MICROSCOPE 6

Nikon Eclipse Ni-U Compound Microscope

TRAINING GUIDE

Instruction for use of Microscope 6. Detailing the techniques available, location of supplies, and requirements for use of this shared resource

3364G Advanced Microscope Suite

Prepared by Georgette Sass and Ashley Vanhouten

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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 report 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, Ashley Vanhouten (Equipment Repair), or Zach Hancock (IT Support) so we may provide support for this space. Examples of when to reach out include, but are not limited to:

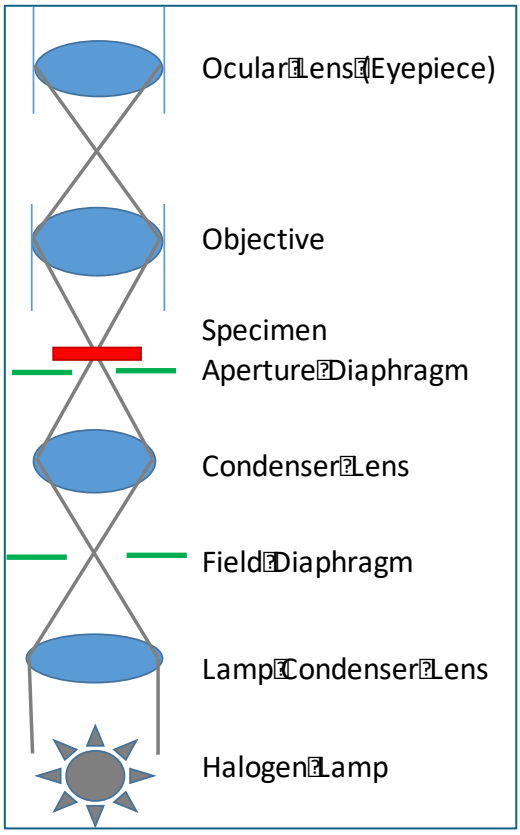
- Bulb outages
- Software calibration issues
- Mechanical focus issues
- Error messages
- Initialization issues

