Nikon Training Notebook

Lab Manager: Dr. Perry Cheung
MSE Fee-For-Service Facility
Materials Science and Engineering
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 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)

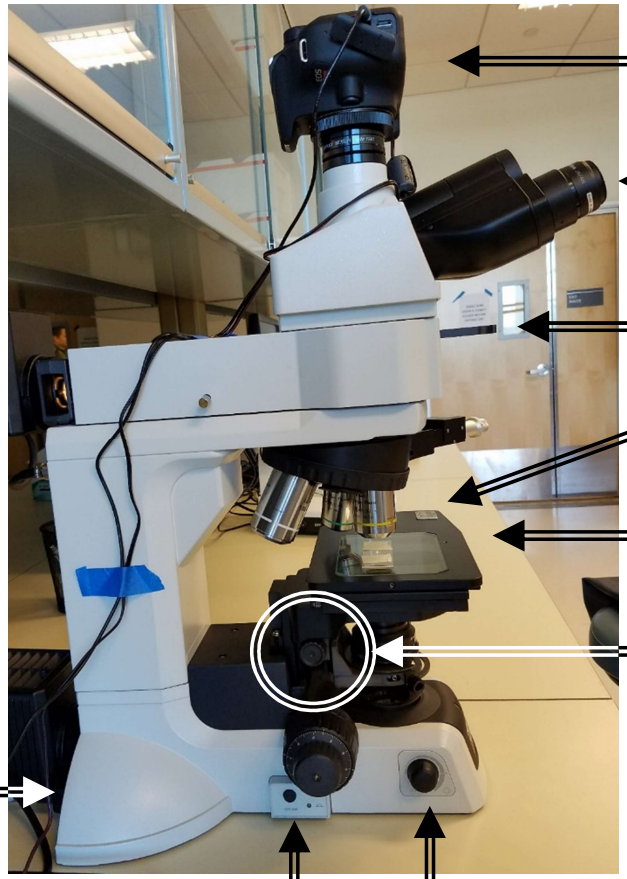
VII. DIA: Bright Field

VIII. Image Capture

IX. Cleanup

X. ImageJ

I. Microscope Layout – 1/1



Camera

Binocular Eyepiece

Analyzer Plate

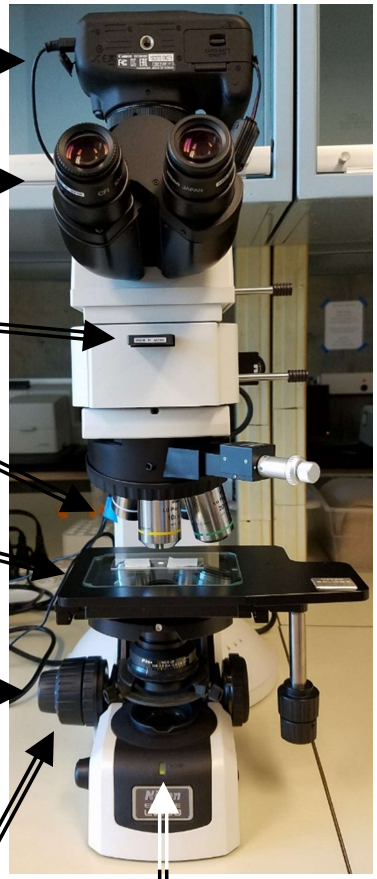
Objectives

Stage

Condenser Focus Knob

Fine Focus Knob

Coarse Focus Knob



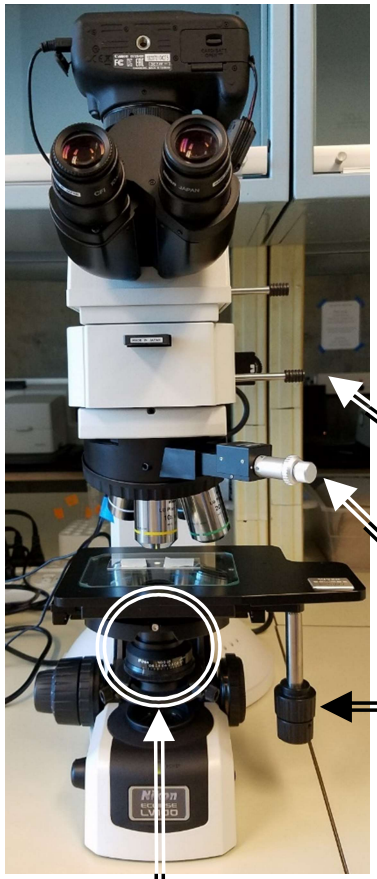
Lamp Power Switch

EPI/DIA Selector

Brightness Control Knob

Power Indicator

I. Microscope Layout – 2/2



Optical Path Selector Lever

Polarizer Slider

Field Diaphragm Lever

Aperture Diaphragm Lever

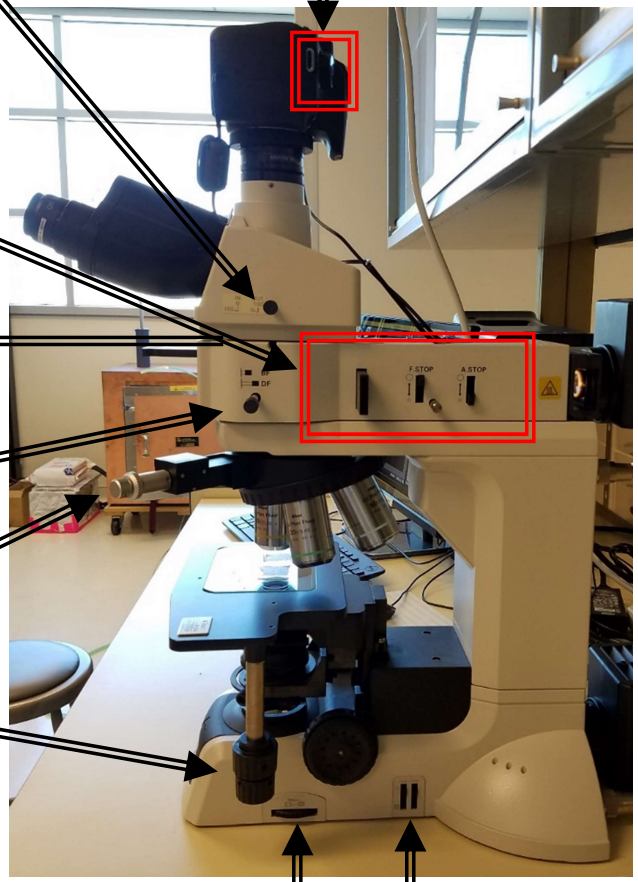
