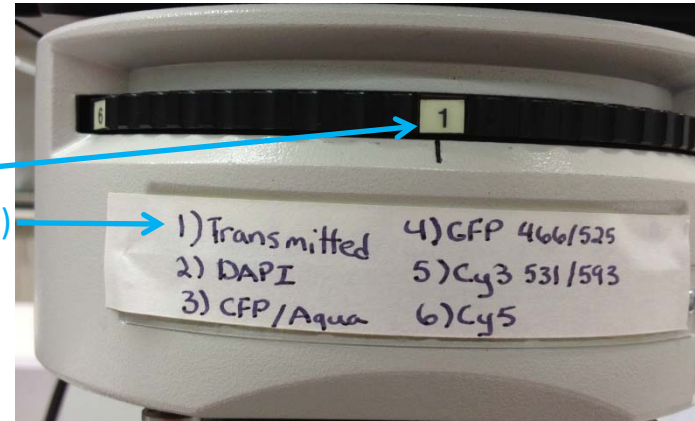


Daylight blue filter makes light bluer

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

- Remove any fluorescent filter cubes from the light path

Rotate to 1  
(Transmitted light)



- Remove all polarizing filters and DIC components

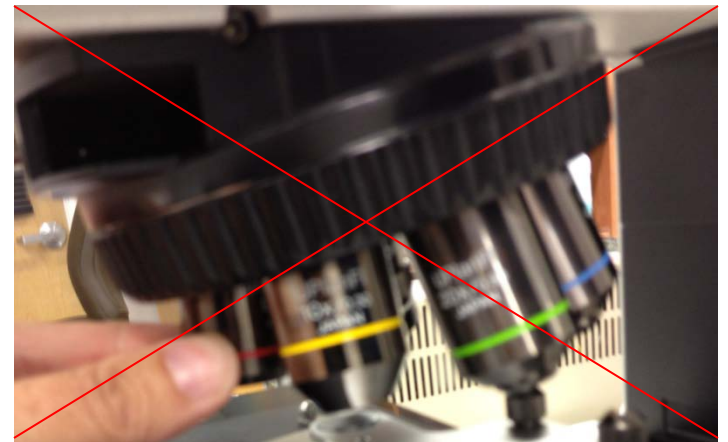
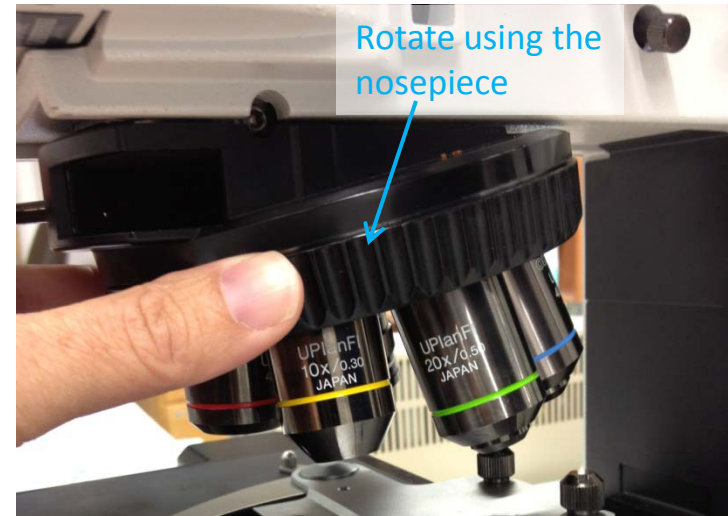
Condenser is set for BF  
(Brightfield)