Microscope Techniques

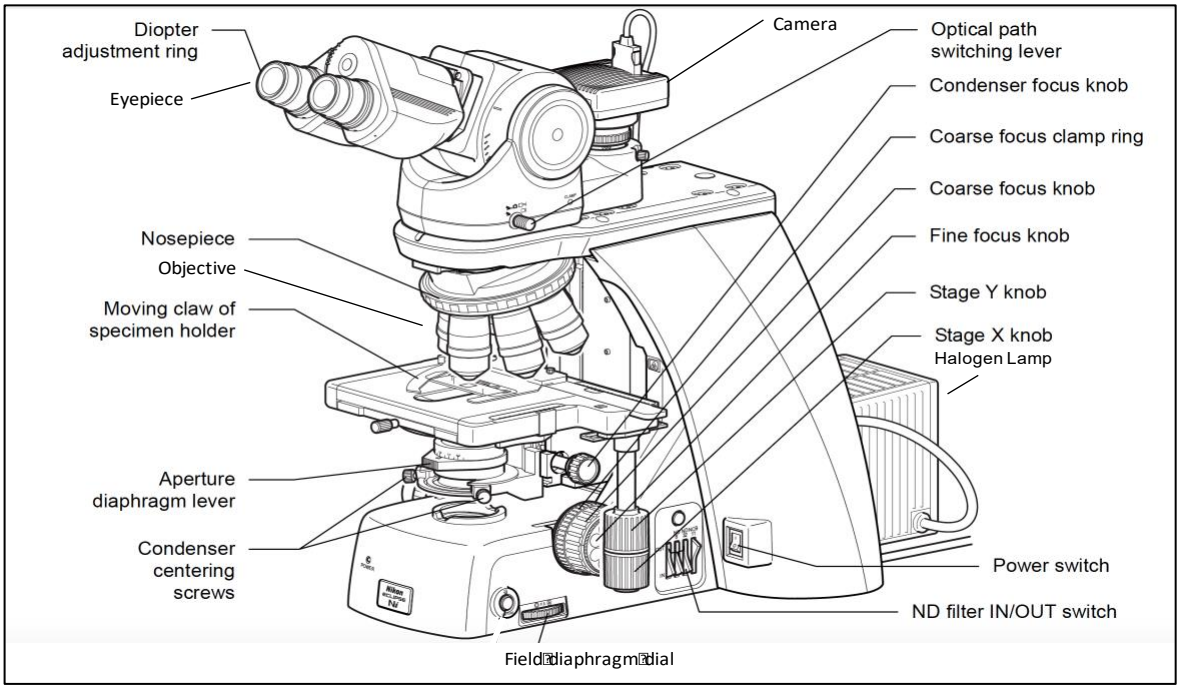
Bright-field Microscopy

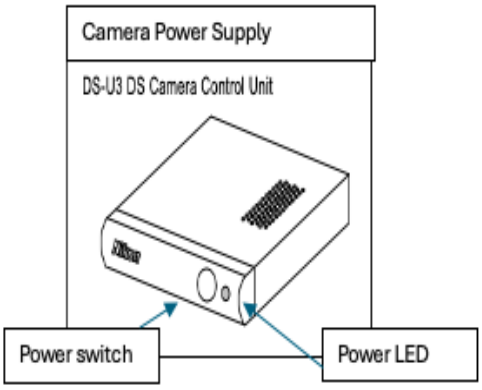
Bright field is the most basic microscopy technique and uses illumination that is passed through your specimen. How the light path is modified (reflected or absorbed) as it passes through your specimen generates the image.

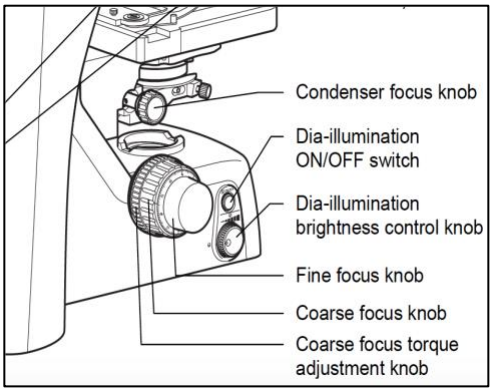
The light path of a bright-field microscope is straightforward (see figure below) with a light source (a halogen lamp) to illuminate the specimen from below, a condenser lens to focus the light on the specimen, an objective lens to provide magnification and collect light from the specimen and an ocular 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



Microscope Detailed Images







Bright-field Microscopy Adjustment

1. Turn on the power.

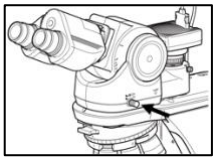
   The Power switch is a rocker switch on the rear portion of the Ni-U main body, on the right side (your right side as you are facing the scope). When you have been successful you will see the Power LED light on the front of the scope illuminated.

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.

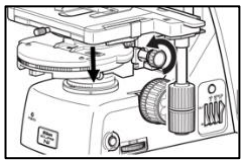
3. Set the optical path of the binocular tube to receive 100% of light to the eyepiece

   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.



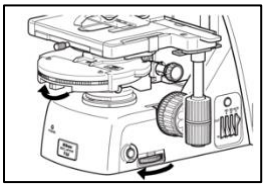
4. Lower the condenser slightly from its uppermost position.

   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.



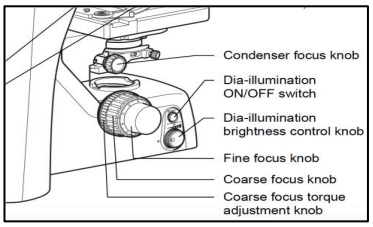
5. Fully open the field diaphragm and fully close the aperture diaphragm.

   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. Adjust Dia-illumination.