Bright field/Dark field Selector Lever

DIC Prism Plate

Stage Movement

Condenser

Camera On/Off Switch



Field Diaphragm Control

Filter Selector Switches

II. Startup – 1/3

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

   Temporary Username/Password: Nikon/camera

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

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

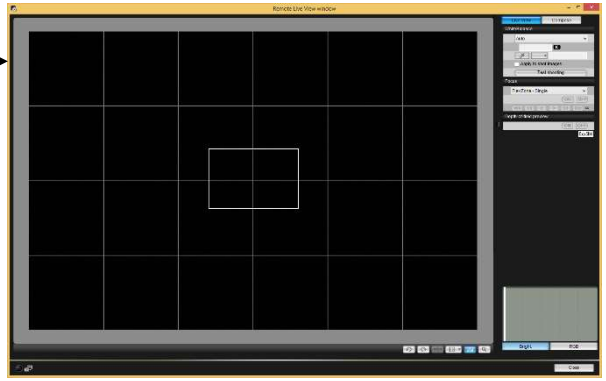
4. Toggle the Camera ***On/Off*** switch to connect it to the computer

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

II. Startup – 2/3

6. Click on ***Live View shoot***

7. Remote Live View window will appear



8. Turn on the lamp at the back of the microscope

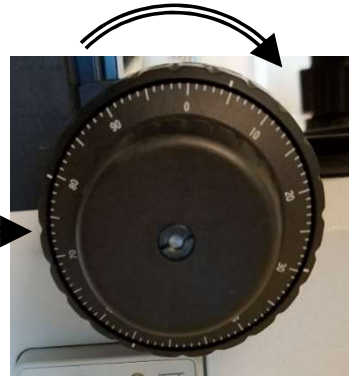
Check that the power indicator is lit showing green or orange

