MICROSCOPE 5

Nikon Eclipse Ni-U DIC/EPI-Fluorescence Compound Microscope

TRAINING GUIDE

Instruction for use of Microscope 5. Detailing the techniques available, location of supplies, and requirements for use of this shared resource

3364G KHS Advance Microscope Suite

Prepared by Georgette Sass and Ashley Vanhouten

Table of Contents

Introduction

Training requirements:

- Training on the use of this instrument is required prior to keycard access.
- First come, first served – if you are planning any time-sensitive analysis remember to reserve your time on the shared calendar to prevent any scheduling delays.
- Please remember to log your time, even retroactively, to provide data of usage.

Calendar information:

- Viewing of the shared calendar is accessible on the Microscopy Suite GVSU page

  https://www.gvsu.edu/clas/labresource/microscopy-facility-13

- Access to the calendar is automatically added with Keycard request
- To add the calendar to your account please follow the steps outlined in "Advanced Microscope Suite Calendar Access"

Supplies available:

- Drawer 11 includes a ready supply of cleaning agents for the microscopes. If low, please email Ashley Vanhouten.
- Sparkle, IPA 70%, and Ethanol are the only cleaning agents approved for use in the suite.
- If there is an advanced issue please contact your PI, Aaron Perry, or Ashley Vanhouten for additional support.

Please communicate issues:

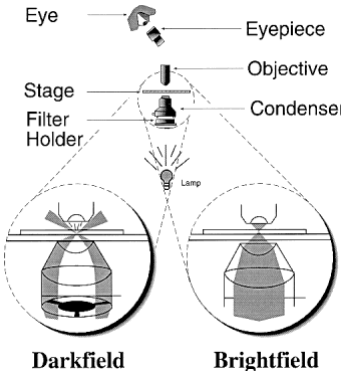
If you encounter a situation where the microscope has become damaged or is malfunctioning in any way, please communicate this issue with your PI. PIs, please communicate issues to Aaron Perry or Ashley Vanhouten so we may provide support on repair of this instrument. As these units age, we anticipate the need for adjustments and repairs, so please don't hesitate to communicate any needs.

Microscope Techniques

Bright-field Microscopy

Bright field is the most basic microscopy technique and uses illumination that is passed through your specimen. How the light path is modified (reflected or absorbed) as it passes through your specimen generates the image.

The light path of a bright-field microscope is straightforward (see figure below) with a light source (a halogen lamp) to illuminate the specimen from below, a condenser lens to focus the light on the specimen, an objective lens to provide magnification and collect light from the specimen and an ocular lens or eyepiece to view the specimen image. Proper adjustments of the light intensity and the light path are critical for a good image. It is always recommended that you optimize the light path, which is accomplished by focusing and centering the condenser



http://public.wsu.edu/~omoto/papers/darkfield.html

Dark-field Microscopy

Illumination technique that allows viewing of unstained, transparent specimens by highlighting edges and surfaces against a black background. This technique is popular for use with samples that have refractive indices very close in value to their surroundings making it difficult to image with conventional brightfield techniques.

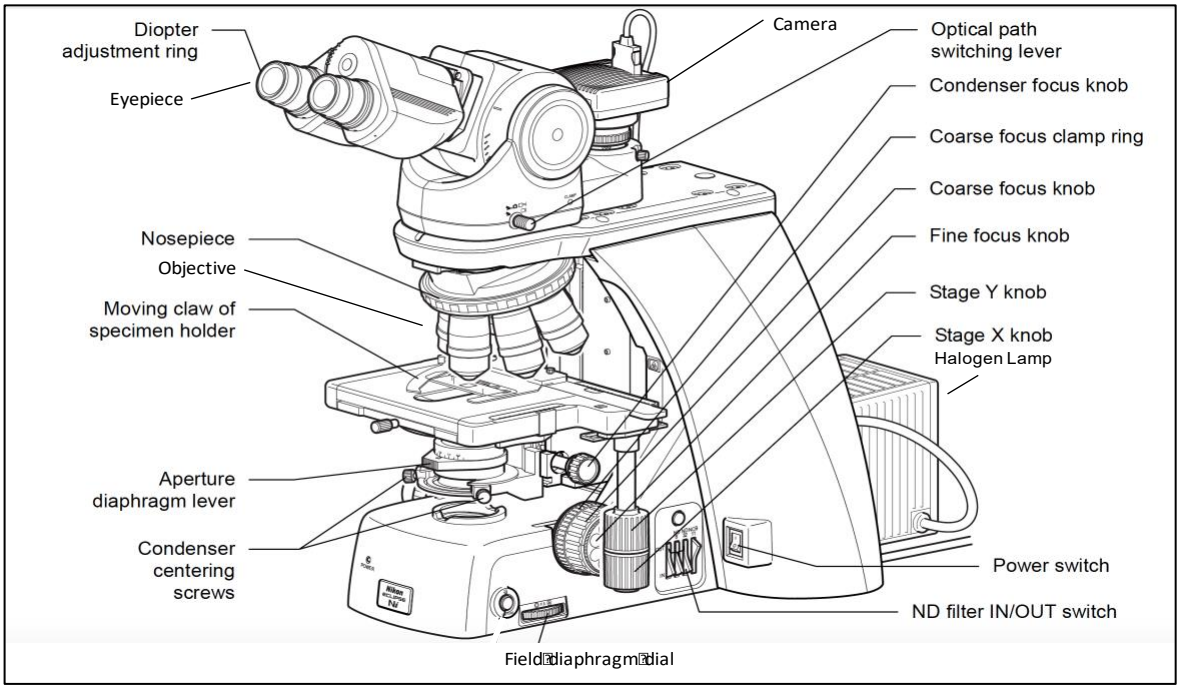
Differential Interference Contrast (DIC) Microscopy