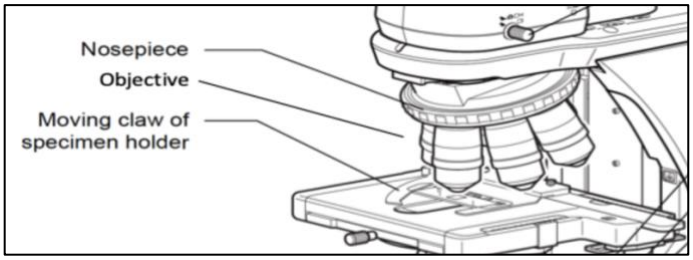
   The Dia-illumination brightness control knob is just below the Dia-illumination button switch. This may need to be adjusted to an intensity that is comfortable for your eyes as you move through the next adjustment steps.



7. Begin with the 4x Objective.

   Bring the desired objective into place by rotating using the nosepiece (it will click into place). Rotate the nosepiece to set the 4x 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.



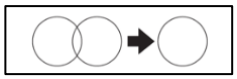
8. Focus on the sample

   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.

9. 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.



10. Diopter Adjustment.

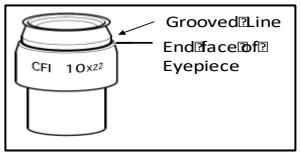
    The diopter adjustment ring on each eyepiece can be adjusted to match the vision in each eye.

    - 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.

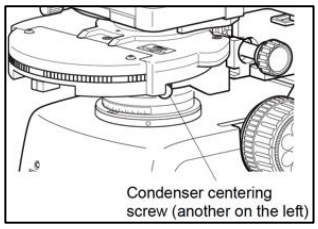
    Note: To avoid using your left eye it is a good idea to cover it with a piece of paper. Do not press on your eye as this changes the shape of your lens!

    - 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.



11. 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.

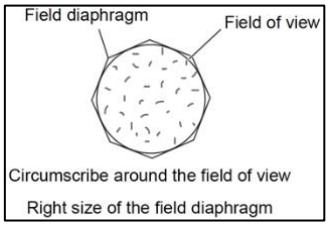
12. Choose your objective and adjust the aperture diaphragm

    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.

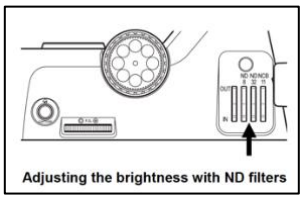
13. Adjust the field diaphragm

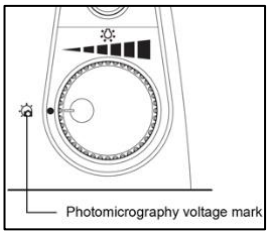


It is important that you adjust the Field Diaphragm for each objective used (See step 5 above). With your sample in focus, turn the field diaphragm dial just to the point where is circumscribes the field of view. Opening the Field diaphragm too far beyond the field of view will compromise your image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

14. 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.

15. 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.

Image Acquisition

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

    a. Turn on the computer.
    b. Turn on the camera.

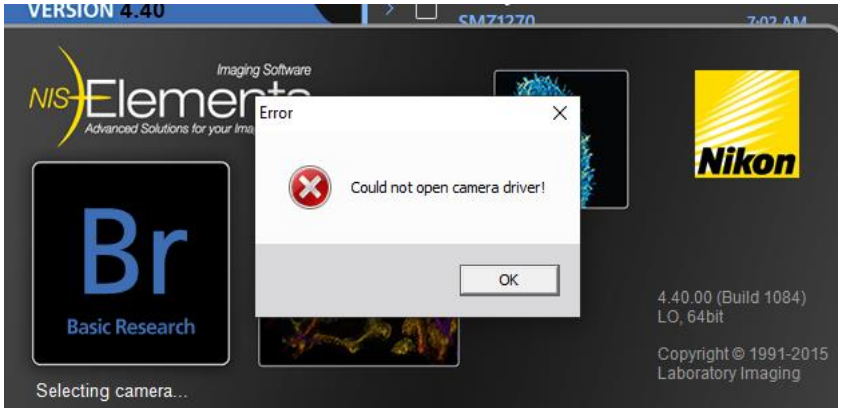
2. Push button located on camera power supply – to the left of the microscope. Power LED at front will light when powered on.

    a. Set the optical path of the binocular tube to distribute 100% to the camera. This is accomplished using the optical path switching lever.