9. To use ***Camera View***: Pull lever completely out

To use ***Binocular Eyepiece***: Push lever completely in

II. Startup – 3/3

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



11. Place sample on microscope stage
12. Rotate and start with the 10X magnification first
13. Pull out the polarizer, analyzer, and DIC prism if inserted

14. Identify which microscope mode you wish to use:

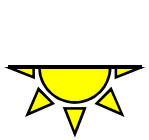
    Episcopic Illumination ()

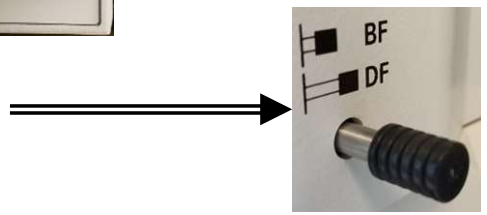
    III. Bright field
    IV. Dark field
    V. Polarization
    VI. Differential Interference Contrast (DCI)

    Diascopic Illumination()

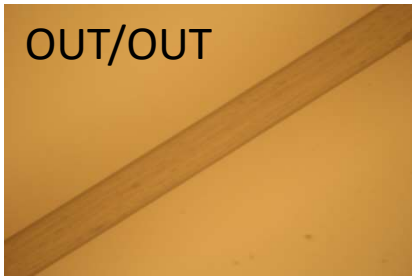
    VII. Bright field

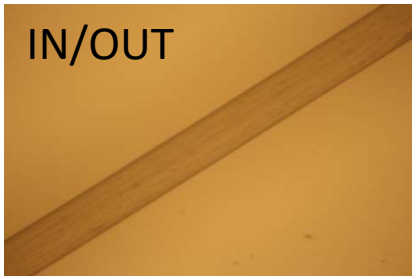
III. EPI: Bright Field – 1/2

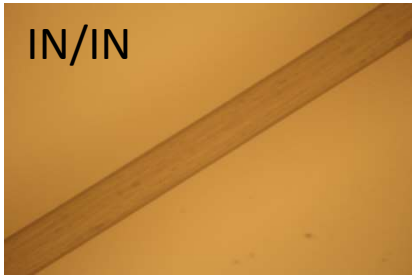
1. Press the ***EPI/DIA*** selector and set to ***EPI***  

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

3. Select any filters you wish to use:
   ND8: changes brightness / ***NCB***: balances color

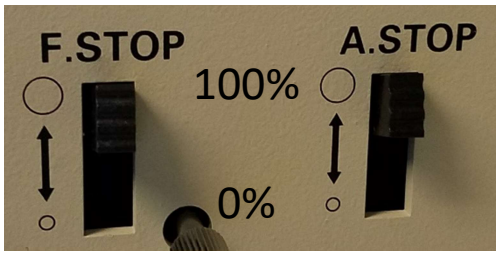






III. EPI: Bright Field – 2/2

4. Adjust the brightness with the ***Brightness Control***
5. Adjust the ***F. STOP*** (field diaphragm) and ***A. STOP*** (aperture diaphragm) by sliding levers up and down from 100% open to 0% open