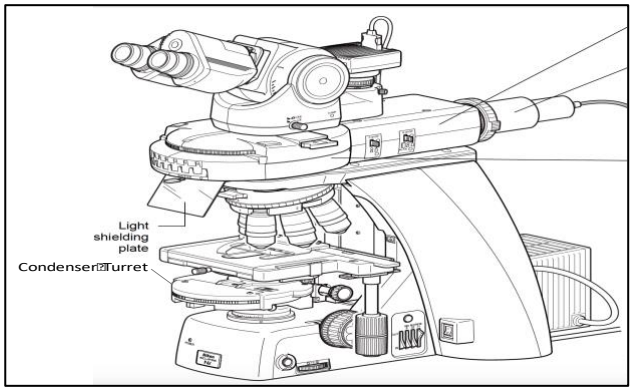
Contrast is the perceived difference in color or light intensity between an object and its surroundings. This is used by the human eye to visualize the world around us. Specimens of biological interest often exhibit little contrast (chloroplasts of plant tissue provide a notable exception) and their inherent transparency does not alter light in ways detectable by the human eye. Differential Interference Contrast (**DIC**) Microscopy is an optical technique that can be used to enhance the contrast in specimens using polarized light.

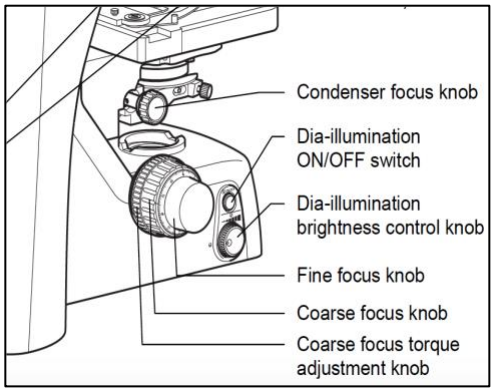
Epi-fluorescence Microscopy

The use of fluorescent molecules in microscopy has revolutionized our ability to study many biological specimens. Fluorescent molecules (or fluorophores) can be used to tag otherwise transparent cellular structures to determine information such as cellular location. Increased sensitivity possible with fluorophores allows the observation of organelles and even molecule-specific signals. Light that illuminates the sample from above is used (thus the term **epi-fluorescence)** and the objective lens acts as both the illumination condenser and the fluorescent light collector. Electrons of a fluorescent molecule can absorb a photon of excitation light and then emit some of the gained energy as a photon with less energy and therefore a longer wavelength. It is this emitted light that is detected during Epi-fluorescence microscopy.

Microscope Detailed Images







Bright-field Microscopy Adjustment

NOTE: All microscopes in the KHS facility are capable of Bright-field Microscopy. You will need to know how to prepare the scope for microscopy as well as how to adjust the focus and optical path on an upright scope to obtain the highest quality Bright-field image. It is recommended to start with Bright-field to obtain the best image using DIC/Epi-fluorescence.

Preparation

1. Turn on the power.

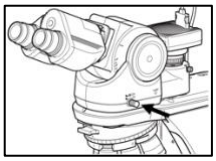
   The Power switch is a rocker switch on the rear portion of the Ni-U main body, on the right side (your right side as you are facing the scope). When you have been successful you will see the Power LED light on the front of the scope illuminated.

2. Turn on Dia-illumination.

   This is a push button switch on the front, left side of the Ni-U main body. When the button switch is pressed – illumination is ON. When the button has been released, illumination is OFF.

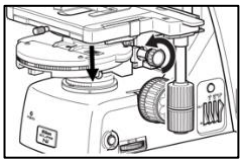
3. Set the optical path of the binocular tube to receive 100% of light to the eyepiece

   This is accomplished using the optical path switching lever. When the lever is pushed in, 100% of the light will pass through the binocular tube to the eyepiece.



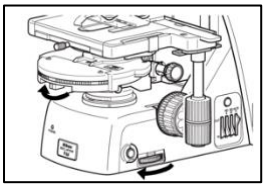
4. Lower the condenser slightly from its uppermost position.

   To do this turn the condenser focus knob until the condenser reaches its upper limit (you will feel it click to a stop). Then, slightly lower the condenser with the knob.



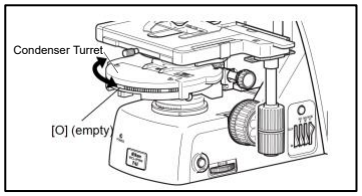
5. Fully open the field diaphragm and fully close the aperture diaphragm.

The field diaphragm dial is on the base of the Ni-U main body on the right side. The aperture diaphragm lever is located just below the condenser turret. Both are moved clockwise to open.



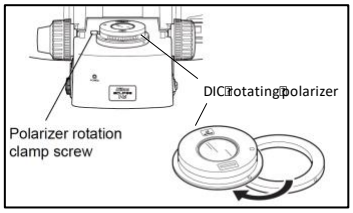
6. Set the Condenser Position.

   Turn the **Condenser turret** until the [0] symbol comes to the front to bring the empty position into the optical path. This is the default position for the microscope.



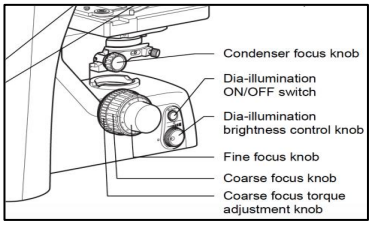
7. Remove the DIC rotating polarizer from the optical path**.**

   The DIC components on this scope will reduce the light intensity to your sample because of their placement in the optical path. For this reason, it is suggested that you remove the **DIC rotating polarizer** from the light path for Bright-field Microscopy. To do this, gently rotate the upper portion of the DIC rotating polarizer clockwise.