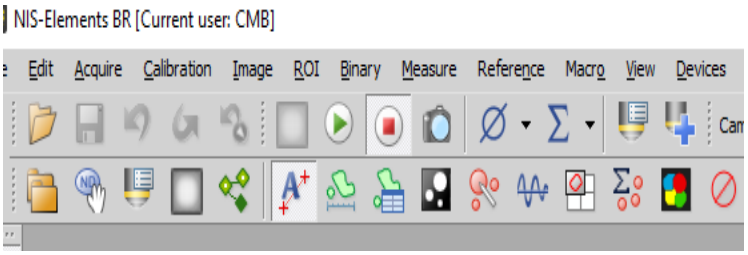
        - [PHOTO] 100% of the light will pass through the binocular tube to the camera pulled out 2 notches.
        - [BINO/PHOTO] When the lever is pulled out by 1 notch, 80% of the light will pass through the binocular tube to the camera and 20% will pass to the eyepiece.
        - [BINO] Lever is pushed in

3. Open the NIS-Elements microscope imaging software

If you open the NIS elements software before camera is fully initialized an error code will pop up (see image below). If that happens, close the error code, exit the NIS elements software and reboot software after camera is powered on.



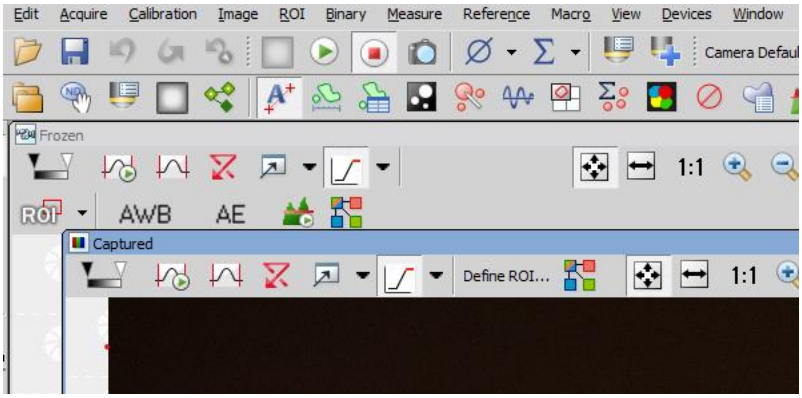
4. Once software is initialized, to capture an image, you will select the Play button located in the top tool bar.



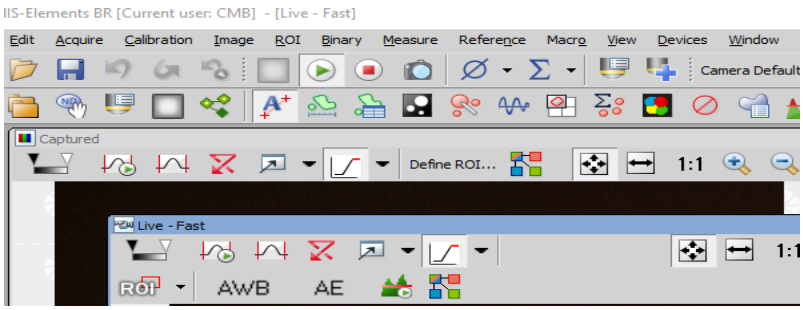
5. This will populate an interior window within the NIS Elements software. The top left of that window will state “Live-Fast” to indicate active camera/live feed of your specimen.

6. 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:

