

Nikon Training Notebook

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University of California, Riverside

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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 Manager 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

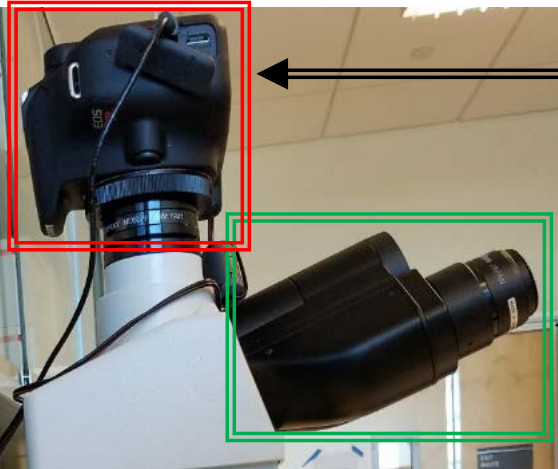
I. Microscope Layout – 1/4

Camera ON/OFF Switch

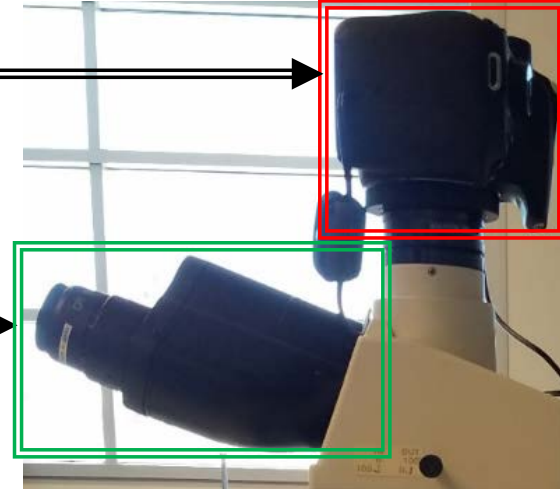


Camera Mode Selector:
M (default)