Polarizing filter out



# Part II: Establish Kohler Illumination

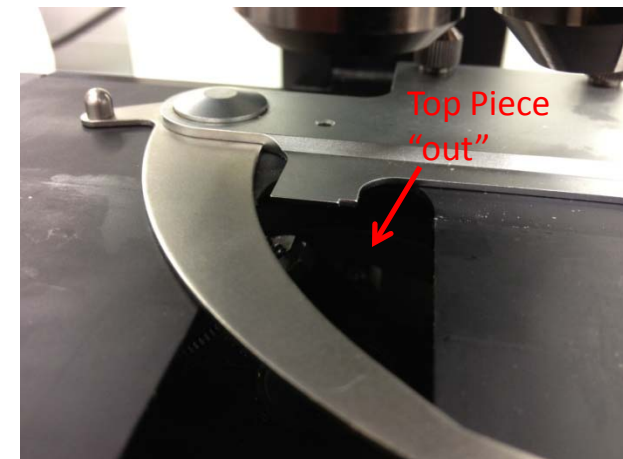
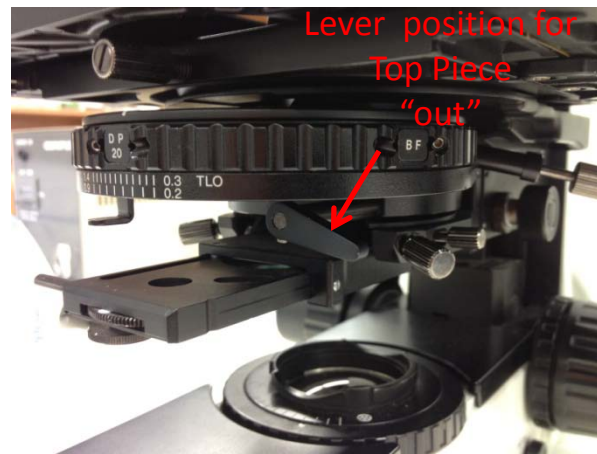
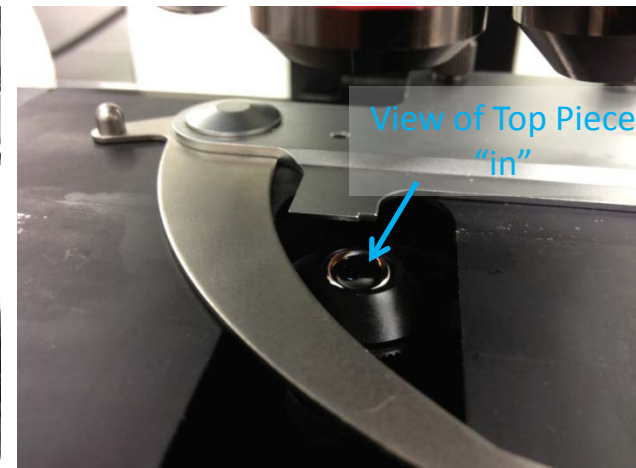
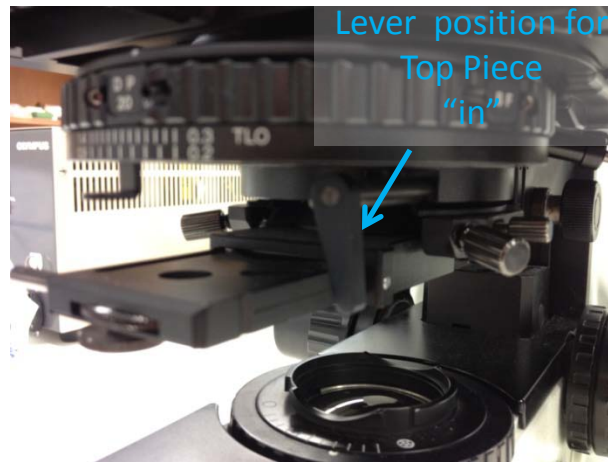
- Place a slide on the stage
- Rotate the 10x objective into position using the nosepiece.