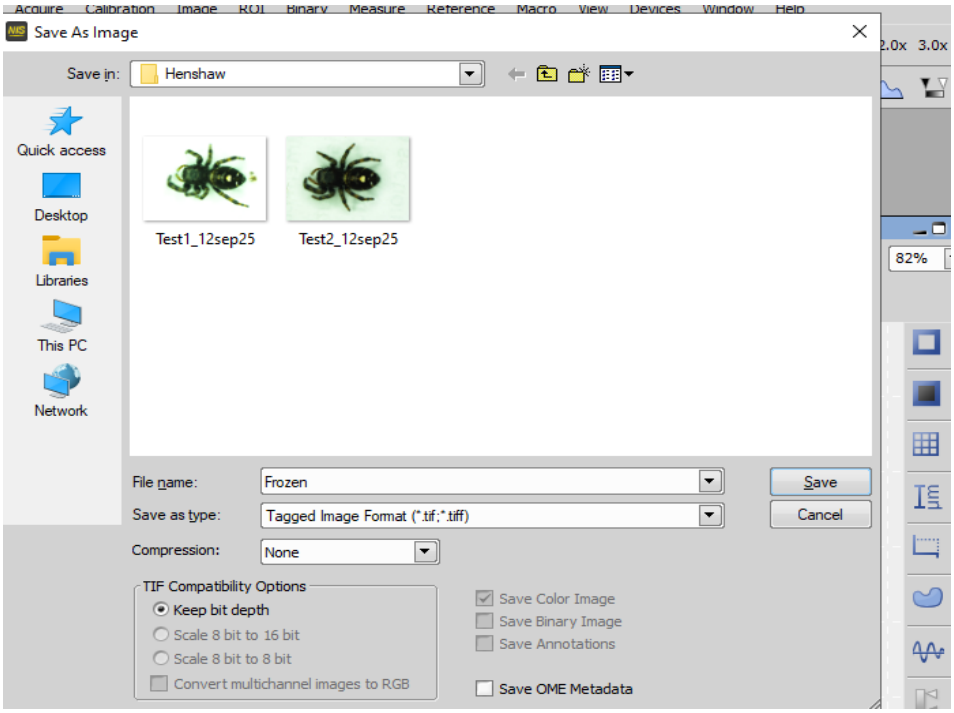
    a. “Frozen” – this is the camera window, the stop icon  is highlighted.
    b. “Captured” – this is your image.



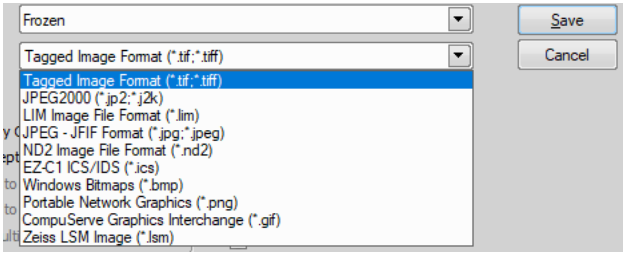
7. To return to your live view, press the play button. The two windows status will be as follows (pictured below):
    a. "Captured” – image captured
    b. “Live-fast” – live view of specimen



8. To save image: go to File > Save as Image



Pay attention to the file format you are saving your image as. The most popular for after capture image analysis is .Tiff and .Jpeg. If uncertain, ask your PI for their preferred method.



9. Note that there are preprogrammed settings for each magnification selection on the zoom body. It is recommended to verify the settings before image acquisition as these are calibrated to provide accurate measurements/annotations later

10. Additional Image Analysis information can be found in the NIS-Elements handbook. There is a copy on each computer with detailed instructions.

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.
10. Cover the microscope.

Technical Specifications

- Microscope Make: Nikon
- Microscope Model: Eclipse Ni-U
- Objectives:
    - 4x: Plan Fluor 4x/0.13 ∞/- WD 17.2
    - 10x: Plan Fluor 10x/0.30 ∞/0.17 WD 16
    - 40x: Plan Fluor 40x/0.75 OFN25 DIC M/N2
    - 100x OIL: 100x/1.30 OIL ∞/0.17 WD 0.16
- Condenser: Achr 0.8 (Aperture 0.0 – 0.8)
- Eyepieces: CFI 10x/22
- Filters:
    - NCB-11
    - ND-8
    - ND-32
- Camera 1:
    - Color camera
    - Make: Nikon
    - Model: DS-Fi2
    - Pixel resolution: 5.0 megapixel maximum resolution 2560x1920 pixels
    - Frame rate: Up to 21 fps (full resolution) or higher in lower resolutions
- Camera controller:
    - Make: Nikon
    - Model: DS-U3
- Software: NIS ELEMENTS
    - Manual available on desktop of units for image acquisition tutorials