# Nikon Training Notebook

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# Before you begin…

- ❑ Complete the required safety training modules on UC Learning
  - ❑ Laboratory Safety Orientation (Fundamentals) 2013
  - ❑ Hazardous Waste Management
  - ❑ Compressed Gas Safety
- ❑ Submit a copy of your Training Transcript to Lab Manager
- ❑ Review the MSE Policies and Regulations
- ❑ Fill out the MSE 150, 250, 309 FAU Authorization Form with PI signature
- ❑ Provide your ENGR username to Lab Manger to set up Faces account
- ❑ Arrange a time for training with Lab Manager
- ❑ Schedule your reservation on Faces for your training

# Nikon Microscope Operation

I. Microscope Layout

II. Startup

III. EPI: Bright Field

IV. EPI: Dark Field

V. EPI: Polarization

VI. EPI: Differential Interference Contrast (DIC)

VII. DIA: Bright Field

VIII. Image Capture

IX. Cleanup

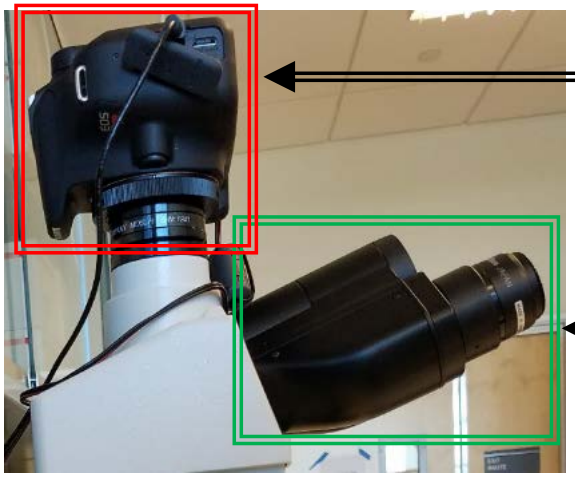
X. ImageJ

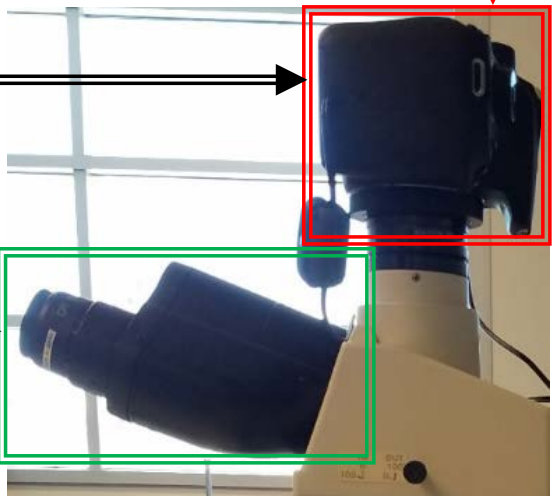
# I. Microscope Layout – 1/4

Camera ON/OFF Switch


Camera Mode Selector:
M (default)