Do not rotate  
by grabbing the  
objectives

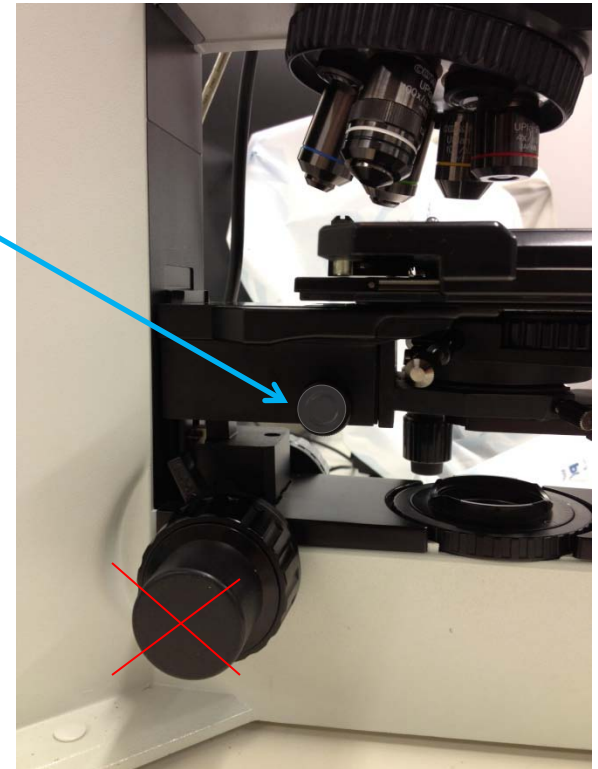
# Part II: Establish Köhler Illumination continued

Position the top piece "in" by rotating the lever on the condenser.



# Part II: Establish Köhler Illumination continued

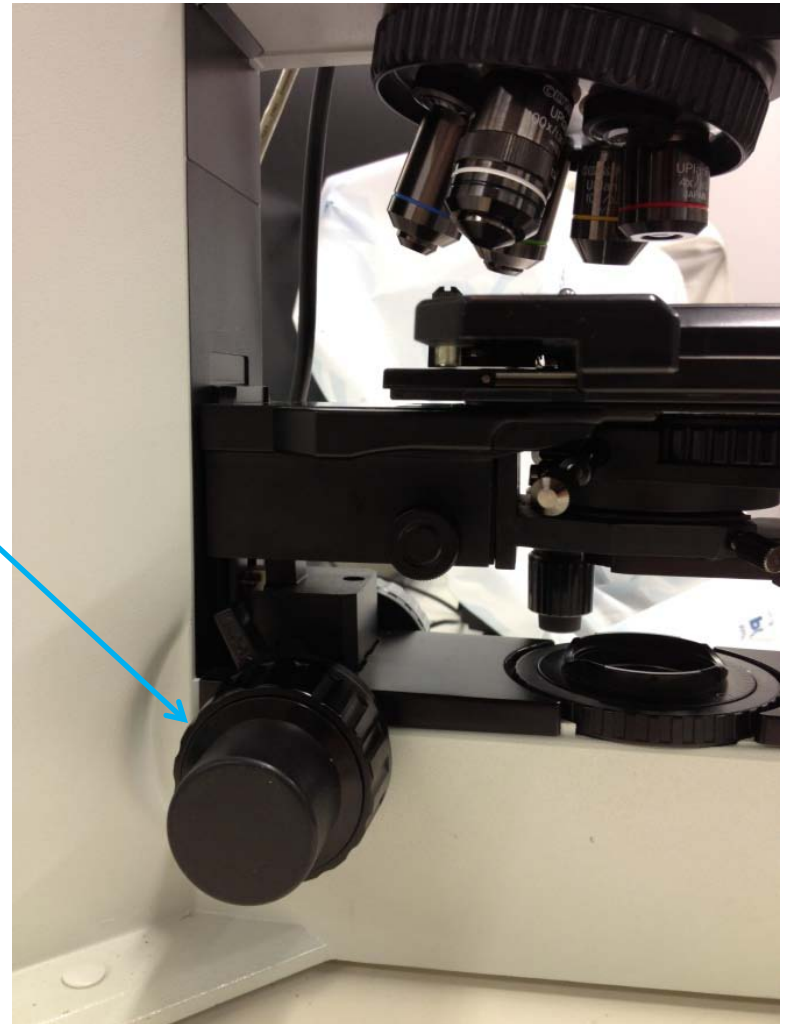
Use the condenser focus knob to position the condenser as close as possible to the slide.