8. Adjust Dia-illumination.

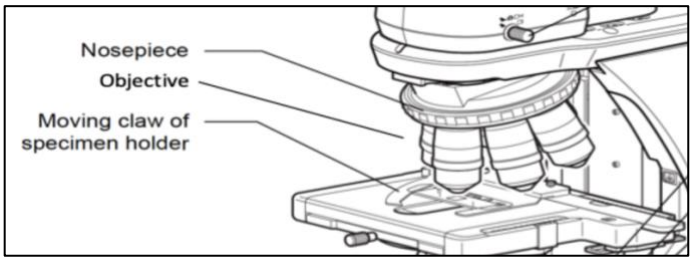
   The Dia-illumination brightness control knob is just below the Dia-illumination button switch. This may need to be adjusted to an intensity that is comfortable for your eyes as you move through the next adjustment steps.



9. Begin with the 10x Objective.

   Bring the desired objective into place by rotating using the nosepiece (it will click into place). Rotate the nosepiece to set the 10x objective in place.

   Do not rotate objectives by holding and rotating the objective itself, as this can damage the objective, use the nosepiece as identified in the image below.



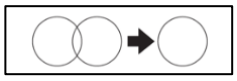
10. Focus on the sample

    Place a specimen on the stage. Turn the Coarse Focus knob to raise the stage to its upper most limit. Look into the eyepiece and lower the stage using the Coarse Focus knob until you can see your specimen. To lower the stage, turn the knob toward the front of the scope. Use the Fine focus knob to get image in acceptable focus. You may need to adjust light intensity.

    - View the microscope from the side when you raise the stage.
    - View the specimen through the eyepiece when you lower the stage to focus.
    - Never rotate the right and left focus knobs in opposite directions.

11. Interpupillary Distance Adjustment.

    When you have the specimen in focus, get the best image possible by looking into the eyepieces using both eyes. Adjust the binocular head such that the distance between your eyes allows the field of view from the left eye and the right eye to coincide. It helps to pretend you are looking into the distance.



12. Diopter Adjustment.

    The diopter adjustment ring on each eyepiece can be adjusted to match the vision in each eye.

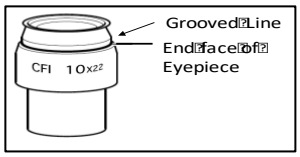
    - Turn the nosepiece to bring the 40x objective in place and focus on your specimen (use the Coarse focus knob if needed then the fine focus knob)

- Return to the 10x objective and look into the right eyepiece with your right eye.

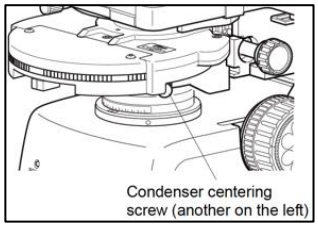
Note: To avoid using your left eye it is a good idea to cover it with a piece of paper. Do not press on your eye as this changes the shape of your lens!

- Focus on your specimen by turning the right Diopter adjustment ring.
- Look into the left eyepiece with your left eye (cover your right eye with a piece of paper). Focus on the sample by turning the left Diopter adjustment ring.
- Repeat the above steps until focus is perfect!

Note: Diopter adjustment increases ease of binocular vision reducing eyestrain and improving imaging. The diopter adjustment ring should always be returned to the diopter adjustment reference position. In this position, the end face of the eyepiece is aligned with a grooved line on the diopter adjustment ring.



13. Focus and Center the condenser.



This step ensures that the light passing through the condenser lens is focused on the surface of the specimen. Follow these steps:

- Return to the 10X Objective
- Focus on your specimen.
- Turn the field diaphragm dial counterclockwise to fully stop down the field diaphragm (see step 5 above).
- You will see the field diaphragm image in your field of view. Use the Condenser focus knob (see Step 4 above) to adjust the field diaphragm image until the outline of the diaphragm image is in sharp focus and colorless.
- If needed, adjust the Condenser centering screws until the field diaphragm image is at the center of the field of view.
- Bring the 40X objective into position. Use the Condenser focus knob to adjust the focus as much as possible. (The 40X objective field diaphragm image cannot be seen quite as clearly at that of the 10x Objective.)

- Adjust as needed to center the field diaphragm image.
- You should focus the field diaphragm image for each objective used.

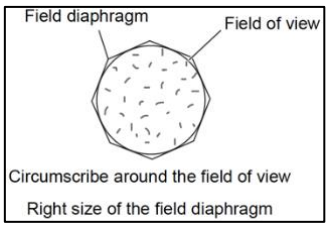
14. Choose your objective and adjust the aperture diaphragm

    It is important that you adjust the Aperture diaphragm (see step 5 above) for each objective used as this affects resolution, contrast, focal depth and brightness of your image. This is accomplished using the Aperture diaphragm lever on the top of the condenser (clockwise opens the diaphragm). The aperture diaphragm setting will be dependent upon the Numerical Aperture (NA) of the Objective chosen.

    NOTE: It is recommended that the proper size of the aperture diaphragm is 70-80% of the Numerical Aperture of the objective. Thus an objective with an NA of 0.75 should be set to a position indicated by 0.525-0.6.

    A small Aperture Diaphragm reduces resolution and brightness but increases depth of focus and contrast whereas a large Aperture diaphragm increases resolution and brightness but reduces depth of focus and contrast.