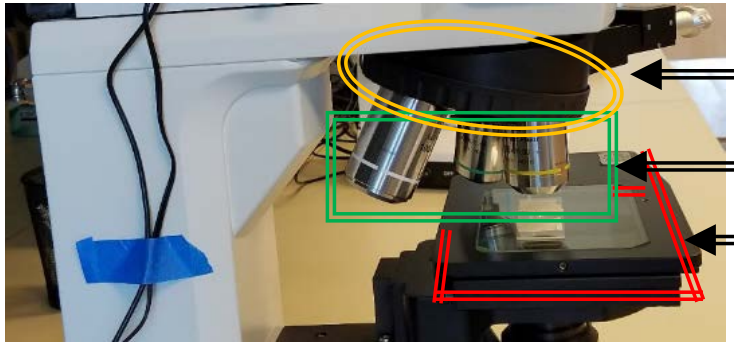
Camera





Binocular Eyepiece

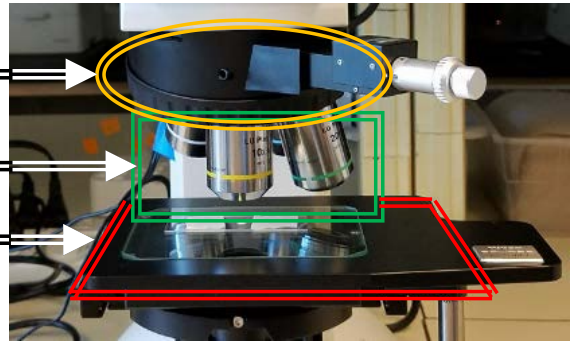
# I. Microscope Layout – 2/4



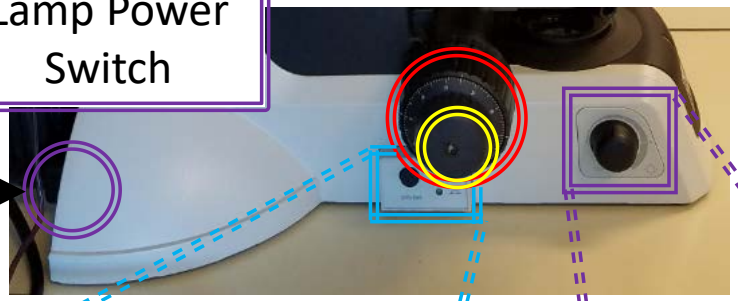
Nosepiece

Objectives

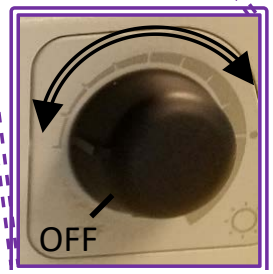
Stage



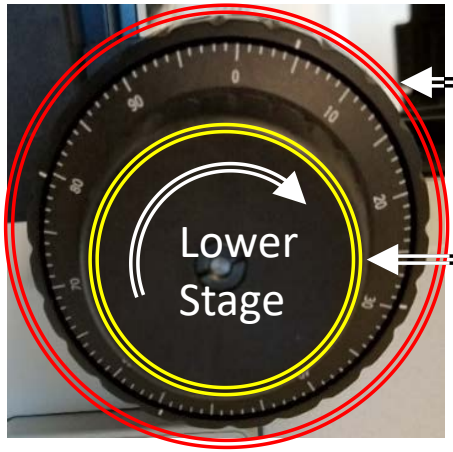
Lamp Power Switch




EPI/DIA Selector




Lamp Brightness Knob




Coarse Focus

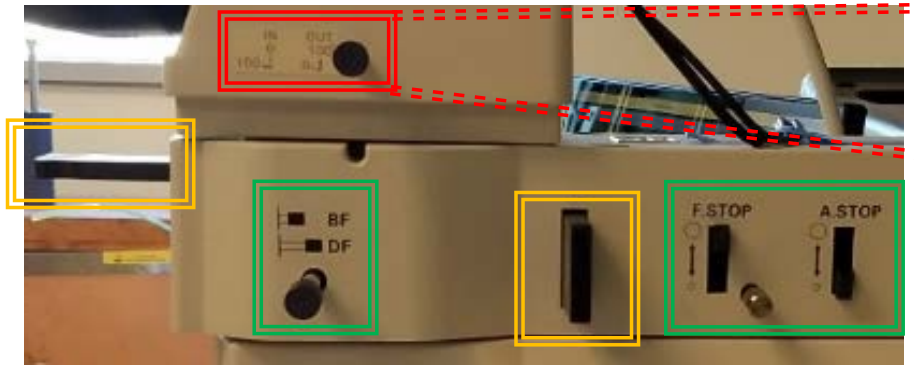
Fine Focus

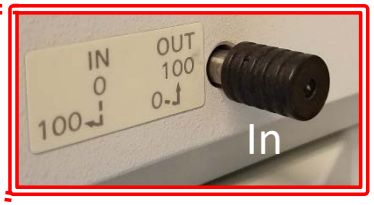
Lamp Indicator

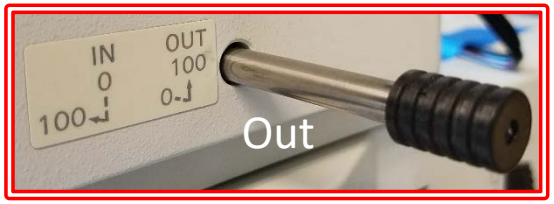
Lamp ON

Lamp OFF

# I. Microscope Layout – 3/4

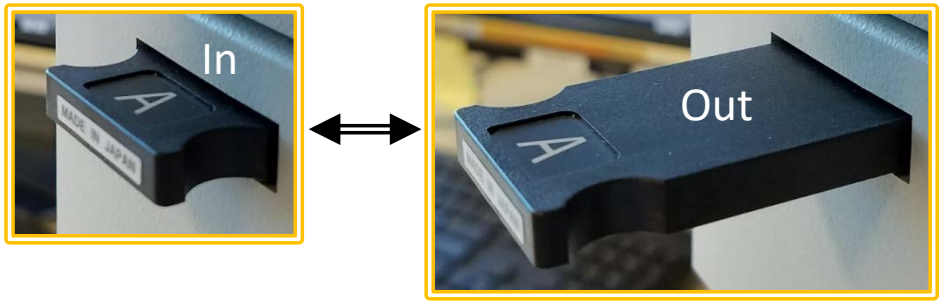


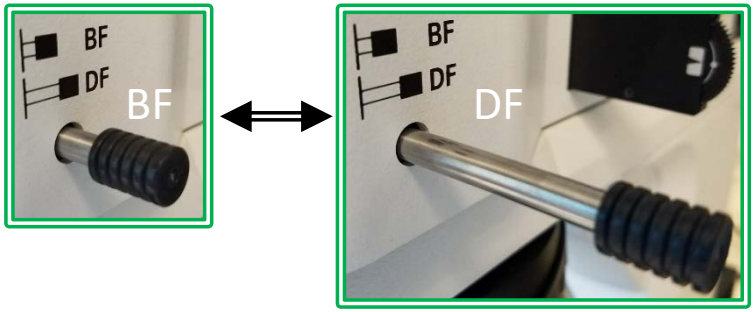




Optical Path Selector Lever

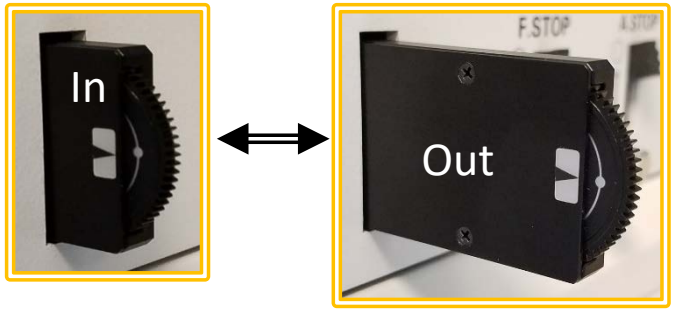
Analyzer Plate



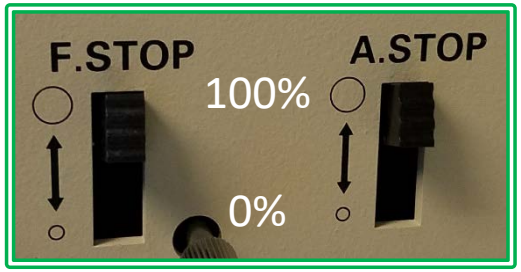

Bright field/Dark field Selector Lever




Polarizer Slider

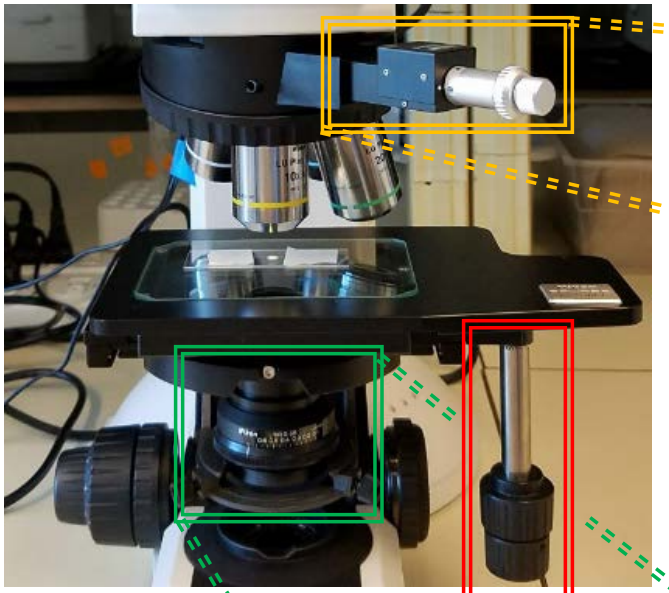



EPI Field Diaphragm Stop

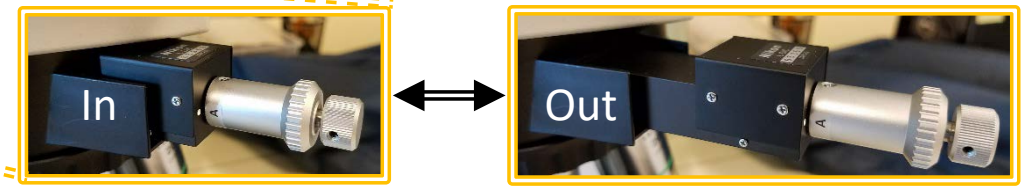



EPI Aperture Diaphragm Stop

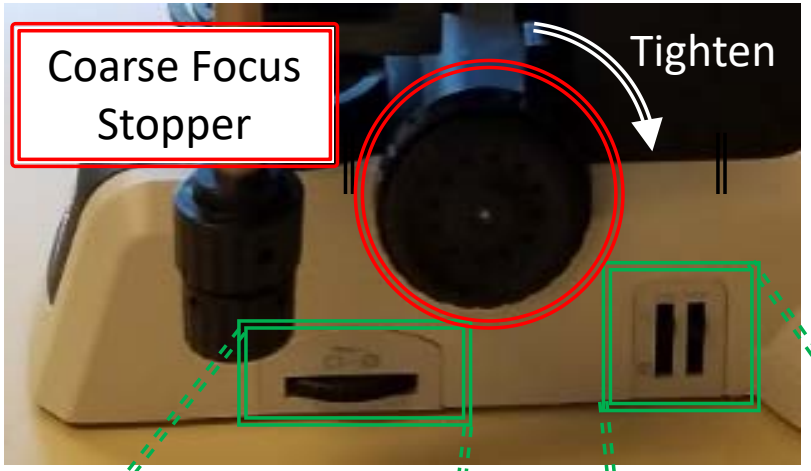
# I. Microscope Layout – 4/4



DIC Prism Plate

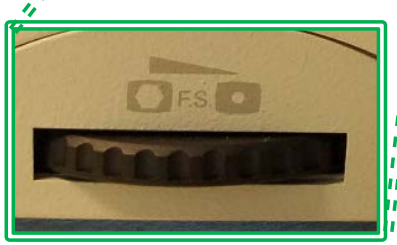



Stage Movement




DIA Condenser Focus Knob


DIA Condenser Aperture




DIA Field Diaphragm Control


Filter Selector Switches

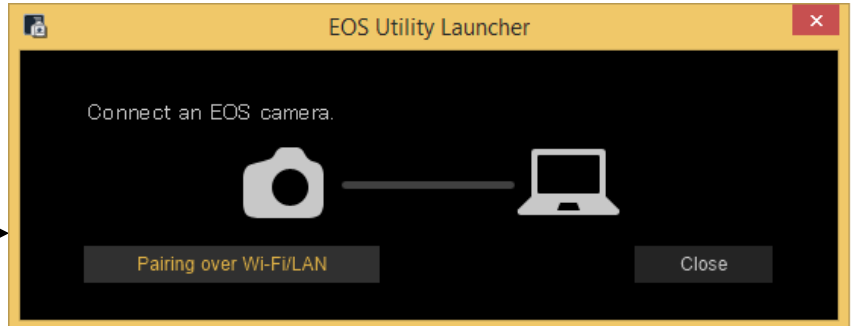
# II. Startup – 1/5

1. Record your time in on the sign-in sheet

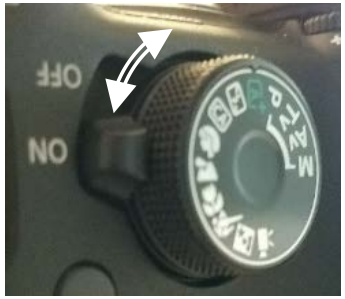
2. Sign-in to the computer with your ***ENGR username*** and ***PW***

3. Double-click on ***EOS Utility*** icon


4. The EOS Utility Launcher may show that the camera is not connected to the computer