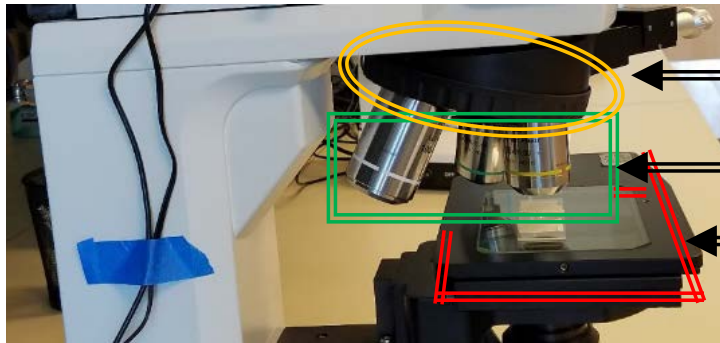
Camera



Binocular Eyepiece



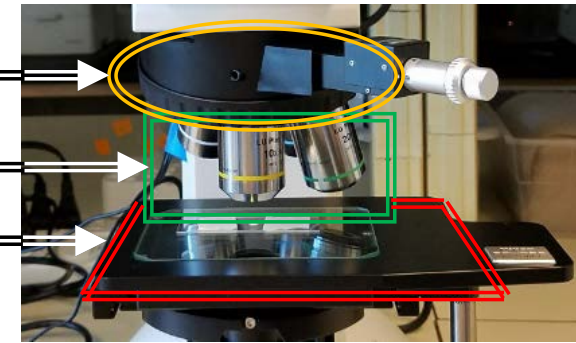
I. Microscope Layout – 2/4



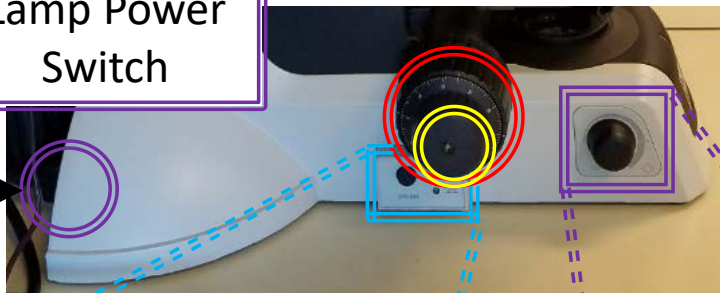
Nosepiece

Objectives

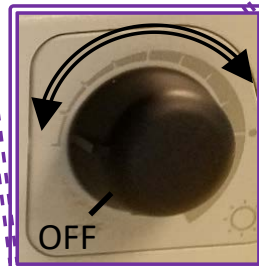
Stage



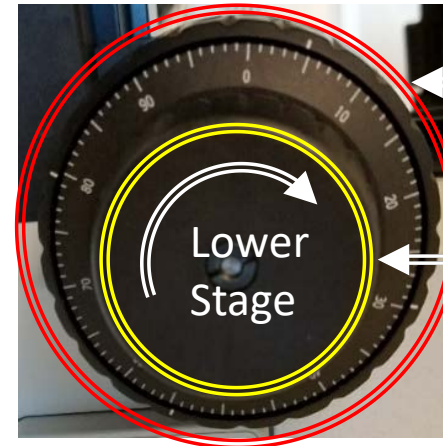
Lamp Power Switch



EPI/DIA Selector



Lamp Brightness Knob



Coarse Focus

Fine Focus

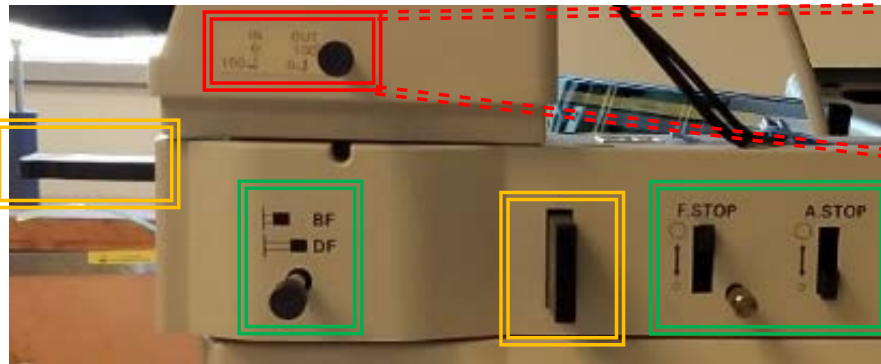
Lamp ON \Rightarrow

Lamp OFF \Rightarrow

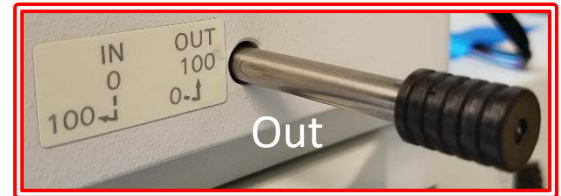
Lamp Indicator



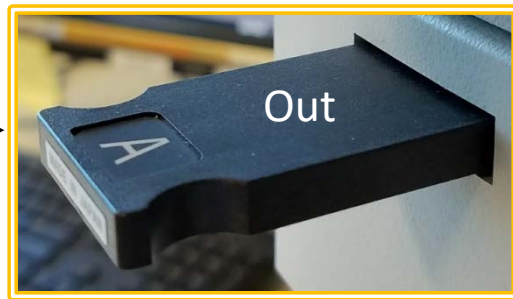
I. Microscope Layout – 3/4



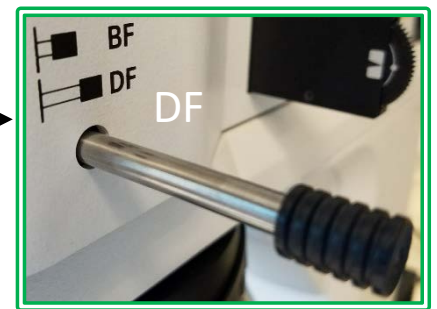
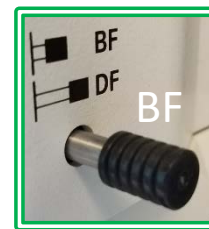
Optical Path
Selector
Lever