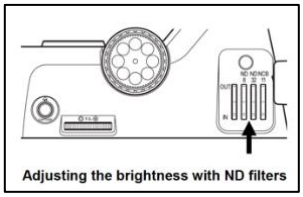
15. Adjust the field diaphragm

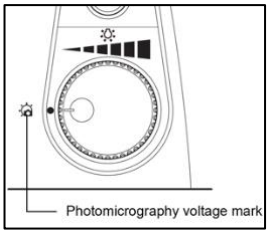


    It is important that you adjust the Field Diaphragm for each objective used (See step 5 above). With your sample in focus, turn the field diaphragm dial just to the point where is circumscribes the field of view. Opening the Field diaphragm too far beyond the field of view will compromise your image quality as it allows stray light to enter the field of view.

    Note: Always check that the field diaphragm is in fact centered! If not, return to step 10 above.

16. Adjust the brightness of the image





The brightness of the image can be adjusted by using a Neutral Density Filter (ND) or by adjusting the Dia-illumination brightness control knob.

Consider how you adjust the brightness when you are capturing an image via a camera because changing the lamp voltage using the Dia-illumination brightness control knob will alter the color balance of an image.

If accurate color reproduction is important, it would be recommended that you set the Dia-illumination brightness control knob to the Photomicrography voltage mark. This can be accomplished by turning the Dia-illumination brightness control knob counterclockwise until it clicks to a stop.

If color reproduction is important, be sure to bring the NCB11 filter into the optical path. Brightness can then be adjusted as needed by the addition of any of the Neutral Density filters.

17. View your specimen

    Use the X stage knob and the Y stage knob to bring a region of your slide into the optical path so that you can view it. The stroke of the stage is X:+/- 78mm, Y:+/- 54mm.

Dark-Field Microscopy

Note: **Preparation** and **Adjustments** for Dark-field microscopy are essentially identical to that described above for Bright field. Exceptions come after you adjust and focus the **Condenser** in **Step 13** for Bright-field Microscopy.

After **Step 13** from above:

1. **Set the Condenser Turret for Dark field.**

   Turn the **Condenser turret** until the [DF] symbol comes to the front to bring the Dark field position into the optical path.

2. **Choose your objective but DO NOT adjust the Aperture diaphragm.**

   The Aperture diaphragm should remain open for Dark-field Microscopy.

3. **Adjust the Field Diaphragm**.

   See **Step 15** for Bright-field Microscopy above.

4. **Adjust the brightness of the image.**

   See **Step 16** for Bright-field Microscopy above.

5. **View your specimen.**

   See **Step 17** for Bright-field Microscopy above.

6. **Image Documentation.**

   See **Step 18** for Bright-field Microscopy above.

Ending your Microscopy Session

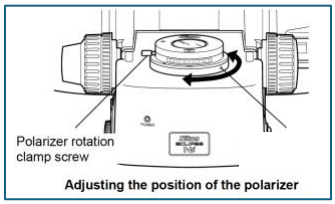
7. You must return the Ni-U to its default condition. Please review the final page of this manual to return settings to default preferences.

Differential Interference Contrast (DIC) Microscopy

Note: **Preparation** and **Adjustment** for DIC microscopy is similar to that described above for Bright field. DIC microscopy begins after the **Condenser** has been focused and centered in **Step 13** above for Bright-field Microscopy.

After **Step 13** from above:

1. **Return the DIC rotating polarizer to the optical path**.



Adjusting the position of the polarizer

   Gently rotate the upper portion of the **DIC rotating polarizer** counterclockwise. It will click in to place to insure that the **DIC rotating polarizer** is set to its default position.

   Loosen the **polarizer rotation clamp screw** and align the ● line to the ♦ symbol. Then tighten the **Polarizer rotation clamp screw.**

2. **Choose a DIC objective.**

   Turn the nosepiece to bring desired objective in position.

3. **Bring the DIC module on the Condenser turret into the optical path.**

   The optical module on the **Condenser turret** that is set for DIC is [N2].

4. **DIC Microscopy requires 3 components** to be present in the optical path.
   Check that each component is in place:
   - The **DIC slider** on the **Objective** (insert completely)
   - An **Analyzer slider for DIC** (insert to second click stop)
   - The **DIC rotating polarizer** (see **Step 1** above)

5. **Adjust the Aperture Diaphragm.**

   See **Step 14** for Bright-field Microscopy.

6. **Adjust the Field Diaphragm**.

See **Step 15** for Bright-field Microscopy.

7. **Adjust the brightness of the image.**

   See **Step 16** for Bright-field Microscopy.

8. **View your specimen.**

   See **Step 17** for Bright-field Microscopy.

9. **Adjust Contrast of Image.**

   Loosen the **Polarizer rotation clamp screw.** While viewing your specimen, rotate the DIC polarizer by sliding the screw either clockwise or counterclockwise. Move until contrast in your specimen has been maximized**.**
   This can be a bit subjective! However, it is best to think in terms of capturing the image with a camera and making sure that the background of your image is a neutral color.

10. **Image Documentation.**

    See **Step 18** for Bright-field Microscopy.

Ending your Microscopy Session

11. You must return the Ni-U to its default condition. Please review final page of this manual to return settings to default preferences.

Epi-fluorescence Microscopy

Note: Even if you think you are interested only in Epi-fluorescence, you should know how to start your microscopy session as described above for Bright field. Nothing that you do with the light path for Bright-field microscopy will impact your fluorescent image. (Illumination for creation of your fluorescent image comes from above the objective while illumination for Bright field/Dark field/DIC comes from below the objective.)

However, it is strongly suggested that you complete all steps through **Step 12** of Bright-field Microscopy before you begin Epi-fluorescence Microscopy. This will optimize microscope configurations (such as focus and eyepiece adjustments) to more easily visualize your fluorescent image. Once you find your specimen and are comfortable with the quality of the image you have obtained you can switch to Epi-fluorescence.

Remember, you will have to return the Ni-U Microscope to the default settings for Bright-field Microscopy when you are finished with Epi-fluorescence!