5. Toggle Camera ***ON/OFF*** switch to connect it to the computer – keep in ***ON*** position




6. Click on ***Camera settings/Remote shooting***

# II. Startup – 2/5

7. Confirm the following ***Camera Settings*** are set:

**Camera**

***M*** = Manual


**Software (right click to change)**

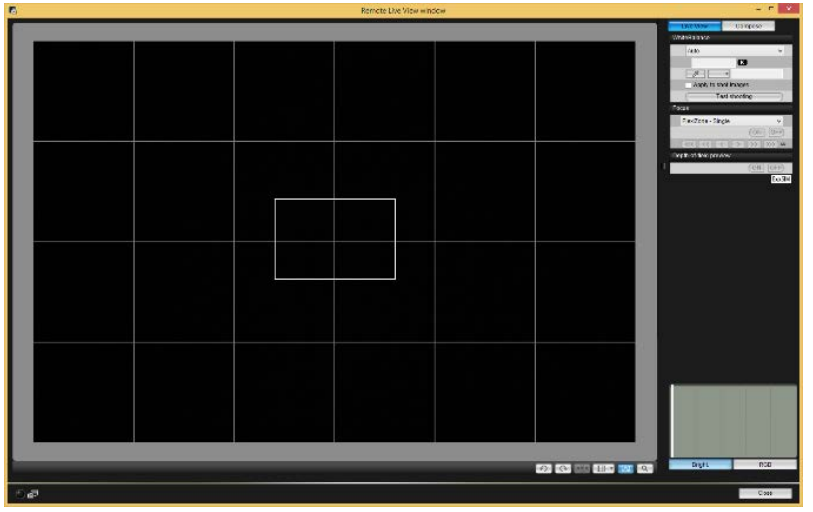
***1/50*** = Shutter Speed

***100*** = ISO

***Tungsten*** = Brightness

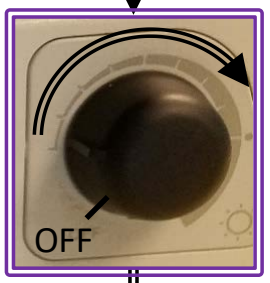
8. Click on ***Live View shoot***

9. Remote Live View window will appear



# II. Startup – 3/5

10. Rotate the ***Lamp Brightness Knob*** until the light indicator goes from ***orange (OFF)*** to ***green (ON)***






11. If light is missing, turn on ***Lamp Power Switch*** on back

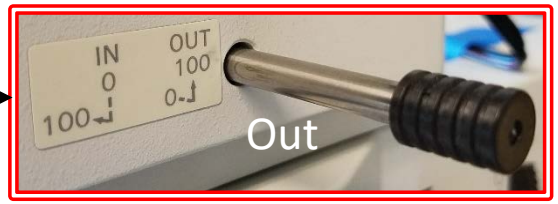

12. For ***Camera View***: Pull lever completely out

    For ***Binocular Eyepiece***: Push lever completely in

**Optical Path Selector Lever**

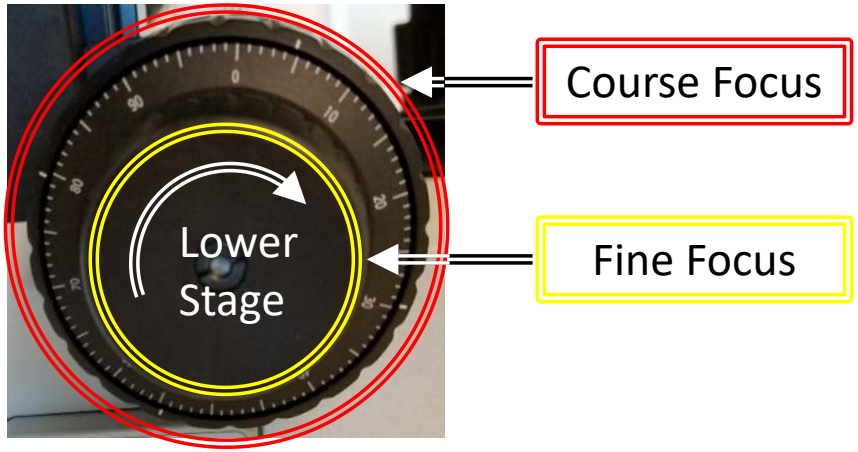
Binocular Eyepiece


Camera View



# II. Startup – 4/5

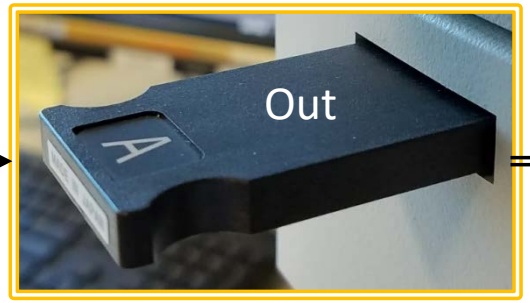
13. Lower stage first by turning ***Coarse Focus*** knob **TOWARD** you

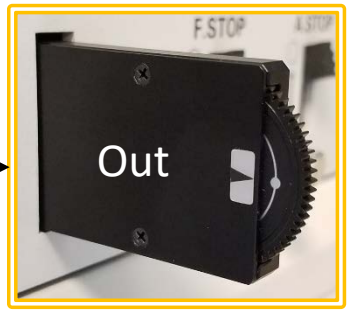



14. Place sample on microscope stage

15. Rotate ***Nosepiece*** and start with the ***10X magnification*** first

16. Pull out ***Analyzer***, ***Polarizer*** and ***DIC Prism*** if inserted

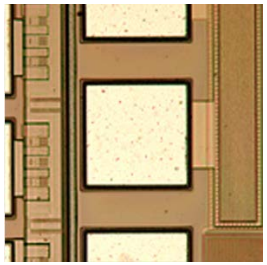




# II. Startup – 5/5

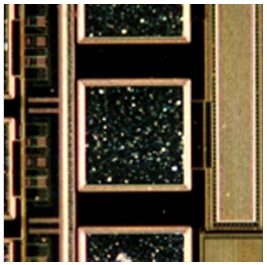
17. Identify which microscope mode you wish to use:

