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 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 – 1/1

- **Brightness Control Knob**
- **EPI/DIA Selector**
- **Coarse Focus Knob**
- **Fine Focus Knob**
- **Lamp Power Switch**
- **Condenser Focus Knob**
- **Camera**
- **Binocular Eyepiece**
- **Analyzer Plate**
- **Objectives**
- **Stage**
- **Fine Focus Knob**
- **Coarse Focus 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
- Field Diaphragm Control
- Filter Selector Switches
- Camera On/Off Switch
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 \textit{EPI/DIA} selector and set to \textit{EPI}.

2. Push \textit{Bright/Dark Field} selector lever to fully in \textit{BF} position.

3. Select any filters you wish to use:
\textit{ND8}: changes brightness / \textit{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%
   - 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: $\text{ND}8$: changes brightness / $\text{NCB}$: 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 \textit{Centering Screws}.

8. Open the \textit{Field Diaphragm Control} until field diaphragm circumscribes the field of view.

9. Focus on specimen by adjusting the \textit{Coarse/Fine Focus} knobs.

10. Open the \textit{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 \textit{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 your 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)
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**