Safety precaution – fluorescence lighting presents a risk to your eyes. Looking directly at the light source can cause damage to your eyes. While utilizing fluorescence lighting, it is recommended to be positioned behind the orange shield and to not look directly at the light source outside of the microscope.

It is safe to look at fluorescence lighting through the instrument because it is designed with a system of filters and mirrors that block harmful excitation light from reaching the users eyes.

Photo-bleaching – fluorescence microscopy requires image setup and analysis quickly as photo-bleaching can occur if specimen is exposed for extended periods. Be mindful of this and remember to shutter the light source when not viewing/capturing images.

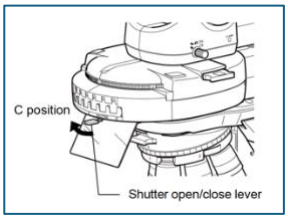
After **Step 12** from above:

1. **Turn off Dia-Illumination.**

   When the button has been released, illumination is OFF.

2. **Close the shutter to block the epi-illumination path.**

   Move the **Shutter open/close lever** on the Epi-fluorescence cube turret to the **C** position to block the optical path. This will prevent damage to the specimen and is a good habit to maintain throughout your session.



3. **Bring the desired filter cube into the optical path.**

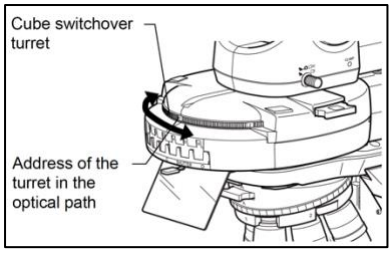
   This is accomplished by turning the **Cube switchover turret** until the address of the desired filter cube is indicated on the front position. Position 1 is open and does not contain a filter cube.

   The filter cube consists of three components

   - an excitation filter
   - a barrier filter
   - a dichroic mirror

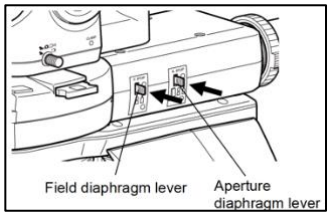
   Each filter cube is specific for a given fluorophore. We have three filter cubes that detect

   - Position 1: Empty
   - Position 2: DAPI – EX 361- 389
   - Position 3: FITC – EX 465-495
   - Position 4: TxRed/mCherry – EX 540-580
   - Position 5: Empty
   - Position 6: Empty



4. Fully open the Field diaphragm and Aperture diaphragm on the Epi-fluorescence attachment.

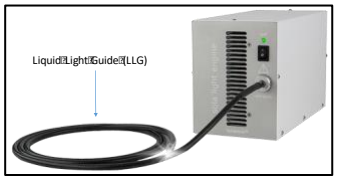
   The open position is achieved when the lever is pushed in**.** These diaphragms should be adjusted for each objective used (See **Step 7** below).



5. **Turn on the Illuminator.**

To turn ON, use the toggle switch on the front of the Illuminator.

We have a lumencor® SOLA Illuminator that provides solid-state white light illumination using a light-emitting diode (LED). Light is delivered via a 3 mm diameter **Liquid light guide** (LLG).



It is critical that the LLG be kept safe so that it is not bent, kinked or pinched! Do not turn off the fan switch at the back, this will operate after use even with power turn off to the LLG to cool the unit.

We are very lucky to have this non-mercury containing light source to use for Epi-fluorescence Microscopy because it allows us to turn on the light as needed without any cool down period between uses. Please take care of this component!

6. **Chose your Objective and open the shutter.**

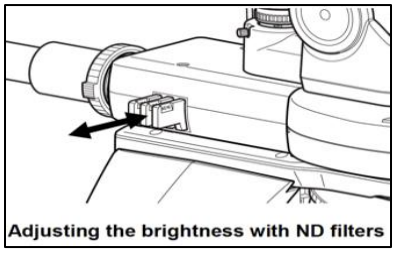
You can now illuminate your specimen. Move the **Shutter open/close lever** on the Epi-fluorescence cube turret to the **O** position. See **Step 2** above.

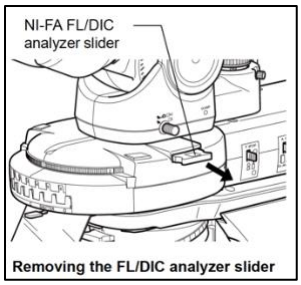
7. **Focus on your sample.** See **Step 15** for Bright-field Microscopy above.

8. **Adjust the Field diaphragm.**

It is best to stop down the **Field diaphragm** so that it just circumscribes the field of view. See image in **Step 15** for Bright-field Microscopy above. This will prevent stray light from entering the field of view and will also reduce the area of your specimen exposed to light that could quench the fluorescent signal.

9. **Adjust the Brightness.**


Adjusting the brightness with ND filters



Removing the FL/DIC analyzer slider

The Neutral Density filters (ND) can be used to adjust brightness**.**

In Epi-fluorescence, the **Aperture diaphragm** on the Epi-fluorescence attachment will also control the brightness of the image. A larger aperture diaphragm size generates a brighter image, but it does so at the expense of increasing stray light in the image.

Note: The Nikon Eclipse Ni-U #5 is capable of concurrent Epi-fluorescence and DIC Microscopy. This is made possible by the presence of the **FL/DIC Analyzer**. When this is placed in the optical path an image that is both fluorescent and DIC can be obtained. However, it will reduce brightness. If this is an issue it can be removed from the optical path. To do this, pull out the Ni-FA **FL/DIC analyzer slider** by 1 click from the pushed in state.

10. **View your specimen.** See **Step 17** for Bright-field Microscopy above.

11. **Image Documentation.** See **Step 18** for Bright-field Microscopy above.