Episcopic Illumination (  )

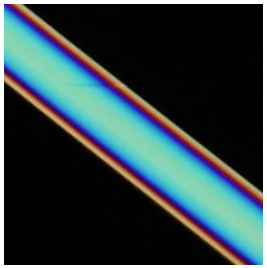
III. Bright field



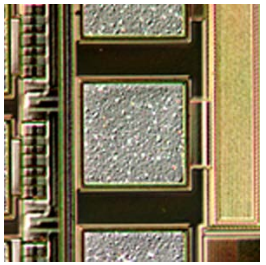
IV. Dark field



V. Polarization

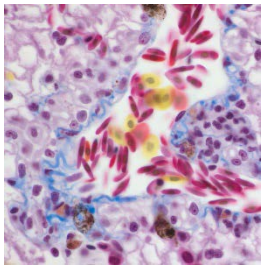


VI. Differential Interference Contrast (DIC)



Diascopic Illumination(  )

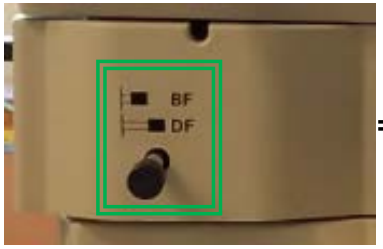
VII. Bright field

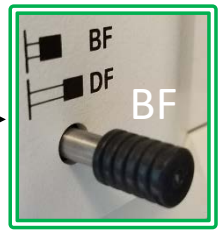


# III. EPI: Bright Field – 1/3

1. Press the ***EPI/DIA*** selector and set to ***EPI***

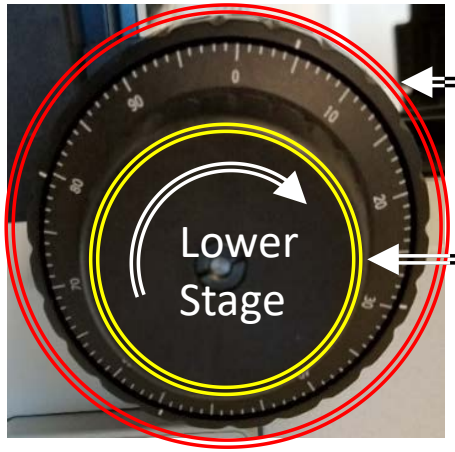

2. Push ***Bright/Dark Field*** selector lever to fully in ***BF*** position




3. Adjust the brightness with the ***Brightness Control*** as necessary


4. Focus on specimen by adjusting the ***Coarse/Fine Focus*** knobs

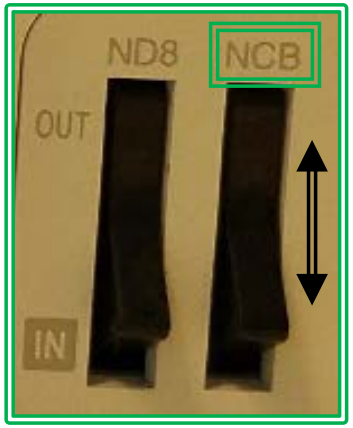


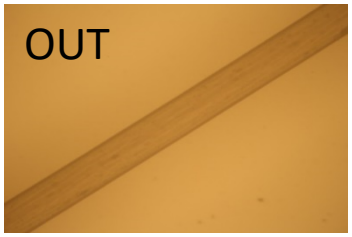

Course Focus

Fine Focus

# III. EPI: Bright Field – 2/3

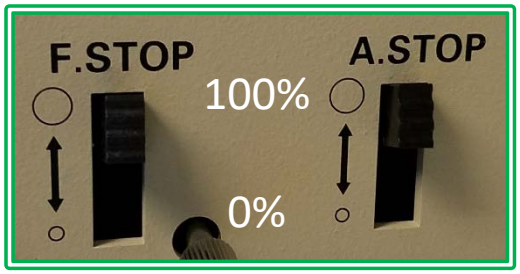
5. Select if ***NCB filter*** (balances color) is desired:

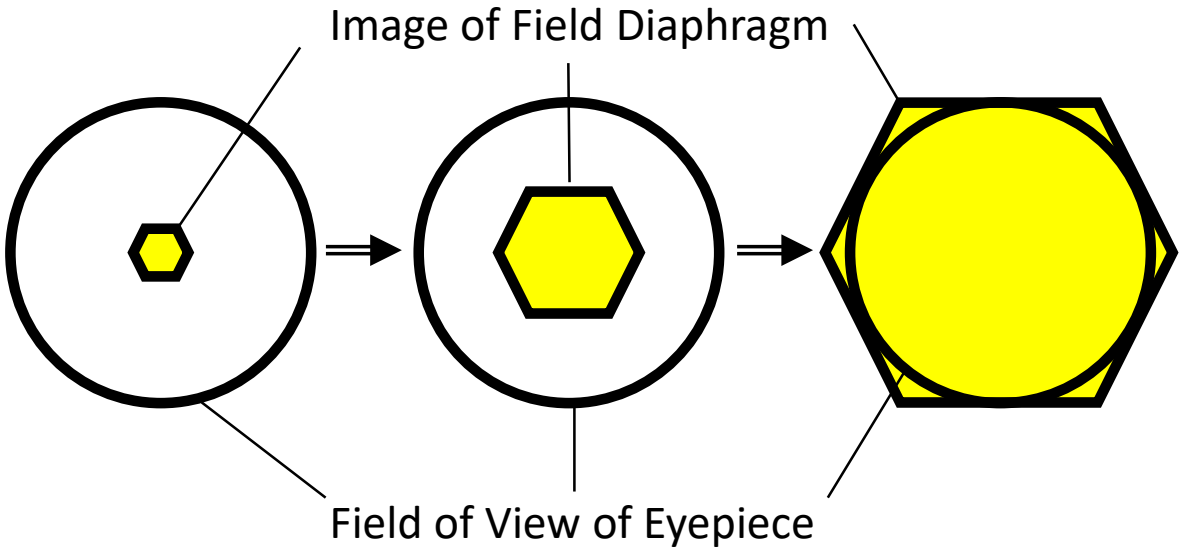


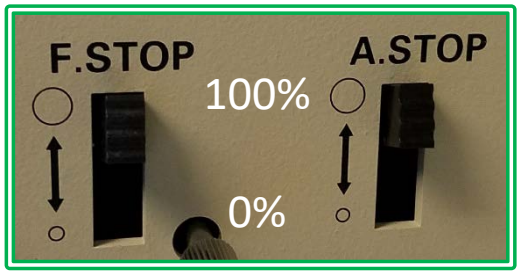


6. Adjust the ***F. STOP*** (field diaphragm) by sliding levers up and down until ***Image of Field Diaphragm*** circumscribes the ***Field of View***