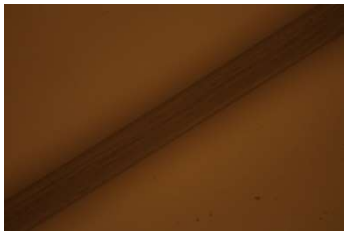
F 100%/A 100%

F 50%/A 100%

F 0%/A 100%

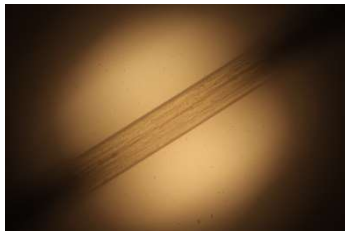
F 100%/A 50%

F 100%/A 0%



F 0%/A 0%

F 0%/A 0%
Brightness Increased



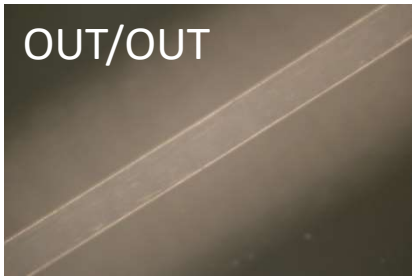
6. Focus on specimen by adjusting the ***Coarse/Fine Focus*** knobs
7. Switch to higher magnification objectives if desired
8. Repeat steps 3-7 until desired magnification and image quality is obtained
9. Go to ***Step VIII. Image Capture*** when ready to acquire image

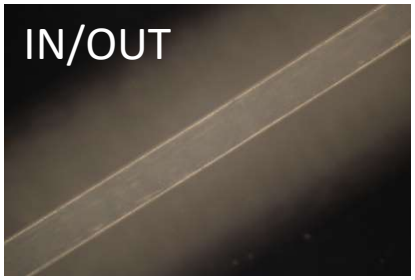
IV. EPI: Dark Field – 1/2

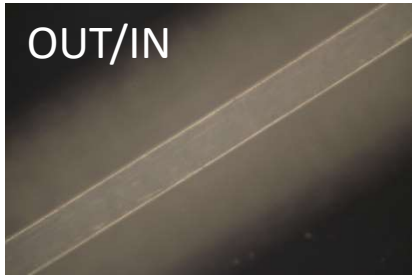
1. Press the ***EPI/DIA*** selector and set to ***EPI*** 

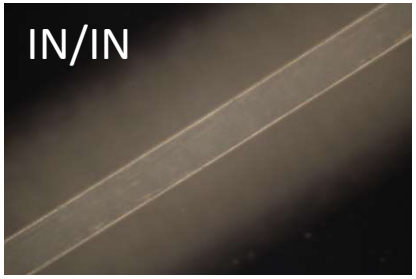
2. Pull ***Bright/Dark Field*** selector lever to fully out ***DF*** position ⟹

3. Select any filters you wish to use:
   ND8: changes brightness / ***NCB***: balances color









IV. EPI: Dark Field – 2/2

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

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

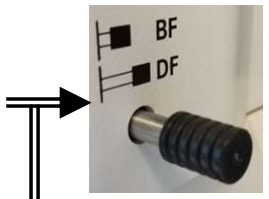
   Levers will have ***NO*** affect

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

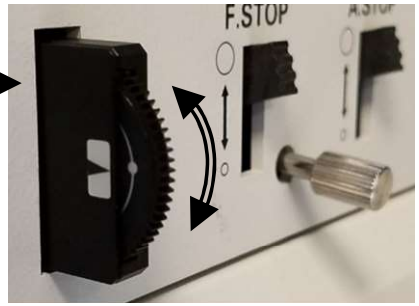
7. Switch to higher magnification objectives if desired
8. Repeat steps 3-7 until desired magnification and image quality is obtained
9. Go to ***Step VIII. Image Capture*** when ready to acquire image

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 any filters you wish to use: 

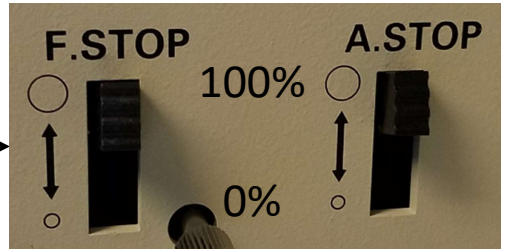
   ND8: changes brightness / ***NCB***: balances color

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

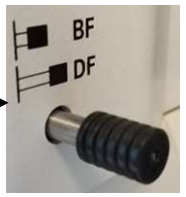


11. Focus on specimen by adjusting the ***Coarse/Fine Focus*** knobs
12. Switch to higher magnification objectives if desired
13. Repeat steps 7-12 until desired magnification and image quality is obtained
14. Go to ***Step VIII. Image Capture*** when ready to acquire image