Ending your Microscopy Session

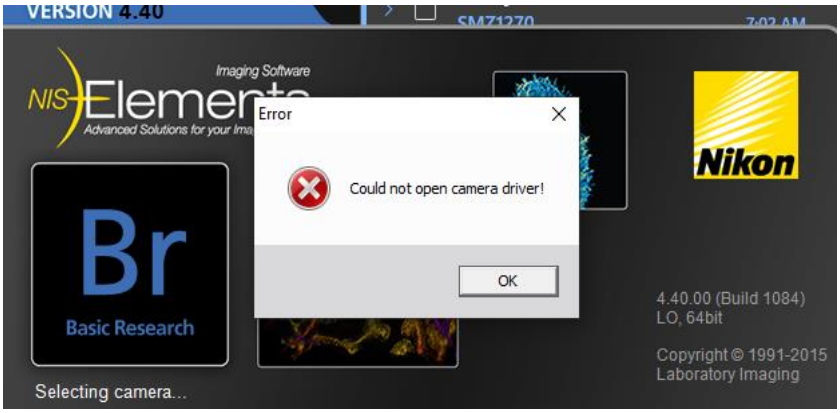
12. You must return the Ni-U to its default condition. Please review the final page of this manual to return settings to default preferences.

Image Acquisition

If you would like to capture an image of your specimen, you will need to:

1. Turn on the computer.
2. Turn on the camera. There are 2 cameras on this microscope

   If you do not power on the camera you wish to you before launching the software, the following error code will pop up.



   If this pops up, close NIS-Elements and verify camera(s) are on and then relaunch NIS-Elements

3. Set the optical path of the binocular tube to distribute 100% light to the camera.

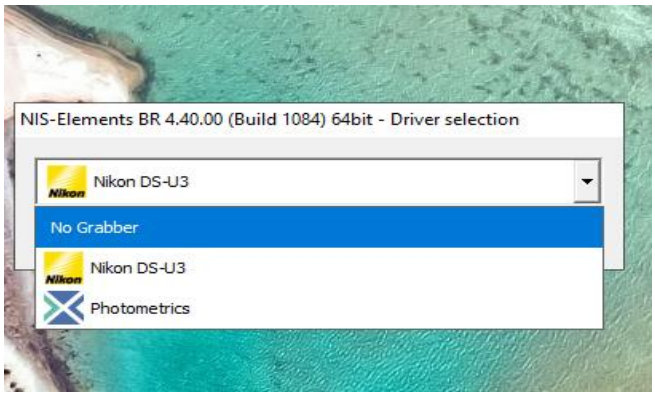
   This is accomplished using the optical path switching lever located on the right-hand side of the microscope head. There are 3 positions:

   - BINO (lever pushed all the way in): 100% of light will go to the eyepieces. 0% light reaching the camera.
   - BINO & PHOTO: (middle position of lever) 80% of the light will pass through the binocular tube to the camera and 20% will pass to the eyepiece
   - PHOTO (lever pulled all the way out):100% of the light will pass through the binocular tube to the camera

4. Select the position of the camera lever (located on the right-hand side of the microscope head)

a. Lever pushed IN =>DS-U3 (color camera) positioned at the rear
b. Lever pulled OUT => CoolSNAP MYO (monochromatic camera) positioned at the top

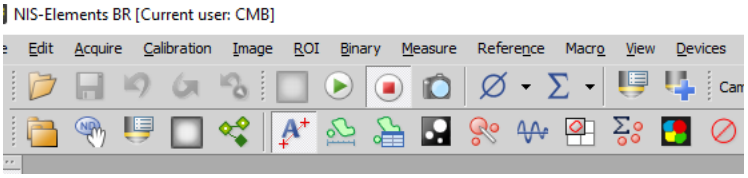
5. Open the NIS-Elements microscope imaging software
6. Driver selection will populate on the screen (camera selection):



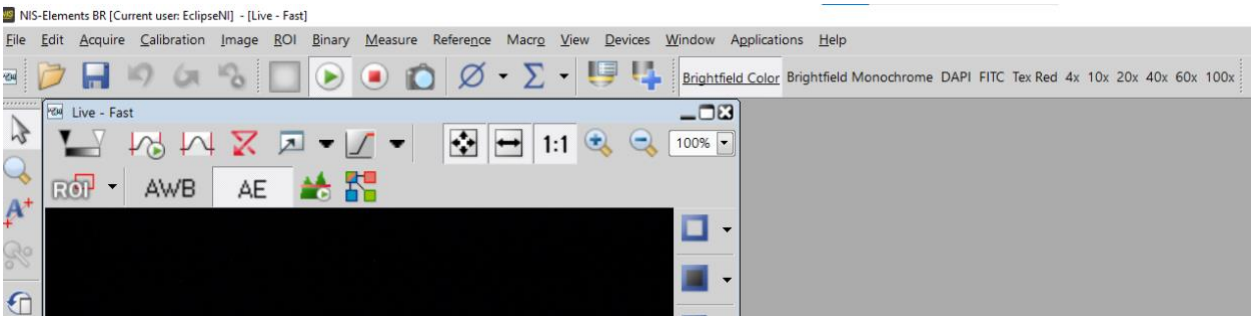
- DS-U3 is your color camera option
- Photometrics is the monochromatic camera option

a. Selected the wrong camera or want to switch? Navigate to the main tool bar in NIS-Elements =>Acquire => Select Driver => Select the camera you want to use