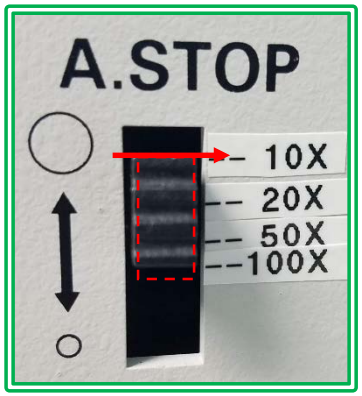


# III. EPI: Bright Field – 3/3

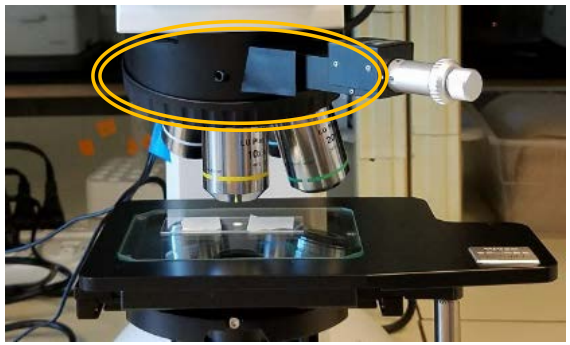



7. Adjust the ***A. STOP*** (aperture diaphragm) by sliding levers up and down to adjust depth of field




8. For each objective, recommended ***A. STOP*** position (top of lever) is shown on markings

9. Switch to higher magnification objectives if desired by rotating nosepiece

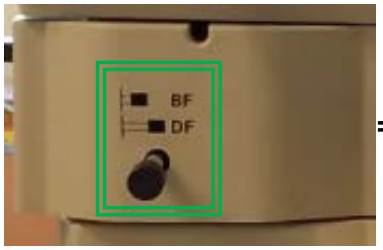


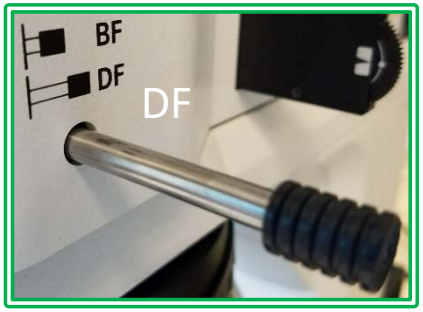
10. Repeat steps 3-9 until desired magnification and image quality is obtained
11. Go to ***Step VIII. Image Capture*** when ready to acquire image

# IV. EPI: Dark Field – 1/2

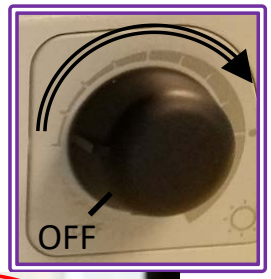
1. Press the ***EPI/DIA*** selector and set to ***EPI***


2. Pull ***Bright/Dark Field*** selector lever to fully out ***DF*** position

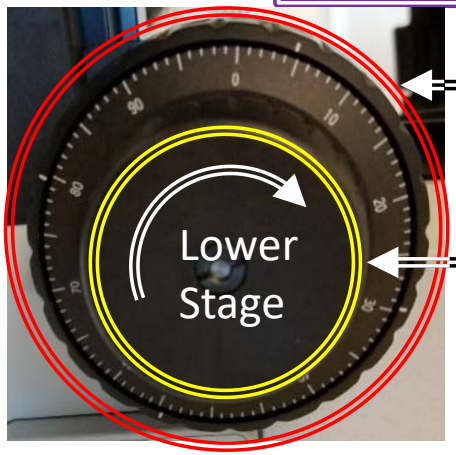



3. Adjust the brightness with the ***Brightness Control*** as necessary




4. Focus on specimen by adjusting the ***Coarse/Fine Focus*** knobs

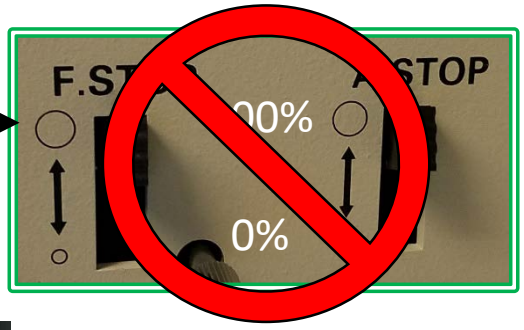



Course Focus

Fine Focus

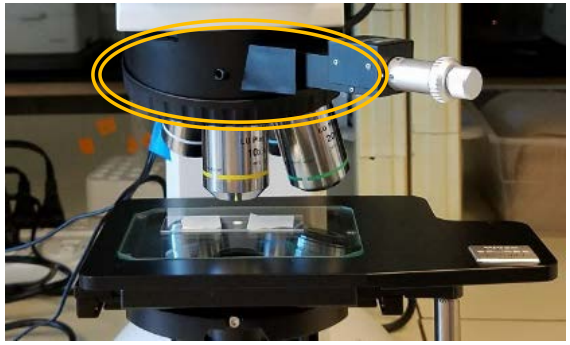
# IV. EPI: Dark Field – 2/2

5. The ***F. STOP*** (field diaphragm) and ***A. STOP*** (aperture diaphragm) are automatically 100% open




   Levers will have ***NO*** affect

6. Switch to higher magnification objectives if desired by rotating nosepiece



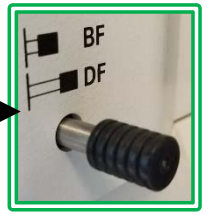
7. Repeat steps 3-6 until desired magnification and image quality is obtained

8. Go to ***Step VIII. Image Capture*** when ready to acquire image

# V. EPI: Polarization – 1/2

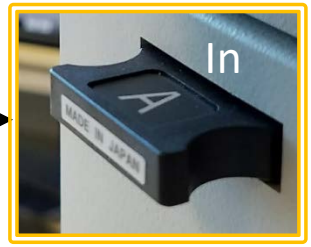
1. Press the ***EPI/DIA*** selector and set to ***EPI***


2. Adjust ***Bright/Dark Field*** selector lever to desired

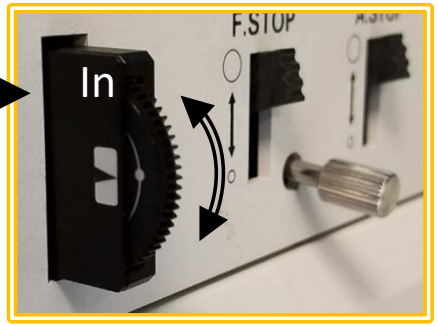




5. Push the ***Analyzer Plate*** in



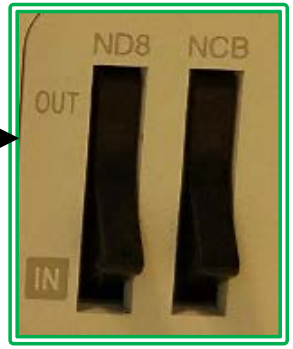

6. Push the ***Polarizer Slider*** in




7. Rotate the polarizer to adjust the polarization from

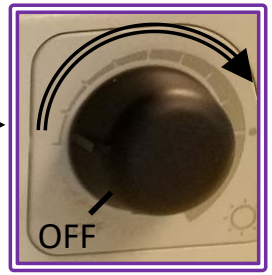
lateral  to vertical 

8. Select ***NCB filter*** (balances color) if desired



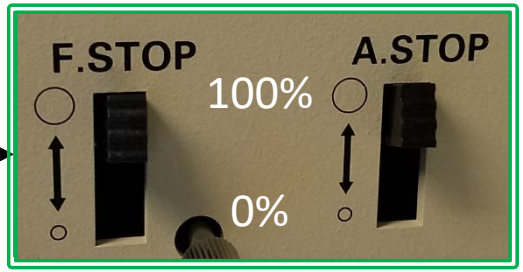
# V. EPI: Polarization – 2/2

9. Adjust the brightness with the ***Brightness Control***

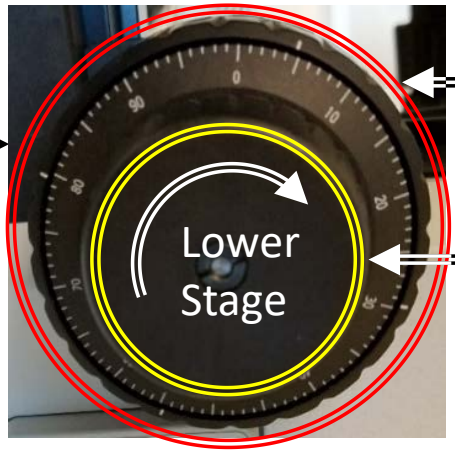



10. Adjust the ***F. STOP*** (field diaphragm) and ***A. STOP*** (aperture diaphragm) by sliding levers up and down from 100% open to 0% open