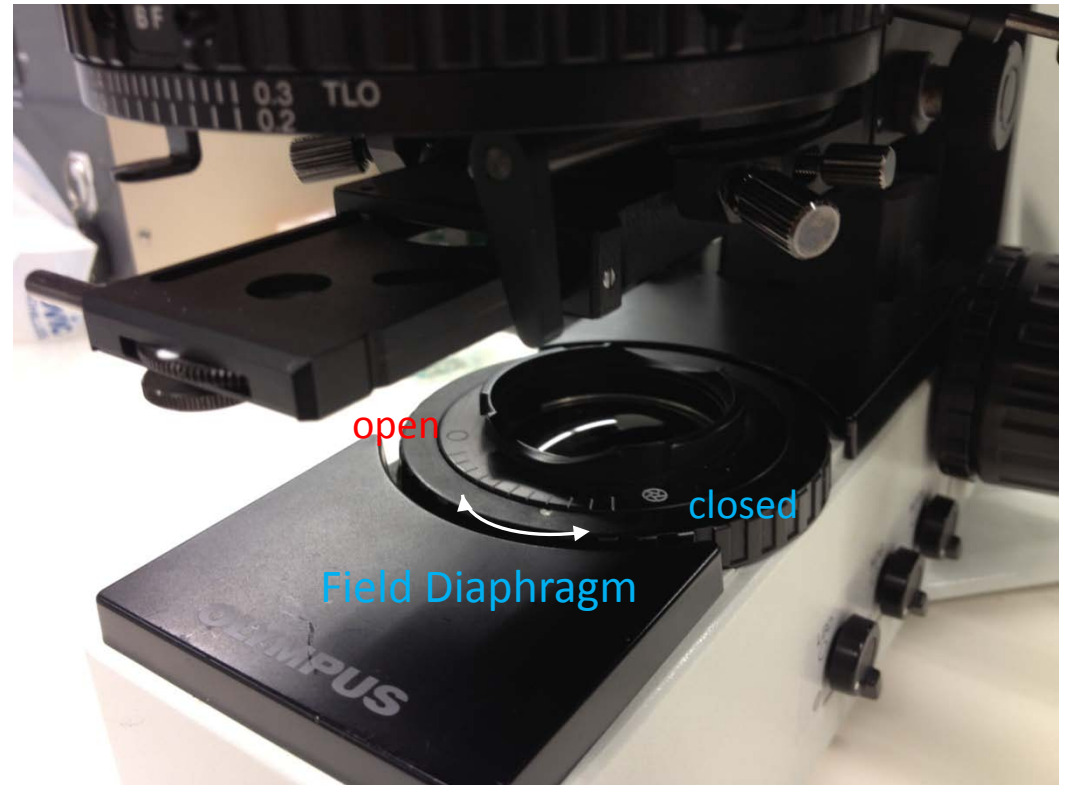
# Part II: Establish Köhler Illumination continued