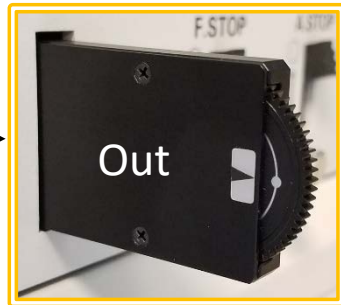
Analyzer Plate



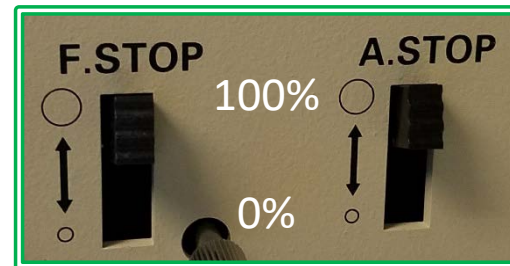
Bright field/Dark field Selector Lever



Polarizer Slider

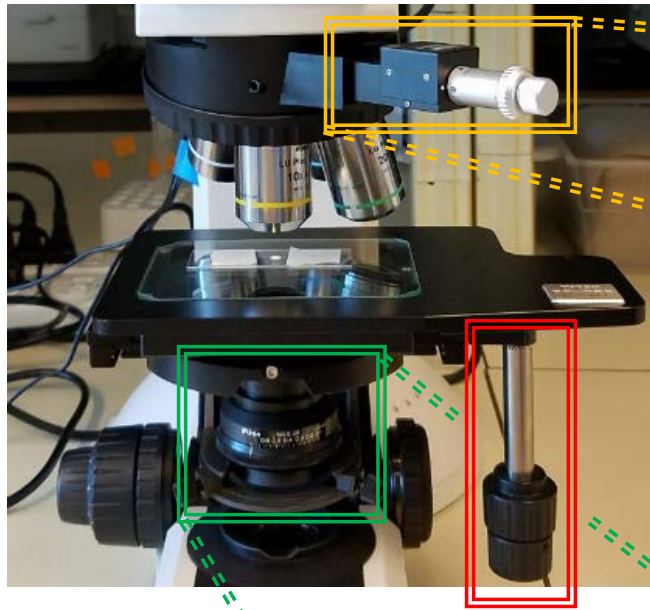


EPI Field
Diaphragm
Stop



EPI
Aperture
Diaphragm
Stop

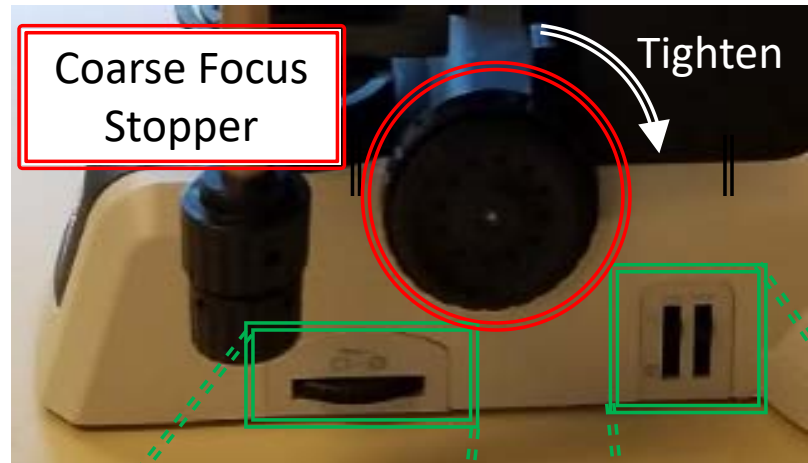
I. Microscope Layout – 4/4



DIC Prism Plate



Stage
Movement



Coarse Focus
Stopper

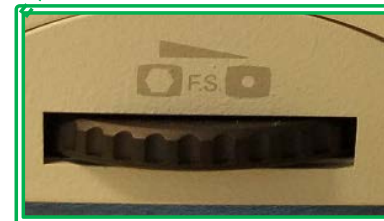
Tighten



DIA Condenser
Focus Knob



DIA Condenser
Aperture



DIA Field
Diaphragm Control



Filter Selector
Switches

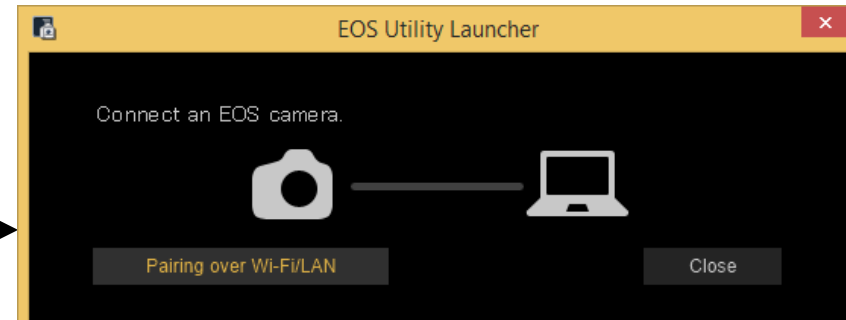
II. Startup – 1/5

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

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



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



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



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



II. Startup – 2/5

6. Confirm the following **Camera Settings** are set:
Camera Software (right click to change)

M = Manual

1/50 = Shutter Speed

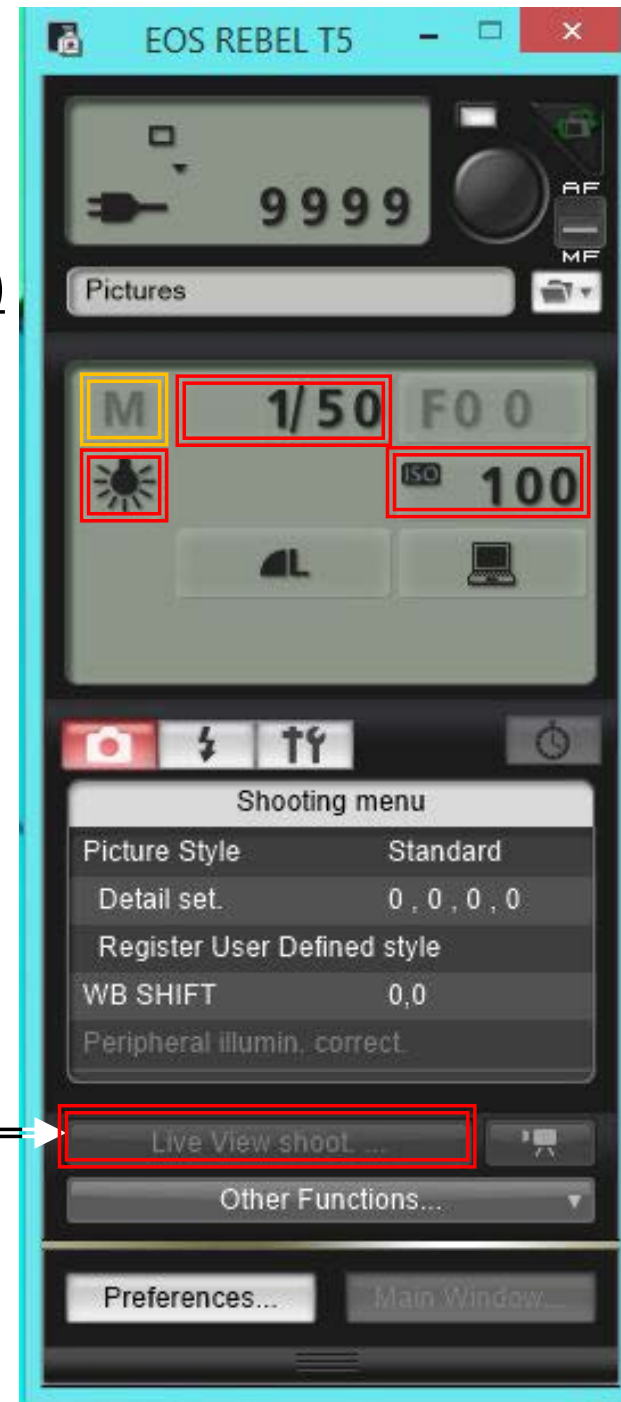
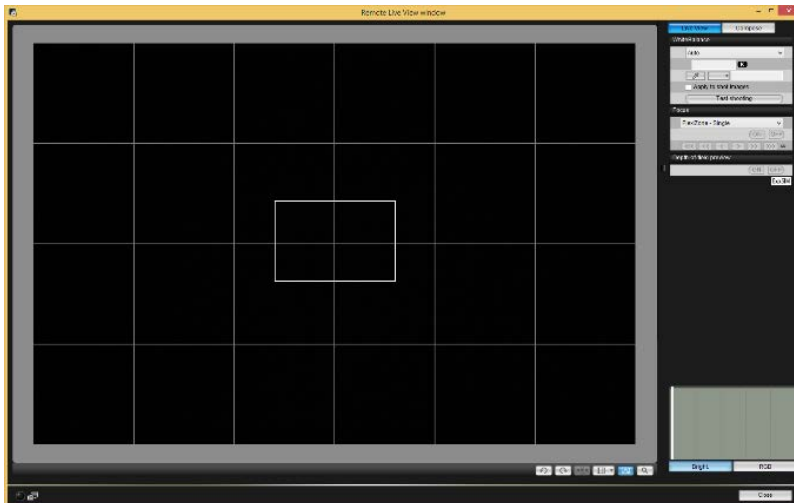
100 = ISO

Tungsten = Brightness



7. Click on **Live View shoot**

8. Remote Live View window will appear

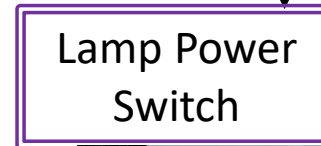


II. Startup – 3/5

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

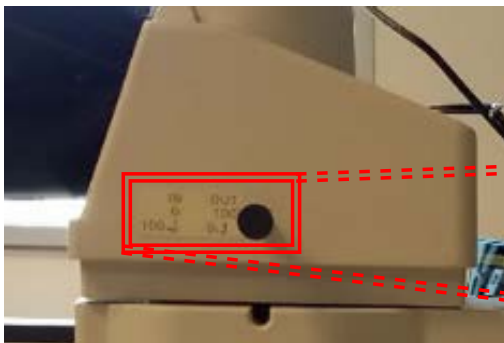


10. If light is missing, turn on **Lamp Power Switch** on back



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

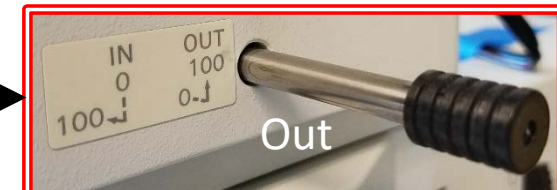
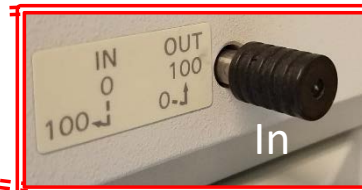
For **Binocular Eyepiece**: Push lever completely in