    Note: ***F. STOP*** and ***A. STOP*** levers will not work if in ***DF*** mode




11. Focus on specimen by adjusting the ***Coarse/Fine Focus*** knobs
12. Switch to higher magnification objectives if desired by rotating nosepiece
13. Repeat steps 7-12 until desired magnification and image quality is obtained

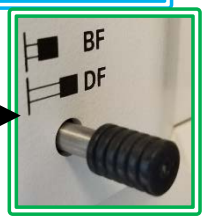



14. Go to ***Step VIII. Image Capture*** when ready to acquire image

# VI. EPI: Differential Interference Contrast – 1/2

1. Press the ***EPI/DIA*** selector and set to ***EPI***

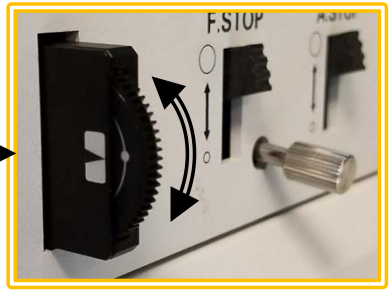

2. Adjust ***Bright/Dark Field*** selector lever to desired





5. Push the ***Analyzer Plate*** in

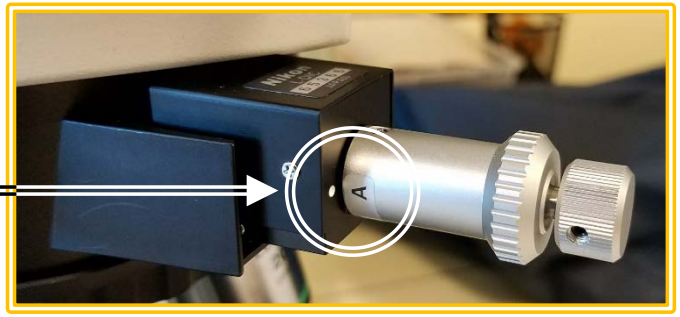
6. Push the ***Polarizer Slider*** in



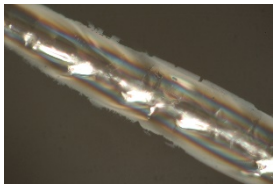
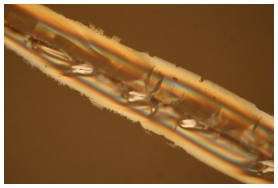
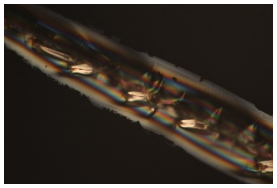
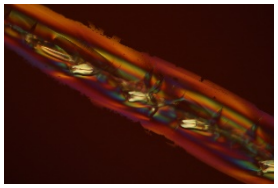
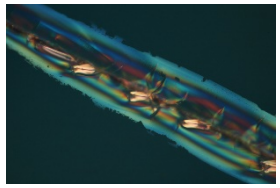
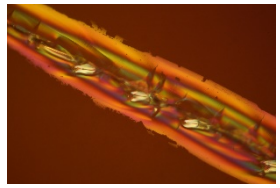

7. Rotate the polarizer to adjust the polarization from

lateral  to vertical 

8. Push the ***DIC Prism*** in and set to ***Position A***



9. Rotate small knob to adjust contrast and color

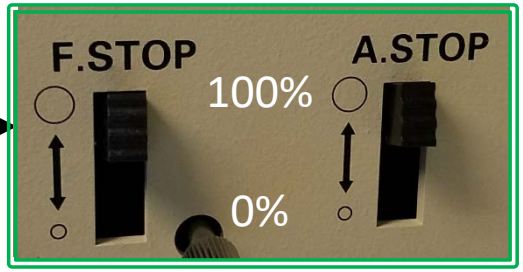







# VI. EPI: Differential Interference Contrast – 2/2

10. Select ***NCB filter*** (balances color) if desired

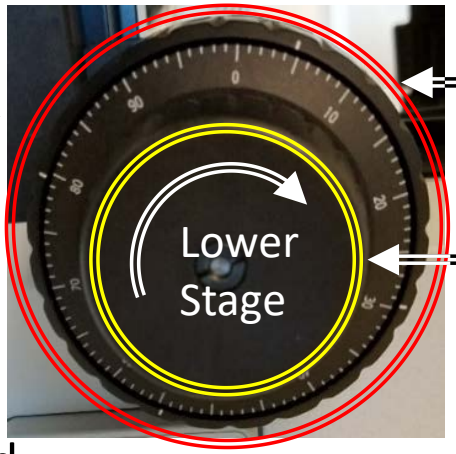

11. Adjust the brightness with the ***Brightness Control***


12. Adjust the ***F. STOP*** (field diaphragm) and ***A. STOP*** (aperture diaphragm) by sliding levers up and down from 100% open to 0% open




Note: ***F. STOP*** and ***A. STOP*** levers will not work if in ***DF*** mode

13. Focus on specimen by adjusting the ***Coarse/Fine Focus*** knobs




Course Focus

Fine Focus

14. Switch to higher magnification objectives if desired by rotating nosepiece

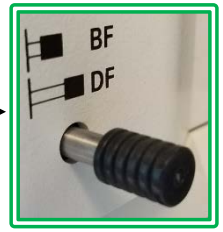
15. Repeat steps 7-14 until desired magnification and image quality is obtained