VI. EPI: Differential Interference Contrast – 1/2

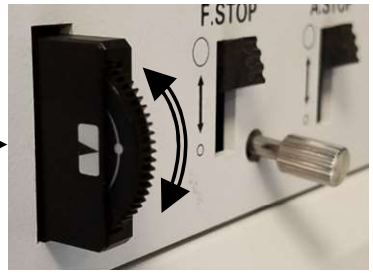
1. Press the ***EPI/DIA*** selector and set to ***EPI***

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

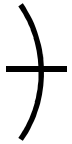


5. Push the ***Analyzer Plate*** in

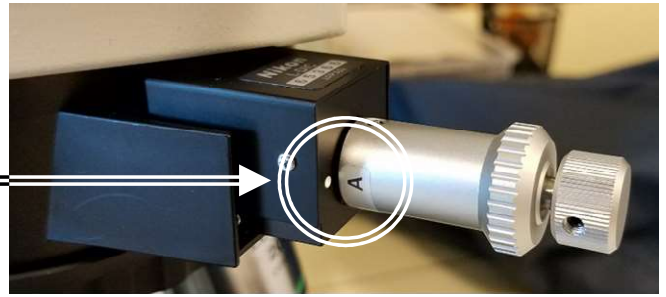
6. Push the ***Polarizer Slider*** in



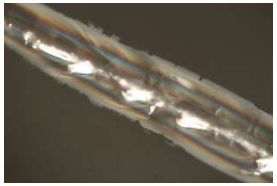
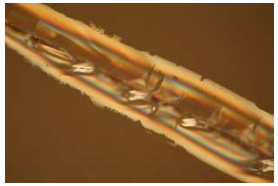
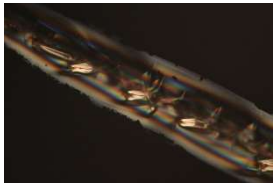
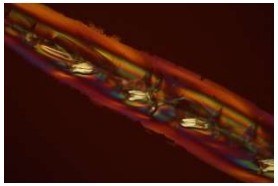
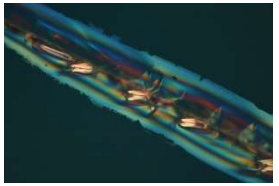
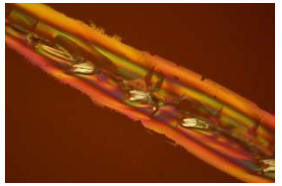
7. Rotate the polarizer to adjust the polarization from

lateral  to vertical 

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



9. Rotate small knob to adjust contrast and color

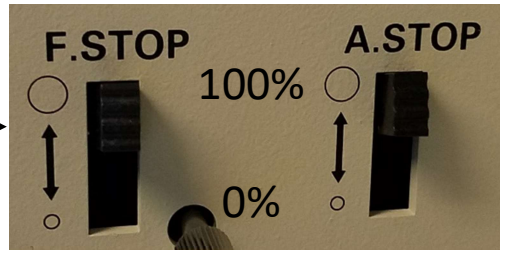
VI. EPI: Differential Interference Contrast – 2/2

10. Select any filters you wish to use: ⟹

ND8: changes brightness / ***NCB***: balances color

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

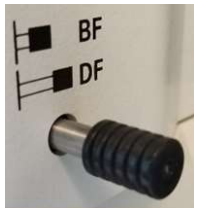
13. Focus on specimen by adjusting the ***Coarse/Fine Focus*** knobs ⟹