Optical Path Selector Lever

Binocular Eyepiece

Camera View



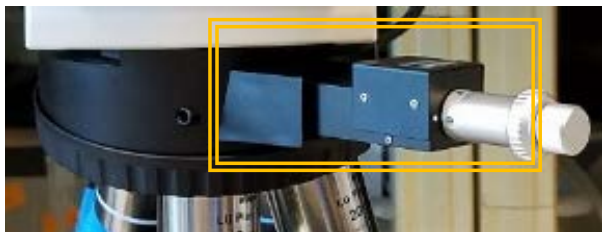
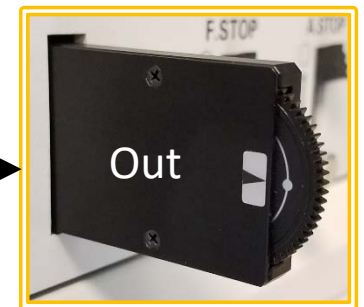
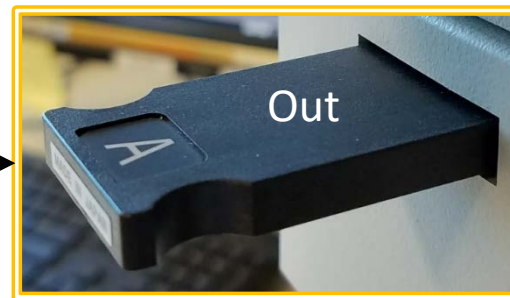
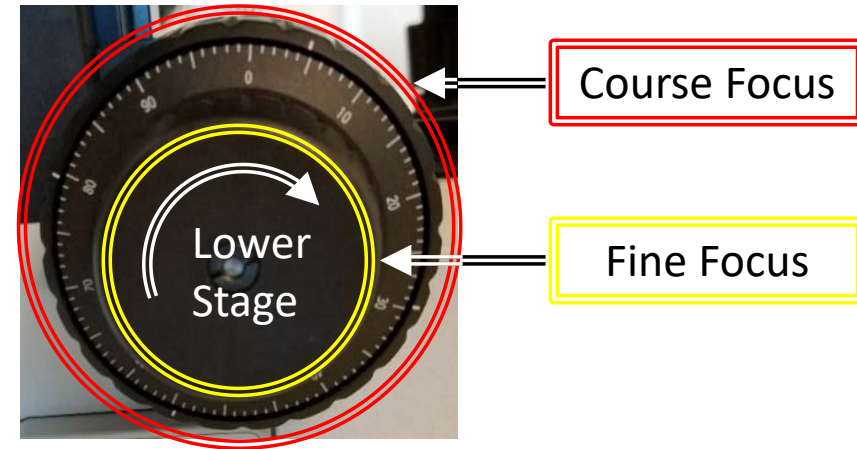
II. Startup – 4/5

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

13. Place sample on microscope stage

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

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

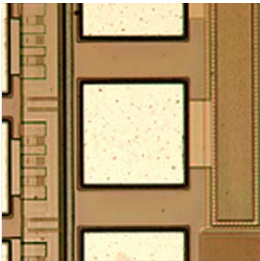


II. Startup – 5/5

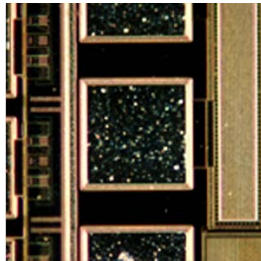
10. Identify which microscope mode you wish to use:

Episcopic Illumination ()

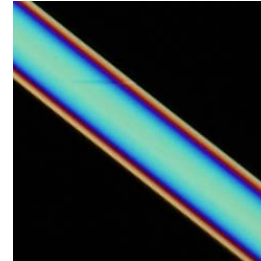
III. Bright field



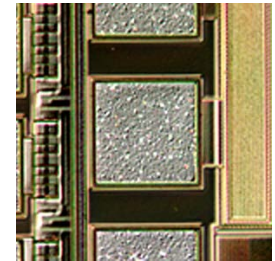
IV. Dark field



V. Polarization

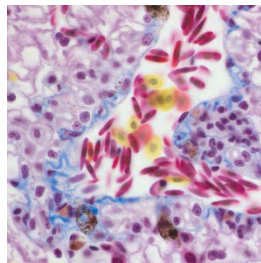


VI. Differential Interference Contrast (DIC)



Diascopic Illumination()

VII. Bright field

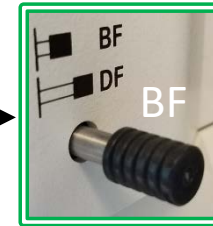


III. EPI: Bright Field – 1/3

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



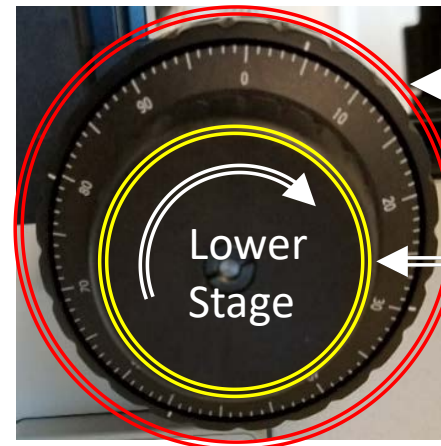
2. Push **Bright/Dark Field** selector lever to fully in **BF** position



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



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

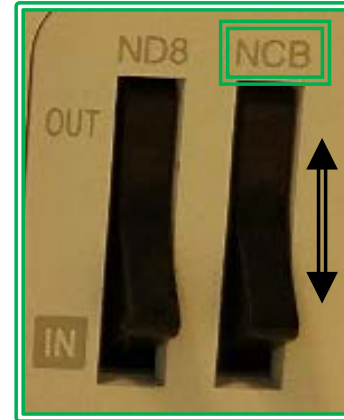


Course Focus

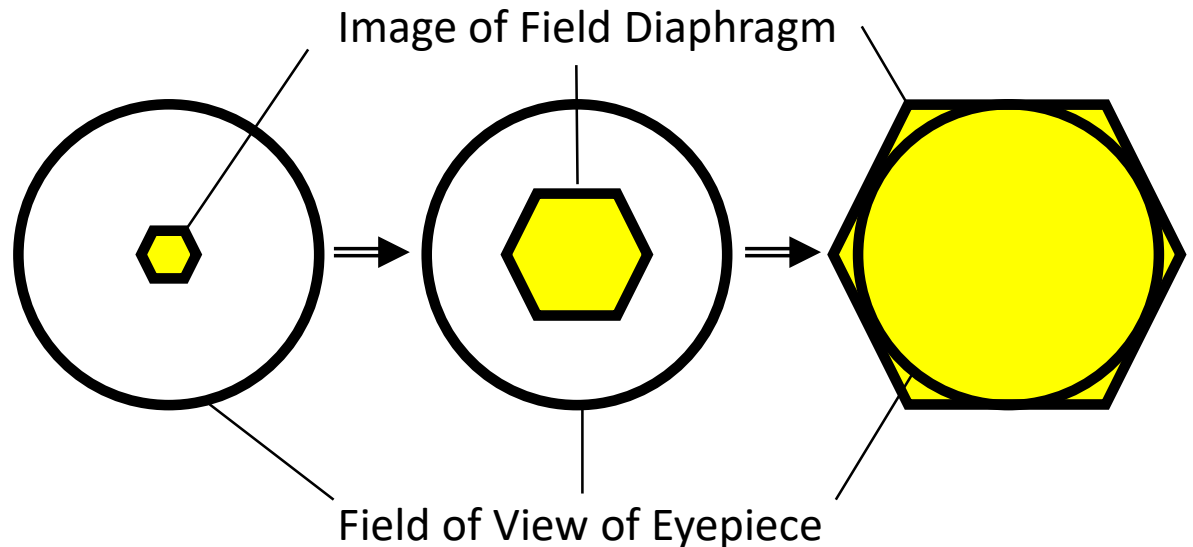
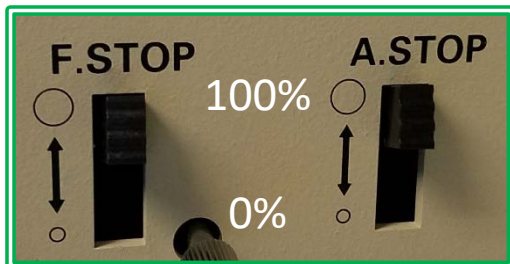
Fine Focus