16. Go to ***Step VIII. Image Capture*** when ready to acquire image

# VII. DIA: Bright Field – 1/2

1. Press the ***EPI/DIA*** selector and set to ***DIA*** 

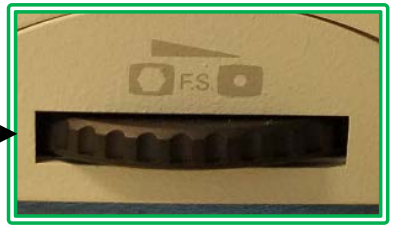

2. Push ***Bright/Dark Field*** selector lever to fully in ***BF*** position



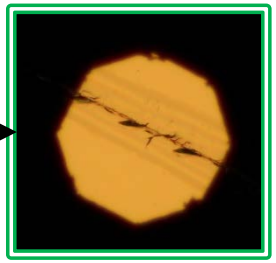

3. Select ***NCB filter*** (balances color) if desired


4. Adjust the brightness with the ***Brightness Control***


5. Adjust the ***Field Diaphragm Control*** to fully closed

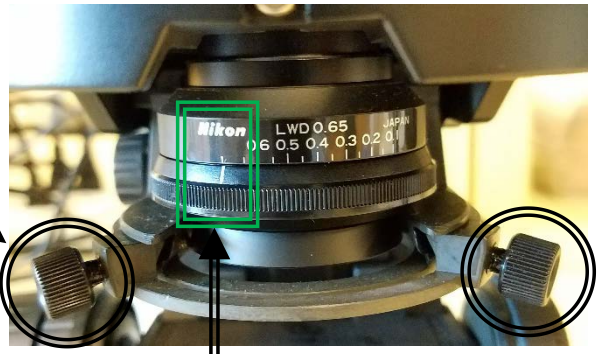



6. Adjust the ***Condenser Height*** until the field diaphragm is focused

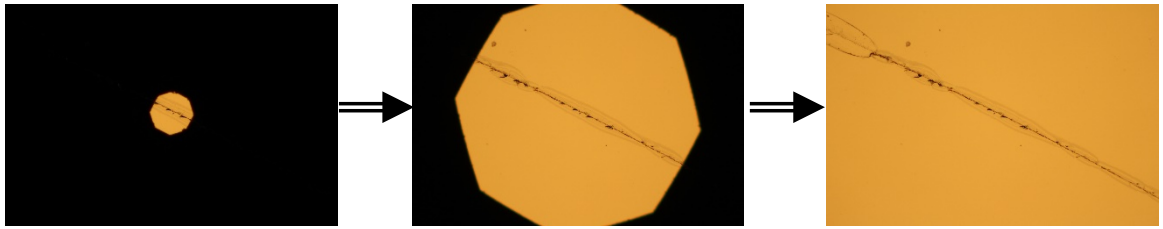


# VII. DIA: Bright Field – 2/2

7. Center the field diaphragm by adjusting ***Centering Screws***




8. Open the ***Field Diaphragm Control*** until field diaphragm circumscribes the field of view



9. Focus on specimen by adjusting the ***Coarse/Fine Focus*** knobs

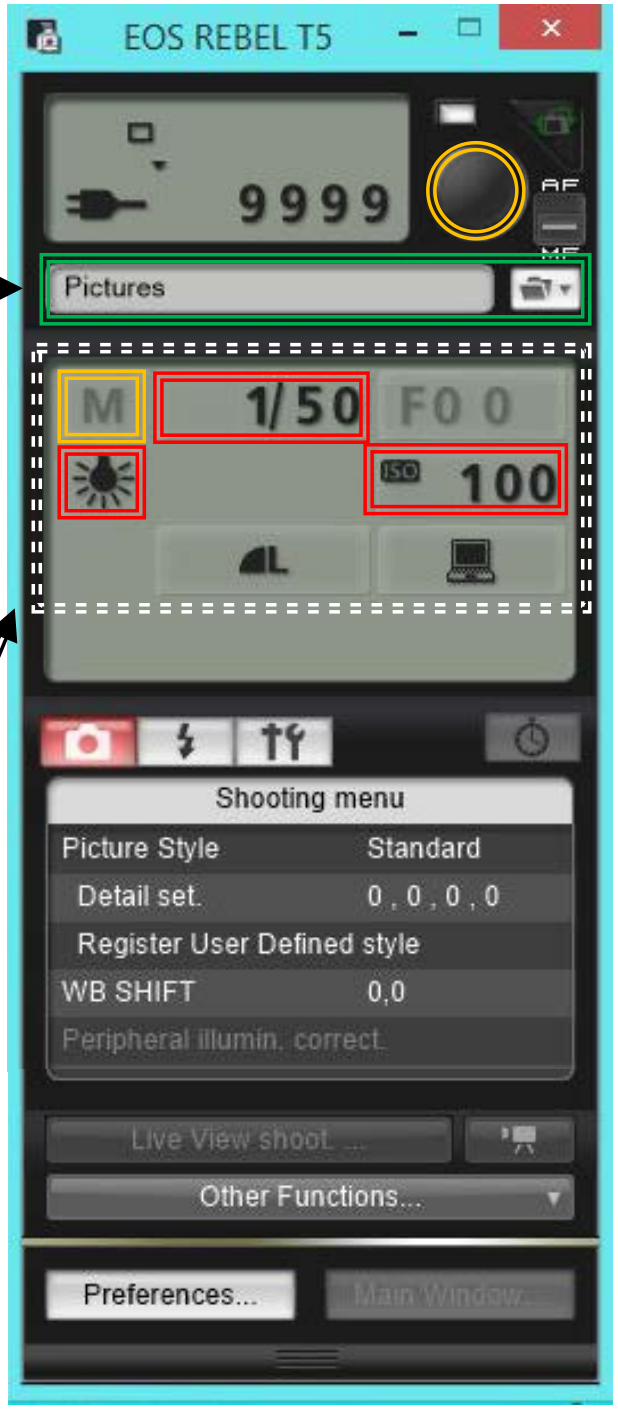
10. Adjust the ***Condenser Aperture*** to match ***Numerical Aperture*** for each objective:

10X = 0.3 20X = 0.45 50X = 0.8 100X = 0.9

10. Switch to higher magnification objectives if desired by rotating nosepiece
7. Repeat steps 3-11 until desired magnification and image quality is obtained
8. Go to ***Step VIII. Image Capture*** when ready to acquire image

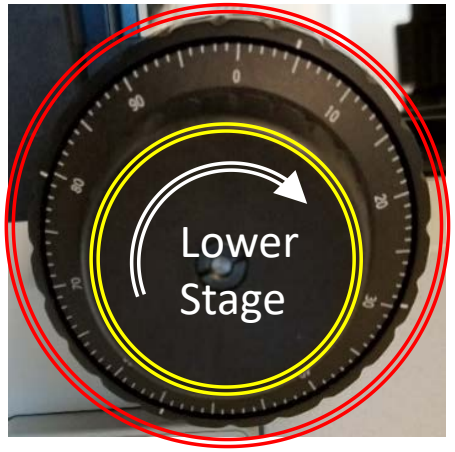
# VIII. Image Capture – 1/1

1. Click on the ***Folder*** icon and select desired folder to store saved pictures in
2. Recommend creating you own personal folder with sub-folders for each sample to help distinguish among them later
3. It is important to record the objective used for ***EACH*** image taken (necessary for scale)
4. Review ***Camera Settings*** before acquiring image
5. Click on the ***Shutter Button*** to acquire your image



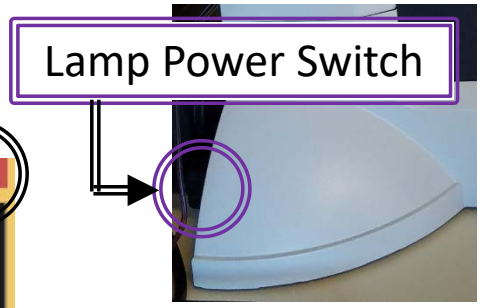
# IX. Cleanup – 1/1

1. Lower the stage away from the objectives by rotating the ***Coarse Focus*** knob **TOWARD** you




2. Rotate nosepiece and place the 10x objective into position

3. Turn off Lamp Power Switch at the back of the microscope



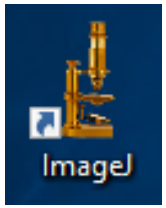

4. Turn off the control software


5. Log out of your ENGR account and record your end time on the sign-in sheet

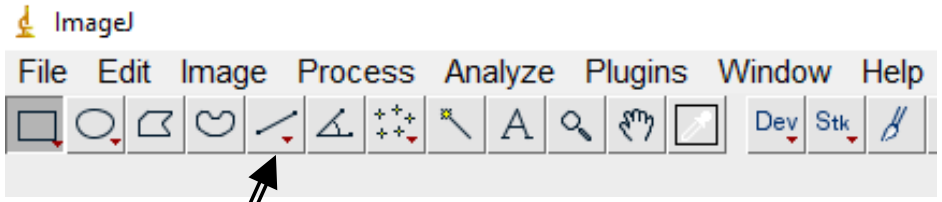
6. Clean up and dispose of any consumables used and return any tools back to its respective containers or bins