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



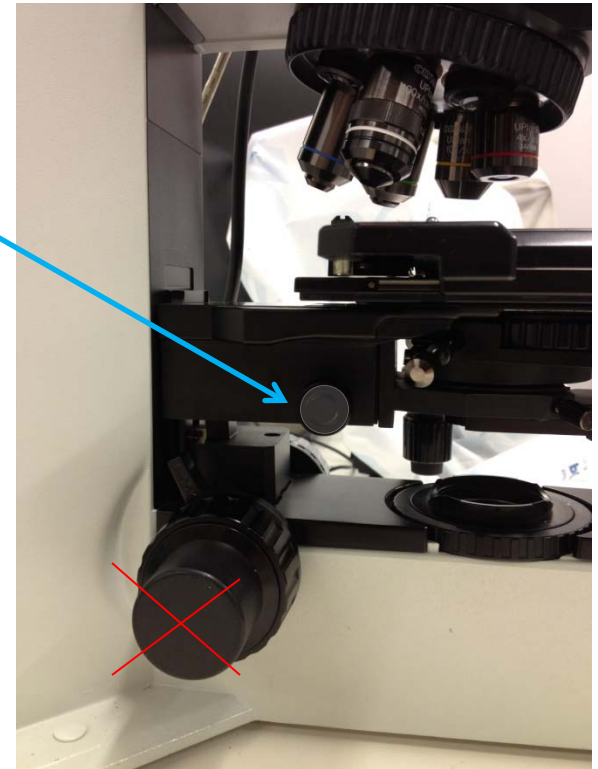
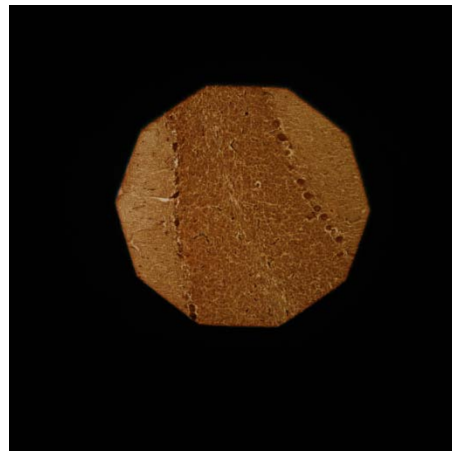
# Part II: Establish Köhler Illumination continued

- Rotate the field diaphragm to the closed position.



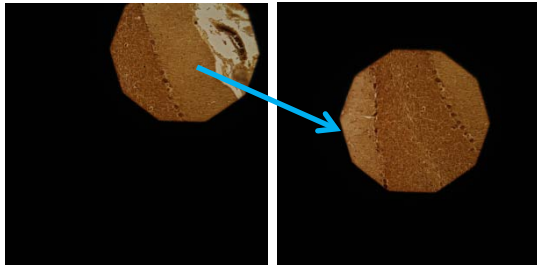
# Part II: Establish Köhler Illumination continued