14. Switch to higher magnification objectives if desired
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

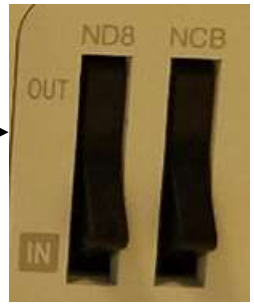
VII. DIA: Bright Field – 1/2

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

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



3. Select any filters you wish to use: ⟹
   ND8: changes brightness / ***NCB***: balances color

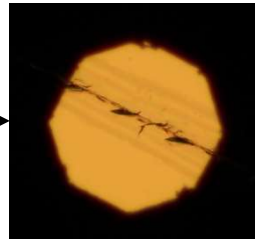


4. Adjust the brightness
   with the ***Brightness Control*** ⟹

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

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

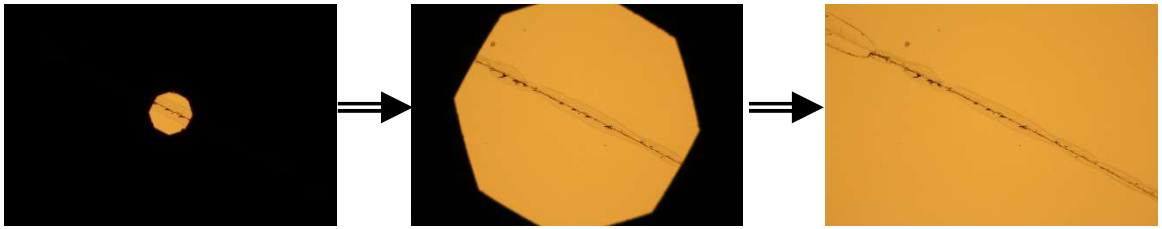
⟹



VII. DIA: Bright Field – 2/2

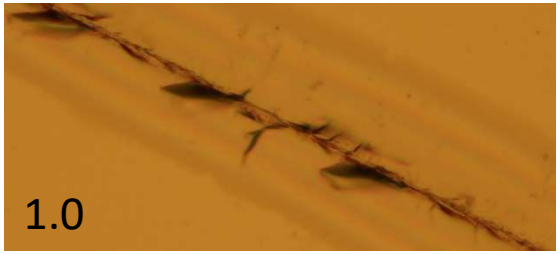
7. Center the field diaphragm by adjusting ***Centering Screws***

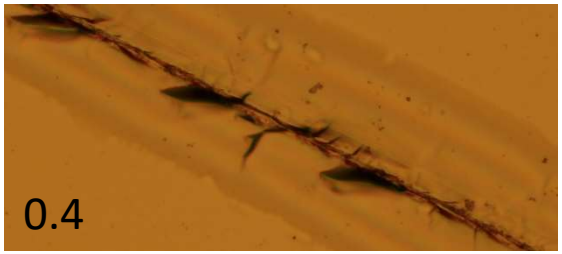
8. Open the ***Field Diaphragm Control*** until field diaphragm circumscribes the field of view



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

10. Open the ***Condenser Aperture*** to achieve desired depth of field





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

VIII. Image Capture – 1/1

1. The images will be saved in the default folder indicated here

2. Click on the ***Folder*** icon, and choose which folder you wish to save your pictures in

3. Recommend creating you own personal folder with sub-folders for each sample to help distinguish among them later

4. Click on the ***Shutter Button*** to acquire your image

IX. Cleanup – 1/1

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

2. Rotate and place the 10x objective into position

3. Turn off the power at the back of the microscope

4. Turn off the control software

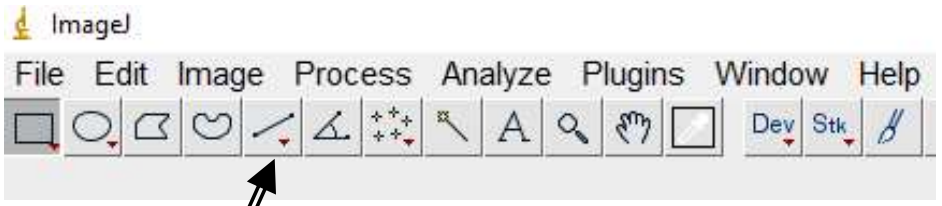
5. Sign-off from your account

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

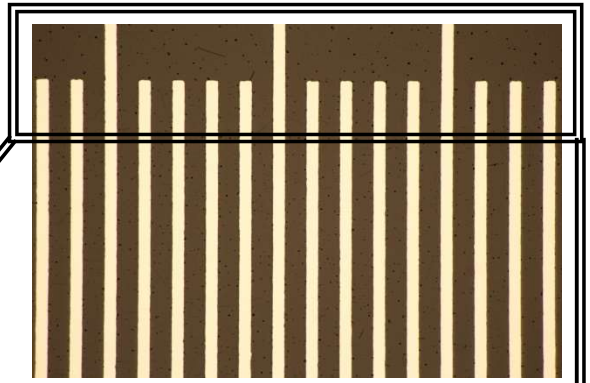
7. Confirm that the microscope is turned **OFF** again (**NO LIGHT!**), then place cover over microscope