III. EPI: Bright Field – 2/3

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

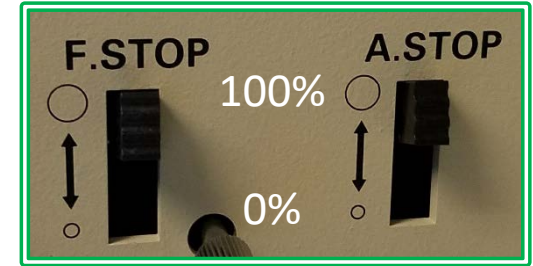


6. Adjust the **F. STOP** (field diaphragm)
by sliding levers up and down until
Image of Field Diaphragm circumscribes the **Field of View**

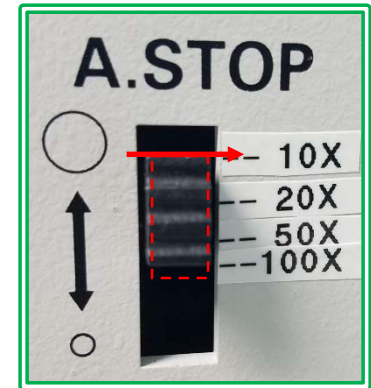


III. EPI: Bright Field – 3/3

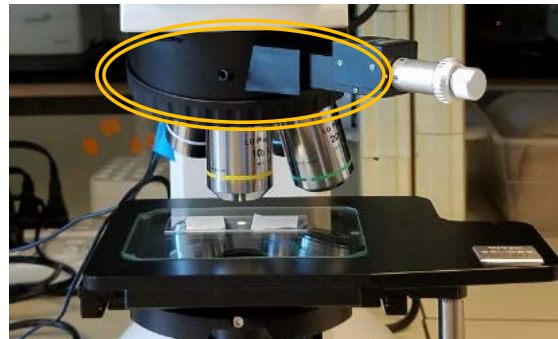
7. Adjust the **A. STOP** (aperture diaphragm) by sliding levers up and down to adjust depth of field



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

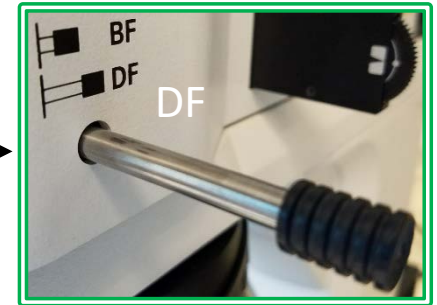
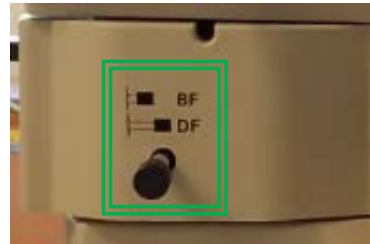
11. Go to **Step VIII. Image Capture** when ready to acquire image

IV. EPI: Dark Field – 1/2

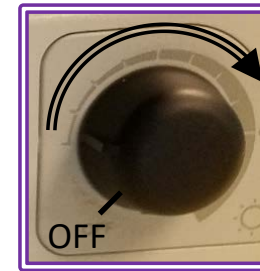
1. Press the **EPI/DIA** selector and set to **EPI**



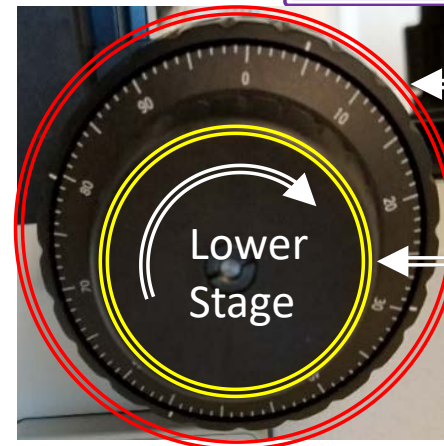
2. Pull **Bright/Dark Field** selector lever to fully out **DF** position



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



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



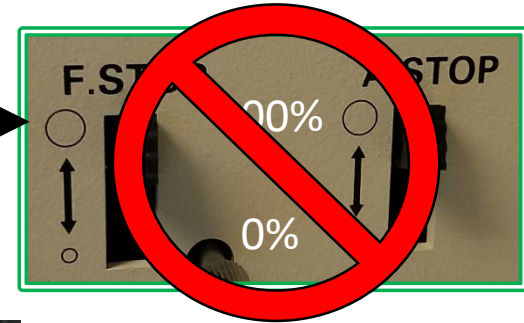
Course Focus

Fine Focus

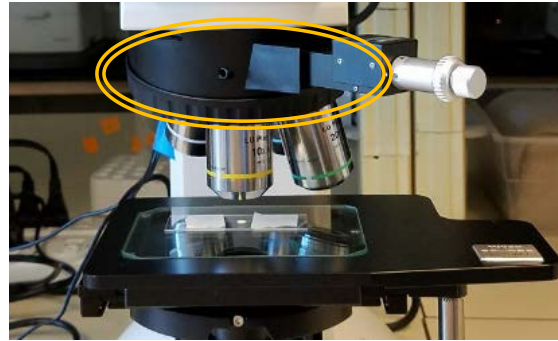
IV. EPI: Dark Field – 2/2

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



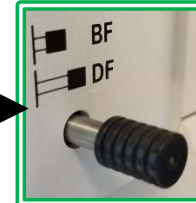
7. Repeat steps 3-6 until desired magnification and image quality is obtained
8. Go to **Step VIII. Image Capture** when ready to acquire image

V. EPI: Polarization – 1/2

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



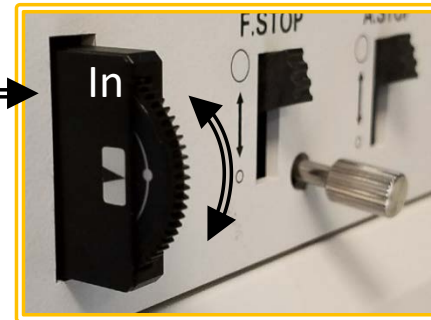
2. Adjust **Bright/Dark Field** selector lever to desired