7. Once software is initialized, to capture an image, you will select the Play  button located in the top tool bar.



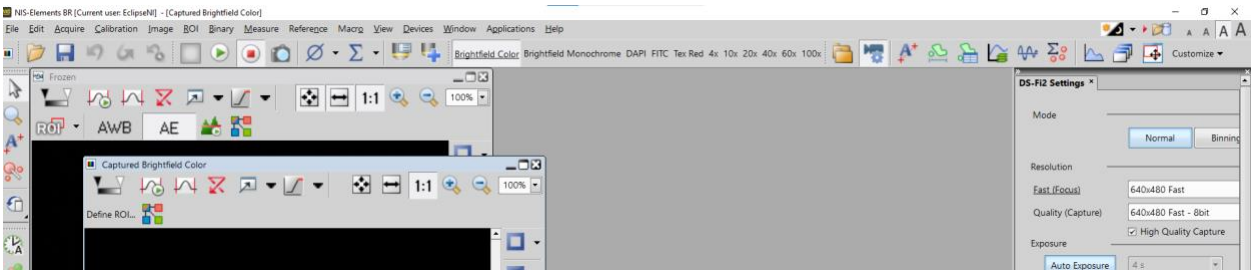
8. This will populate an interior window within the NIS Elements software. The top left of that window will state “Live-Fast” to indicate active camera/live feed of your specimen.



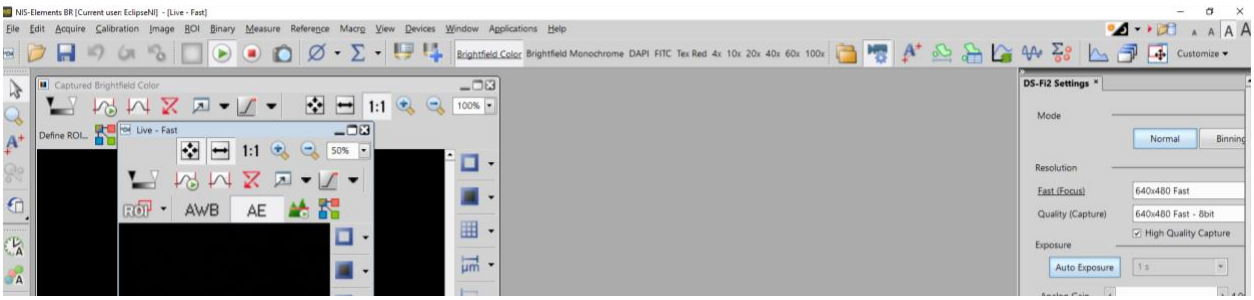
9. Verify settings prior to image capture. There are calibrated options provided at the top of the page for each objective and filter options. Correct selection will make image analysis later (i.e. measurements) easier.

   There are many options to improve image quality – consult the NIS-ELEMENTS guide located at the desktop of each computer for more detailed information.

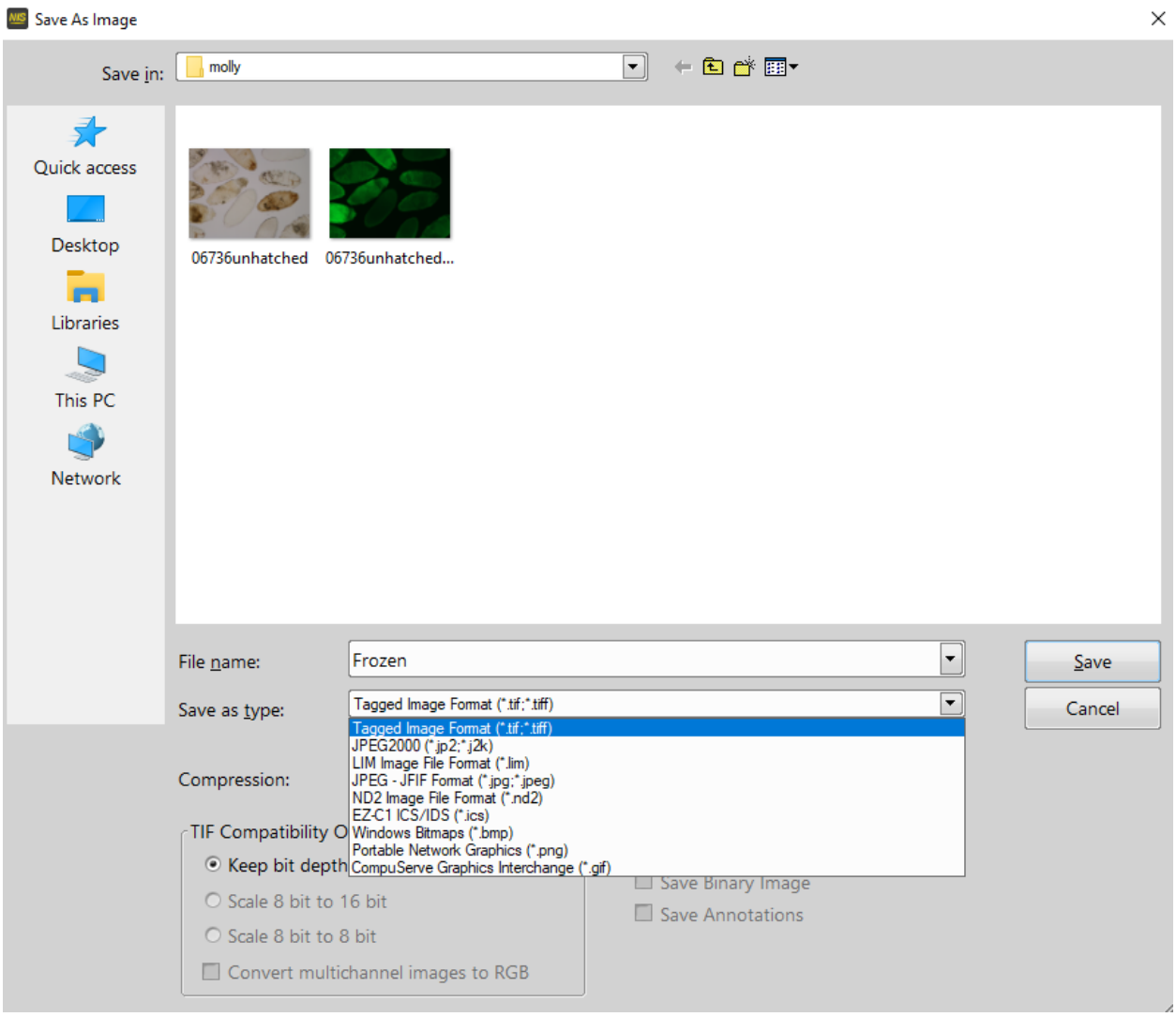
10. To capture an image from “Live-Fast” select the “capture” button  . This action will open a second interior window within NIS – Elements (as pictured below). Viewing the top left corner of each window:
    a. “Frozen” – this is the camera window, the stop icon  is highlighted.
    b. “Captured” – this is your image.