7. Confirm that the microscope is turned **OFF** again (**NO LIGHT!**), then place cover over microscope

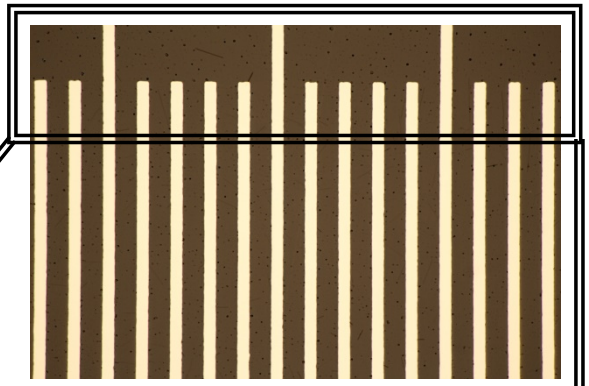
# X. ImageJ – 1/3




1. Double-click on ***ImageJ*** icon

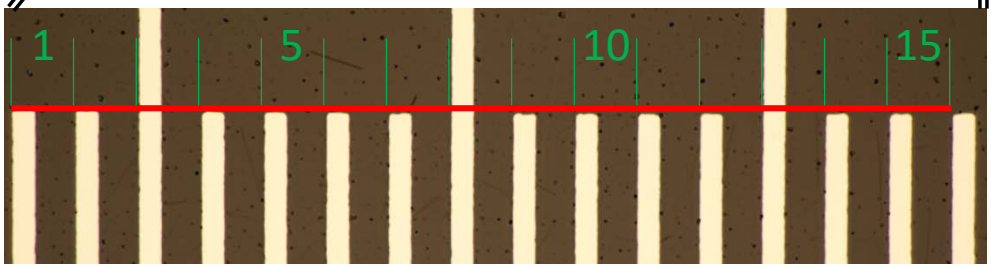



2. Click ***File > Open***
3. Locate the ***Scale Bar Images*** folder
4. Select the ***Magnification*** of the image you wish to measure (e.g. 100X) and ***Open***
5. Click the ***Segment Tool*** and select ***Straight Line***



6. Draw a line that contains the maximum number of tick marks

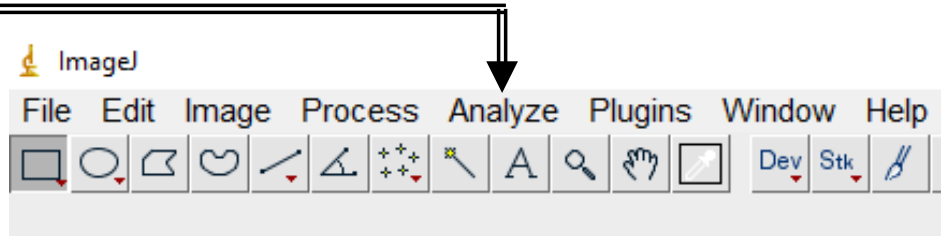
   **Note:** It matters where you start and end the line!




7. Count the number of tick marks contained (e.g. 15)
8. Each division is 0.01 mm (or 10 μm)

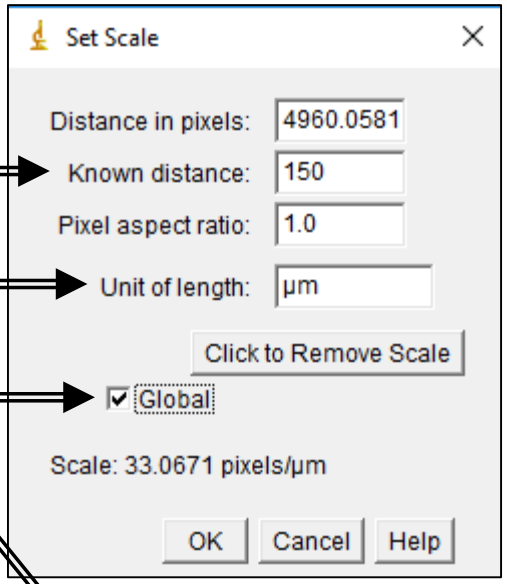
# X. ImageJ – 2/3

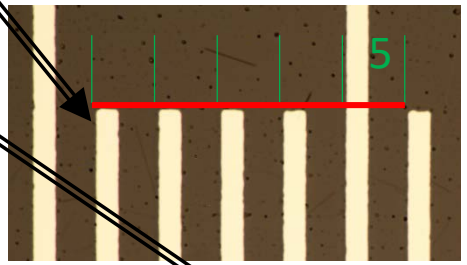
9. Click ***Analyze > Set Scale***



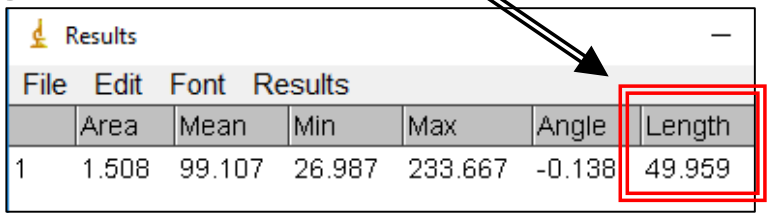

10. Enter the ***Known Distance*** (e.g. 150 μm) based on the number of tick marks and each division = 0.01 mm (or 10 μm)
11. Enter the ***Unit of Length*** to desired unit (e.g. mm)
12. Check ***Global*** to set scale for all images
13. Confirm your scale by drawing a new ***Straight Line***
14. Click ***Analyze > Measure*** and check value

    If incorrect, repeat steps 5 – 13



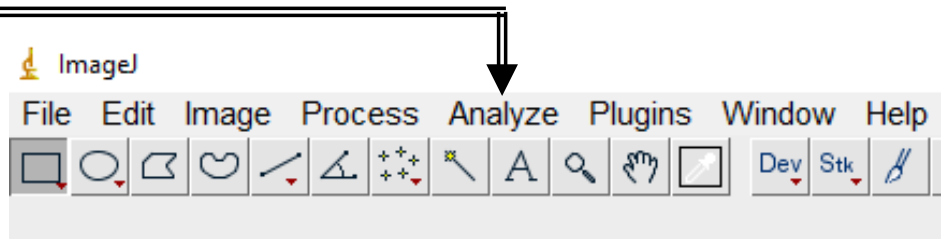




15. Click ***File > Open*** and select your image(s) of interest
16. Draw ***Straight Lines*** and click ***Analyze > Measure***
17. Repeat steps 4 – 16 for other ***Magnifications***



# X. ImageJ – 3/3

9. Click ***Analyze > Tools > Scale Bar***




10. Enter ***Width in um*** (e.g. 50 µm)
    based on the length of scale bar desired

11. Enter ***Height in pixels*** for desired scale bar thickness

12. Enter ***Font size*** for desired text size

13. Identify ***Color*** of the scale bar

14. Identify ***Background*** color (if desired)

15. Identify ***Location*** where ***Scale Bar*** to be placed