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



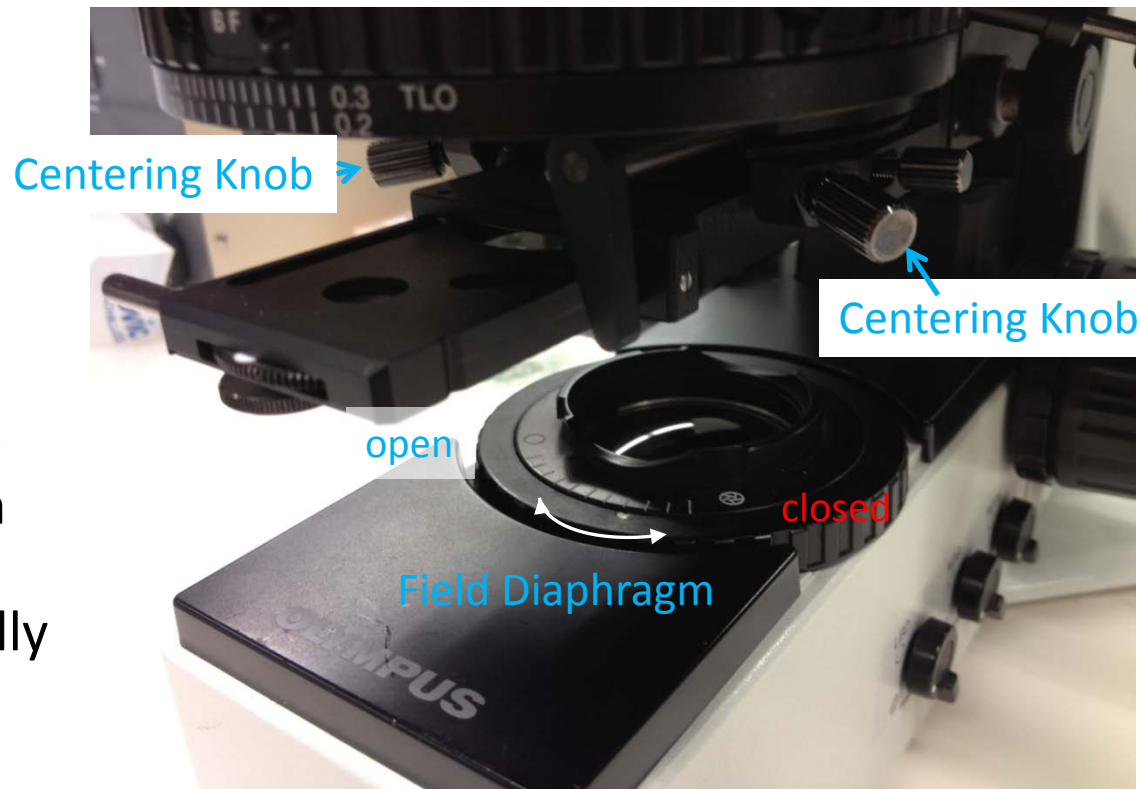
# Part II: Establish Köhler Illumination continued

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



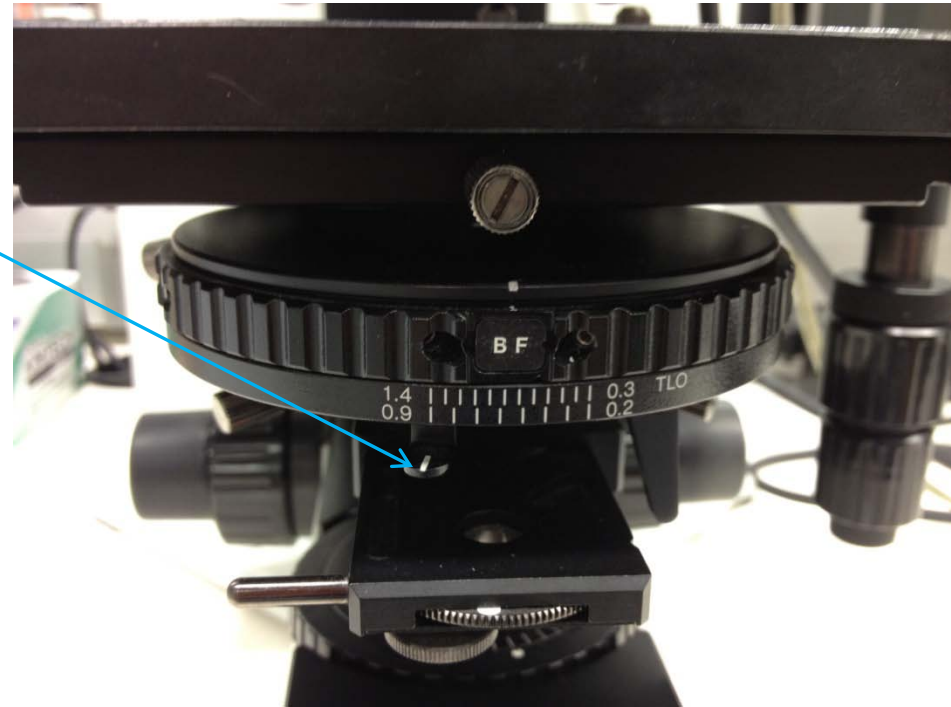
# Part II: Establish Köhler Illumination continued

- Rotate 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.