11. To return to your live view, press the play button. The two windows status will be as follows (pictured below):
    a. "Captured” – image captured
    b. “Live-fast” – live view of specimen



12. Once you've captured your image and you want to save select the  or go to File =>Save As



Remember this is a shared resource, so be mindful of where/how you store your data so you can easily find your data again later. There are multiple login options, make sure you know which profile you are logged in under.

Also, note the formatting you save your image as - .TIFF and .JPEG are popular file formatting options for later annotation.

13. Additional annotation/measurements and image capture instructions can be found within the NIS-Elements user guide. A copy can be found on the desktop of any computer in the Advanced Microscope Suite.

Ending your Microscopy Session

It is a courtesy to all to return settings to our “DEFAULT SETTING” to allow the next user to efficiently and easily examine their specimens. It is also setup to prevent any accidental damage to the microscopes, so please follow these instructions.

Return the Ni-U microscope to its default settings

1. Turn off accessory equipment (camera & computer) if no longer in use.
2. Set the optical path switching lever so that 100% of the light is directed to the eyepiece (push the lever in).
3. The microscope stage should be in its most centered position.
4. Remove all filters (ND of NCD) from the optical path.
5. Return the Lowest objective to the optical path.
6. Return the diopter adjustment rings to their reference position
7. Turn the dia-illumination brightness control knob clockwise until it is at its lowest illumination setting, but not set for constant voltage.
8. Turn off Dia-illumination. Accomplished by releasing the button switch.
9. Turn Power off. Right-hand side rocker switch, power LED at front base of microscope should go dark.
10. Cover the microscope.

Technical Specifications

- Microscope Make: Nikon
- Microscope Model: Eclipse Ni-U
- Objectives:
    - 10x: Plan Fluor 10x/0.30 OFN25 DIC L/N1
    - 20x: Plan Fluor 20x/0.50 OFN25 DIC N2
    - 40x: Plan Fluor 40x/0.75 OFN25 DIC M/N2
    - 60x: Plan Apo λ 60x/1.40 OIL OFN25 DIC N2
    - 100x: Plan Apo λ 100x/1.45 OIL ∞/0.17 WD 0.13
- Condenser: NI-CUD
    - [0] – Brightfield
    - [DF] -Darkfield
    - [N2] – DIC with 20x, 40x, and 60x objectives
- Eyepieces: CFI 10x/22 NIKON with groove reference zero position
- Brightfield/Darkfield/DIC filters:
    - NCB11 (Neutral Color Balance) – removes the warm yellow hue produced by light sources, making the illumination appear white and neutral
    - ND8 (Neutral Density); Filter that reduces light reaching specimen to 1/8 of the original.
    - ND32 (Neutral Density); Filter that reduces light reaching specimen to 1/32 of the original.
    - Note: Neutral Density filters are used to manage illumination, color temperature reproduction, prevention of overexposure, enable clearer images, etc.
- DIC Components
    - No Lambda Plate
    - Analyzer filter
    - Polarizer
    - Objective DIC Slider (located above objective)
    - DIC Objective
- Fluorescence cube information:
    - 1 – Empty
    - 2 – DAPI
        - EX 361 – 389
        - DM 400
        - BA 435 – 485
    - 3 – FITC/Cy2
        - EX 465 – 495
        - DM 505
        - BA 515 – 555
    - 4 – TxRed/mCherry

        - EX 540-580
        - DM 600
        - BA 605 – 695
    - 5 – Empty
    - 6 – Empty
- Epifluorescence filters (push -IN, pull -OUT)
    - These filters are ND (Neutral Density). Neutral Density filters are used to manage illumination, color temperature reproduction, prevention of overexposure, enable clearer images, etc.
    - ND 16 – Neutral Density filter that cuts the optical path light to 1/16 of the original.
    - ND8 – Neutral Density filter that cuts the optical path light to 1/8 of the original.
    - ND4 – Neutral Density filter that cuts the optical path light to ¼ of the original.
- Camera 1:
    - Color camera
    - Make: Nikon
    - Model: DS-Fi2
    - Controller: DS-US
    - Pixel resolution: 2560 x1920 (maximum), 1280x960, 640x480
    - Frame rate: Max 21 fps (at 1280x960), Max 37 fps (at 1280x480 or ROI mode)
    - Exposure time: 120 μs to 60 seconds
- Camera 2:
    - Monochromatic camera
    - Make: Photometrics
    - Model: CoolSNAP MYO
    - Pixel resolution: 2.98 megapixel imagery, 4.54 μm pixel pitch
    - 75% peak quantum efficiency
    - Frame rate: 6.2 frames per second
- Software: NIS ELEMENTS
    - Manual available on desktop of units for image acquisition tutorials