5. Push the **Analyzer Plate** in



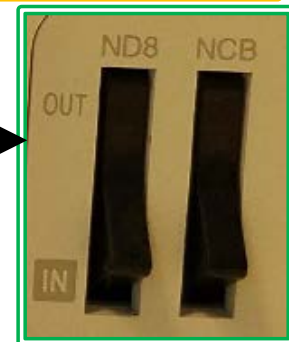
6. Push the **Polarizer Slider** in



7. Rotate the polarizer to adjust the polarization from

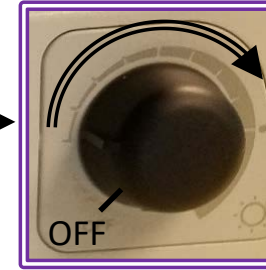
lateral  to vertical 

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

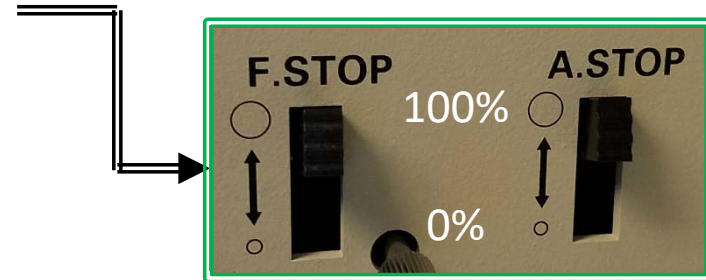


V. EPI: Polarization – 2/2

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



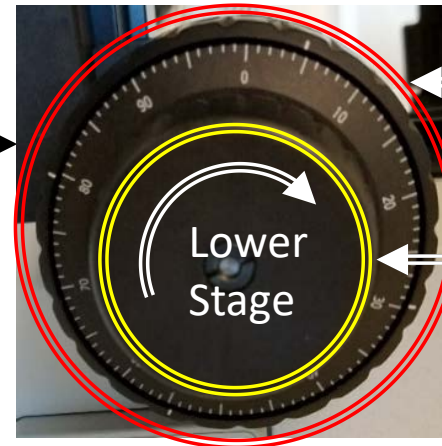
Note: **F. STOP** and **A. STOP** levers will not work if in **DF** mode

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



Course Focus

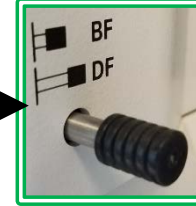
Fine Focus

VI. EPI: Differential Interference Contrast – 1/2

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



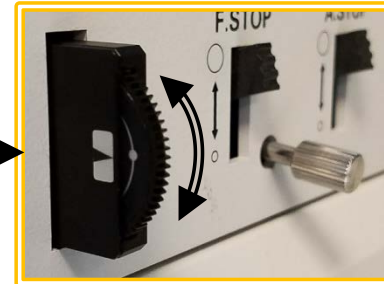
2. Adjust **Bright/Dark Field** selector lever to desired





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



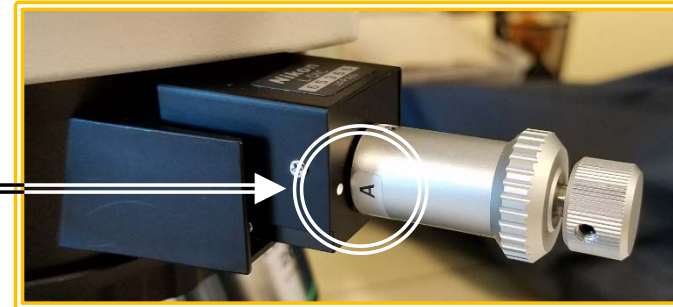
6. Push the **Polarizer Slider** in \Rightarrow



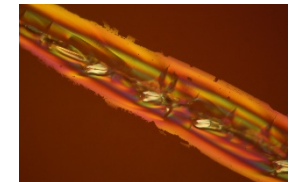
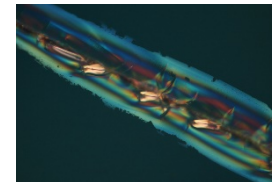
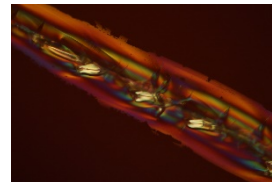
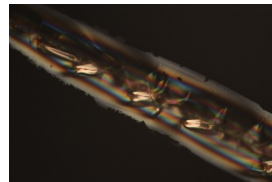
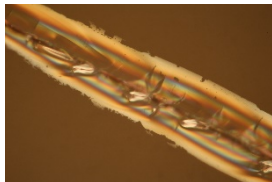
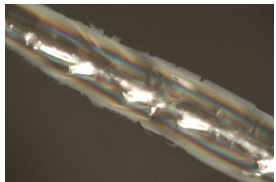
7. Rotate the polarizer to adjust the polarization from

lateral  to vertical 

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

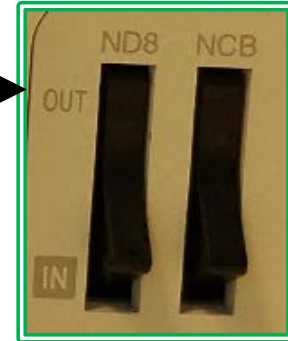


9. Rotate small knob to adjust contrast and color

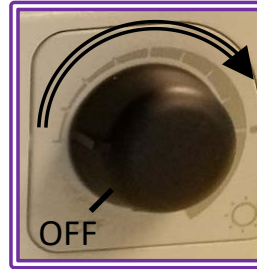


VI. EPI: Differential Interference Contrast – 2/2

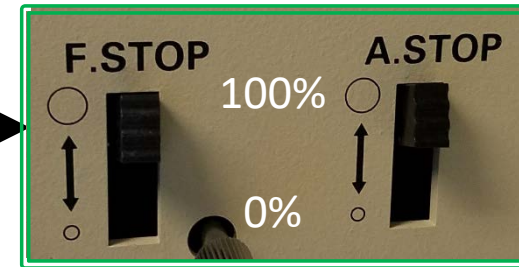
10. Select **NCB filter** (balances color) if desired



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

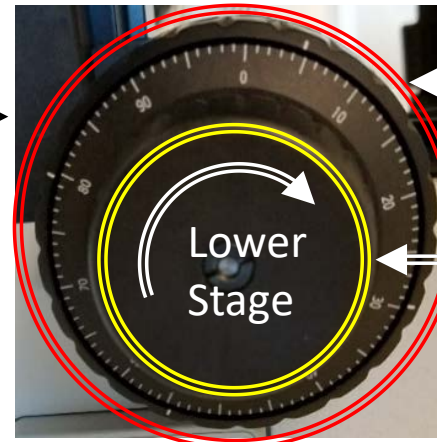


12. Adjust the **F. STOP** (field diaphragm) and **A. STOP** (aperture diaphragm) by sliding levers up and down from 100% open to 0% open



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

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

Fine Focus

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

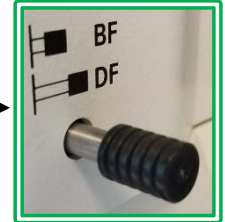
16. Go to **Step VIII. Image Capture** when ready to acquire image