# Part II: Establish Köhler Illumination continued

- 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



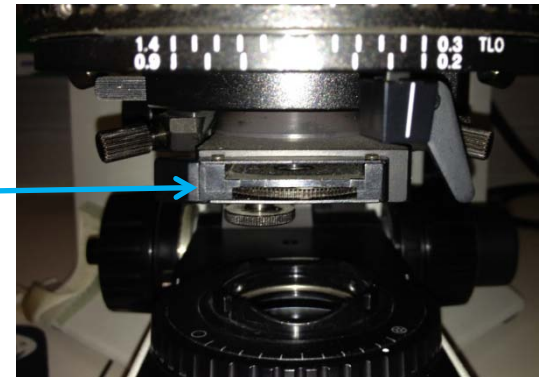
# Part II: Reestablish Köhler Illumination

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

# Part III: DIC

## (Differential Interference Contrast)

- Place the Polarizing Filter into the lightpath
- Rotate the correct DIC prism (corresponds to the objective being used) into position in the condenser.
- Place the analyzer into the lightpath above the objectives.





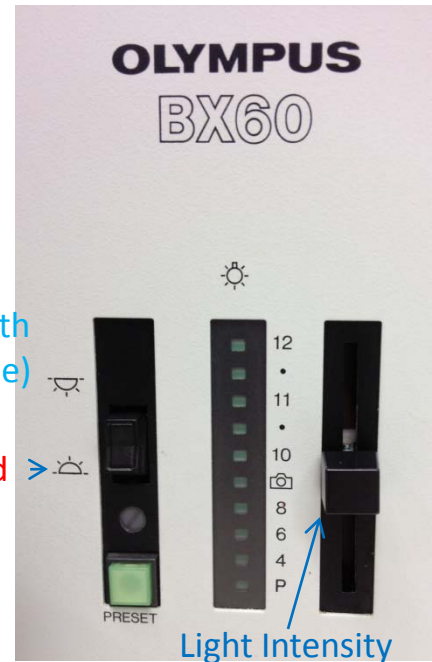
# Part IV: Preparing the microscope for fluorescence imaging

- Direct transmitted illumination to the reflected path if you are alternating between transmitted and fluorescence imaging.
- Otherwise, turn-off or leave off the transmitted light using the microscope power switch on the back of the microscope.



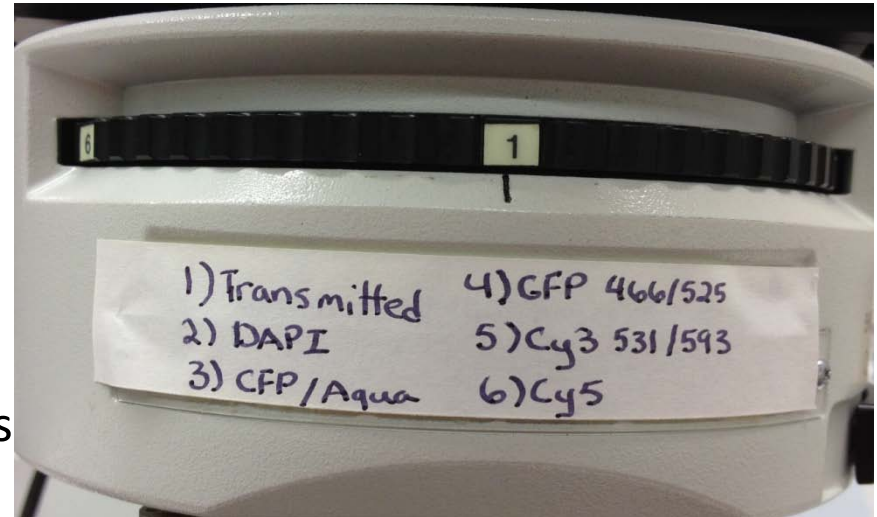
Reflected Path  
(Not available)