X. ImageJ – 1/1

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

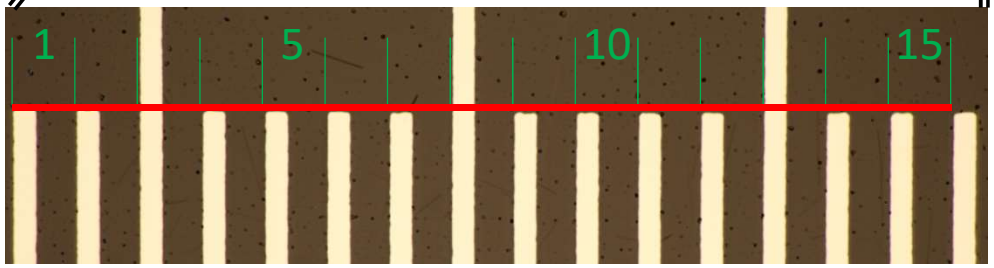


2. Click ***File > Open***
3. Locate the ***Scale Bar Images*** folder
4. Select the ***Magnification*** of the image you wish to measure (e.g. 100X) and ***Open***
5. Click the ***Segment Tool*** and select ***Straight Line***



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

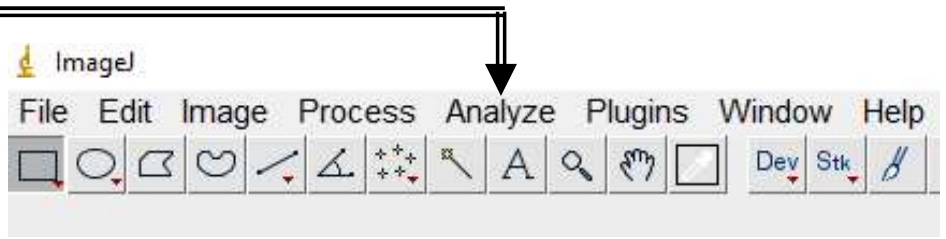
   Note: It matters where you start and end the line!



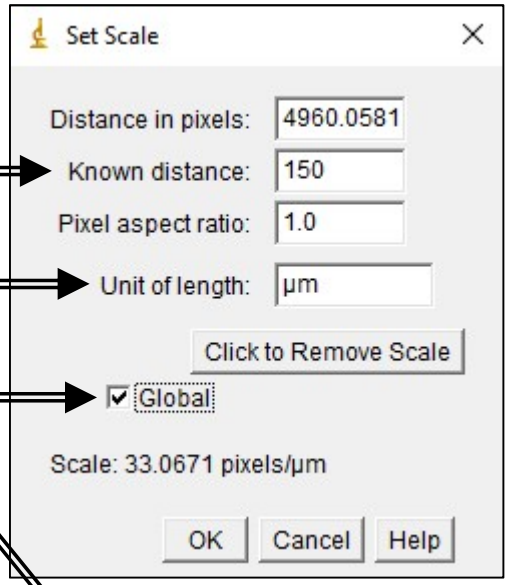
7. Count the number of tick marks contained (e.g. 15)
8. Each division is 0.01 mm (or 10 μm)

X. ImageJ – 2/2

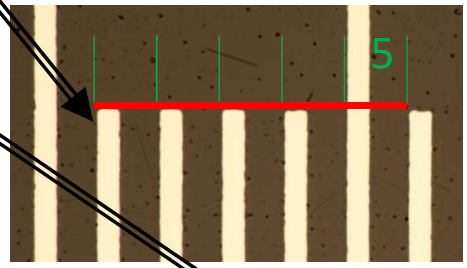
9. Click ***Analyze > Set Scale***



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***