VII. DIA: Bright Field – 1/2

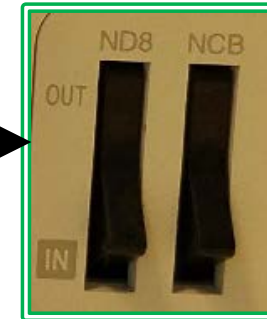
1. Press the **EPI/DIA** selector and set to **DIA** 



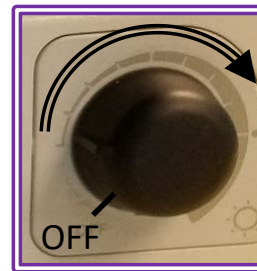
2. Push **Bright/Dark Field** selector lever to fully in **BF** position



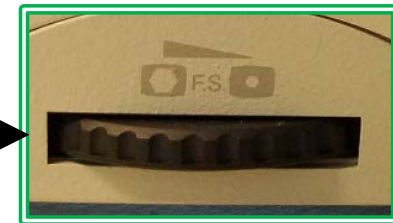
3. Select **NCB filter** (balances color) if desired



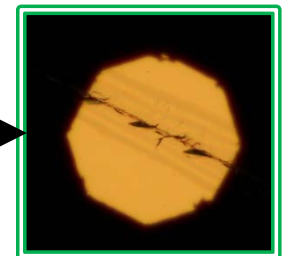
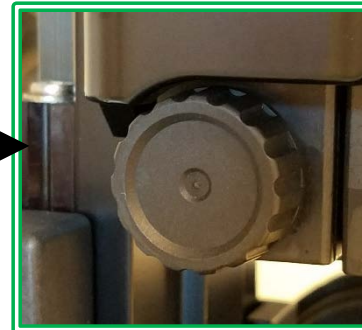
4. Adjust the brightness with the **Brightness Control**



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

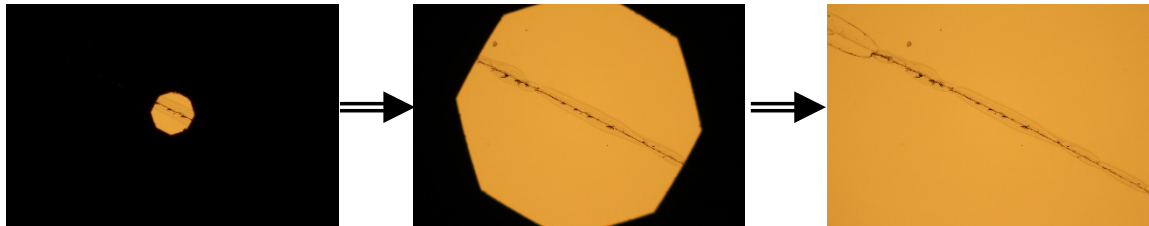


6. Adjust the **Condenser Height** until the field diaphragm is focused



VII. DIA: Bright Field – 2/2

- Center the field diaphragm by adjusting **Centering Screws**
- Open the **Field Diaphragm Control** until field diaphragm circumscribes the field of view



- Focus on specimen by adjusting the **Coarse/Fine Focus** knobs
- Adjust the **Condenser Aperture** to match **Numerical Aperture** for each objective:



10X = 0.3

20X = 0.45

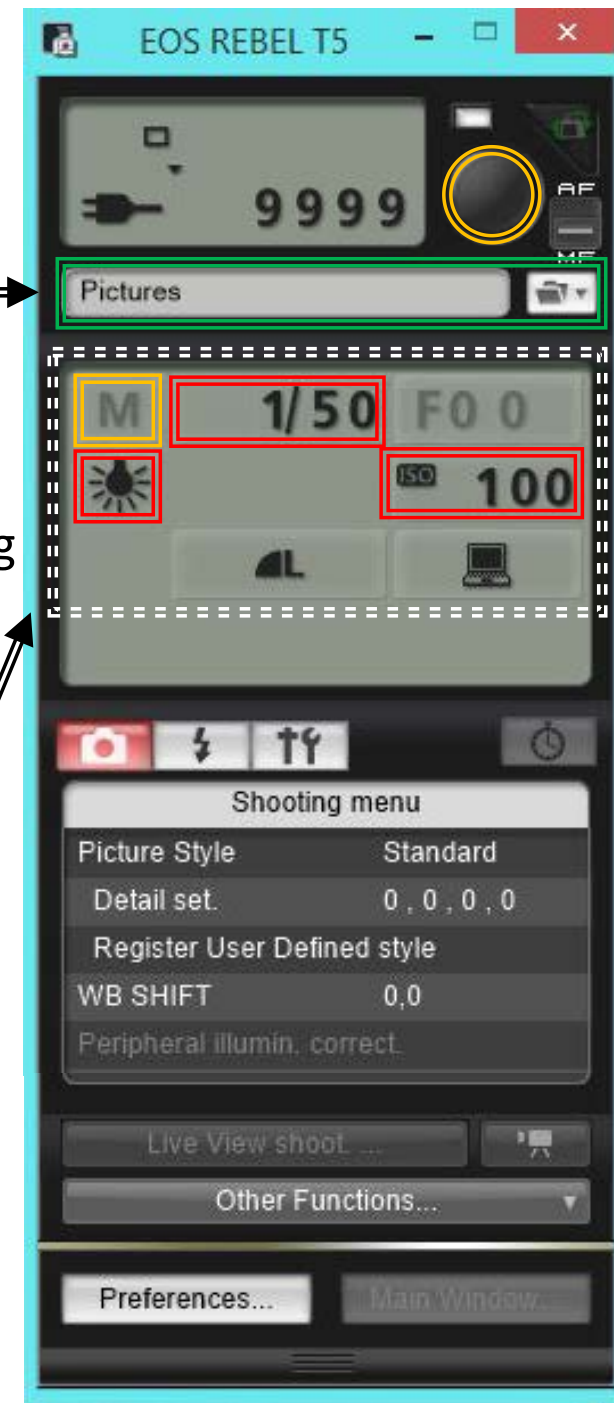
50X = 0.8

100X = 0.9

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

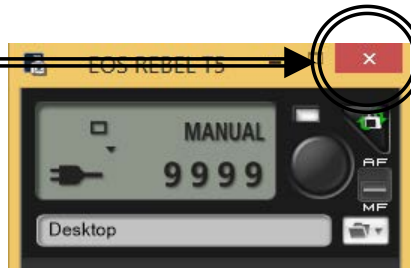
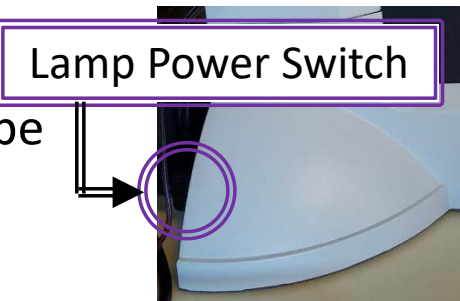
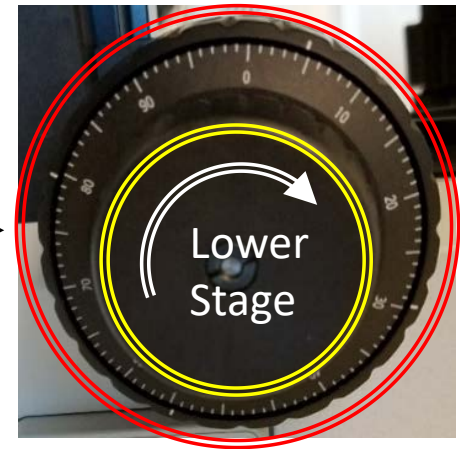
VIII. Image Capture – 1/1