Transmitted



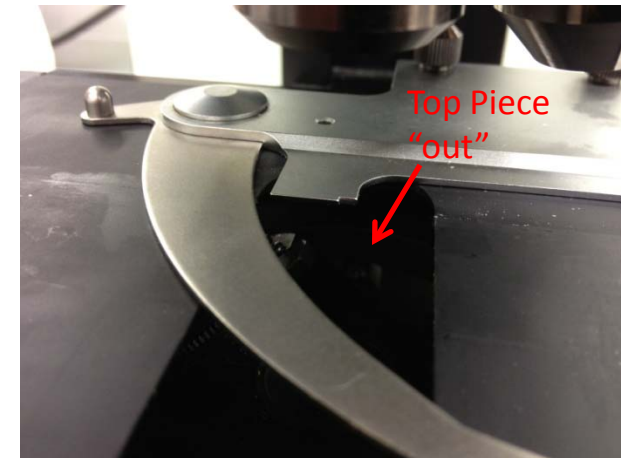
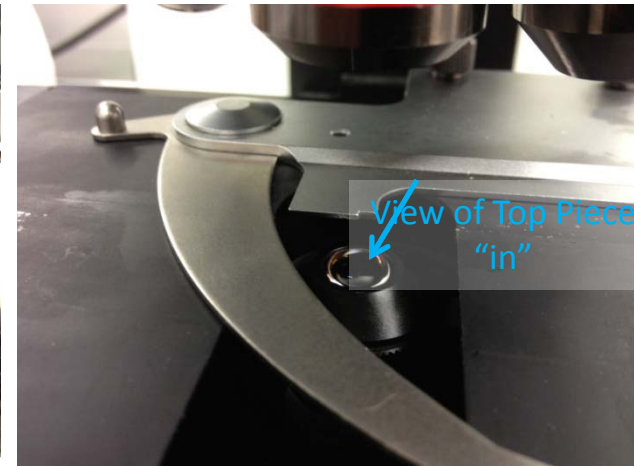
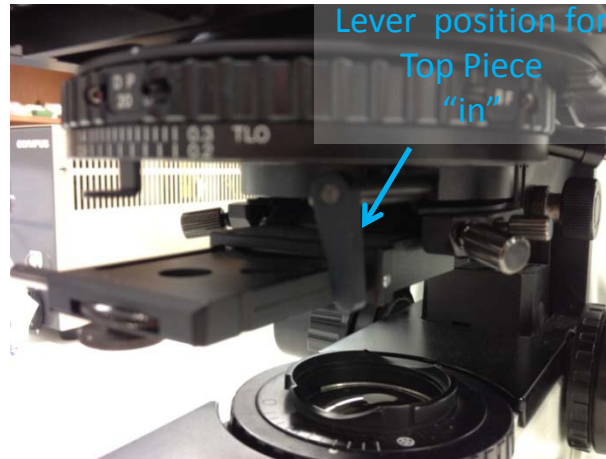
# Part IV: Filter Turret

- Position the desired filter cube in the light path.
  - DAPI and other blue appearing probes excited by near UV.
  - CFP/[Aqua](#) 438/24-25 excitation, 483/32-25 emission
  - [GFP](#) – GFP, FITC, Cy2, Alexa488 and other green appearing probes excited by blue light -466/40-25 excitation, 525/50-25 emission.
  - [Cy3](#) for TRITC, Cy3 and other red appearing probes excited by green light 531/40-25 excitation, 593/40-25 emission
  - [Cy5](#) , 628/40-25 excitation and 692/40-25 emission



# Part IV: Position the top piece “out”

Position the top piece “out” by rotating the lever on the condenser. This reduces light reflection during fluorescence imaging.



# Part IV: Open the Shutter

- Push the Shutter Slider in to open the light path.
- Close the shutter whenever you are not viewing or imaging by pulling on the Shutter Slider.
- The Sliders marked FS and AS should always be open (in).



# 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
  - Return to Mono setting
  - 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