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 the Lamp Power Switch at the back of the microscope
4. Turn off the control software
5. Sign-off from your account
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

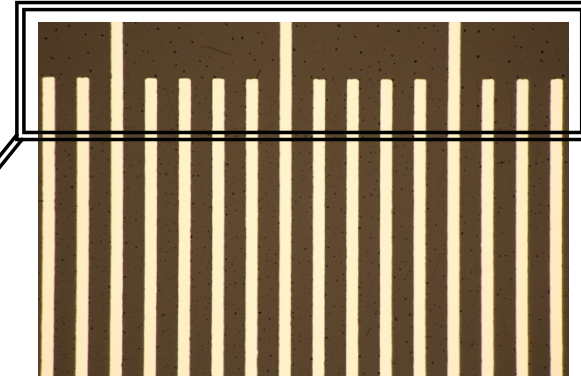
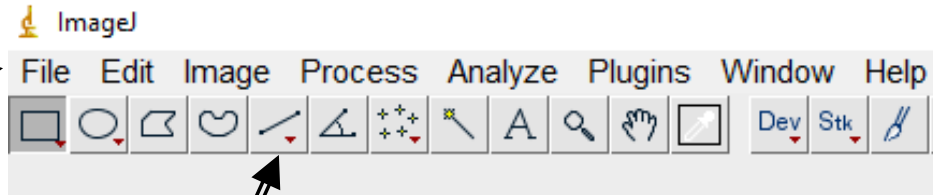


X. ImageJ – 1/3



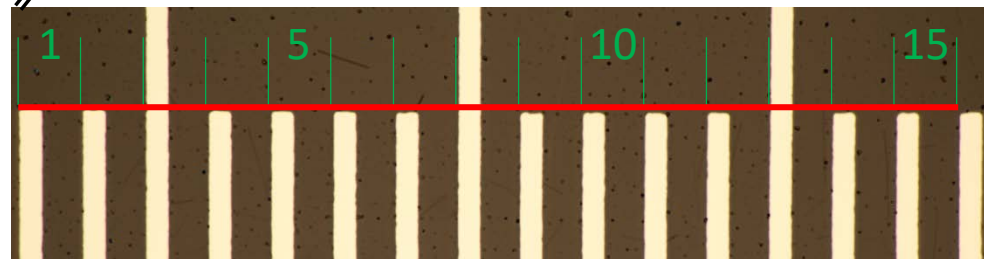
1. Double-click on *ImageJ* icon

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



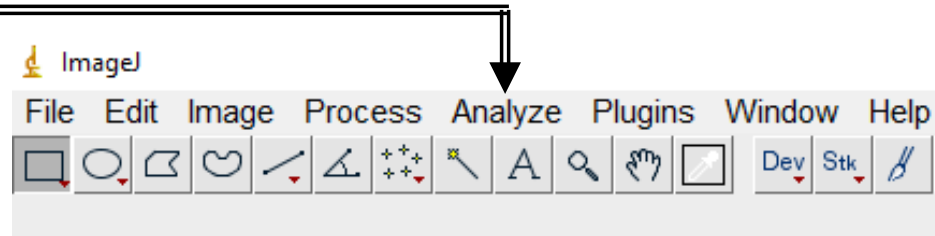
Note: It matters where you start and end the line!

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**



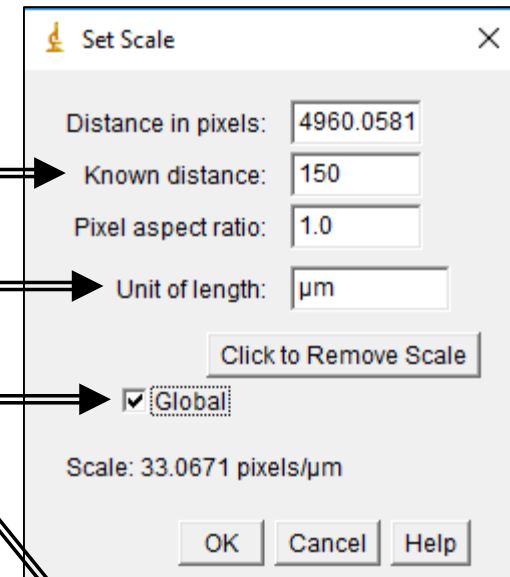
10. Enter the **Known Distance** (e.g. 150 μm) based on the number of tick marks and each division = 0.01 mm (or 10 μm)

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

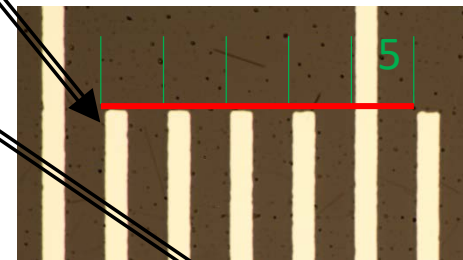


If incorrect, repeat steps 5 – 13

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**

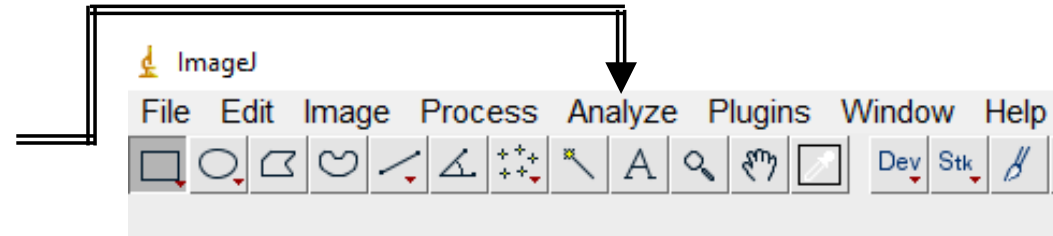


Results						
File	Edit	Font	Results			
	Area	Mean	Min	Max	Angle	Length
1	1.508	99.107	26.987	233.667	-0.138	49.959

A screenshot of the 'Results' window in ImageJ. It shows a table with 7 columns: File, Edit, Font, Results, and three unlabeled columns. The 'Results' column is highlighted. The table contains one row of data. A red box highlights the 'Length' column and its value '49.959'. A double-lined arrow points from step 14 to this value.

X. ImageJ – 3/3

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



10. Enter Width in μm (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

