MICROSCOPE 2

Nikon SMZ18 Stereomicroscope

TRAINING GUIDE

Instruction for use of Microscope 2. Detailing the techniques available, location of supplies, and requirements for use of this shared resource

3364G KHS Advanced Microscope Suite

Prepared by 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 straight away.

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

Microscopy Techniques

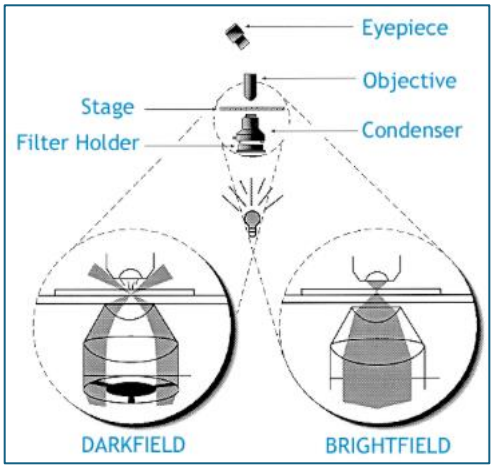
Bright-Field is the most basic microscopy technique and uses illumination that generates a path of light that will pass through your specimen. How the light path is modified (reflected-episcopic or transmitted -diascopic) as it passes through your specimen generates the image.

Reflected (Episcopic) Illumination versus Transmitted (Diascopic) illumination

Opaque specimens most commonly are best viewed using reflected light, while translucent and transparent specimens typically provide the best results under some variation of transmitted illumination. This is not always the case, other variables should be taken into consideration while forming a lighting strategy, including the following:

- Basic physical characteristics of the specimen (geometrical profile, topography, morphology, etc.)
- Type of information looking to extract from the examination of the specimen
- Photographing requirements
- How the information will be used

Dark-Field restricts the illumination (light path) in such a way that only the light that is scattered by your specimen is used to generate an image.



Using Darkfield Microscopy To Enhance Contrast:
An Easy and Inexpensive Method
https://public.wsu.edu/~omoto/papers/darkfield.html

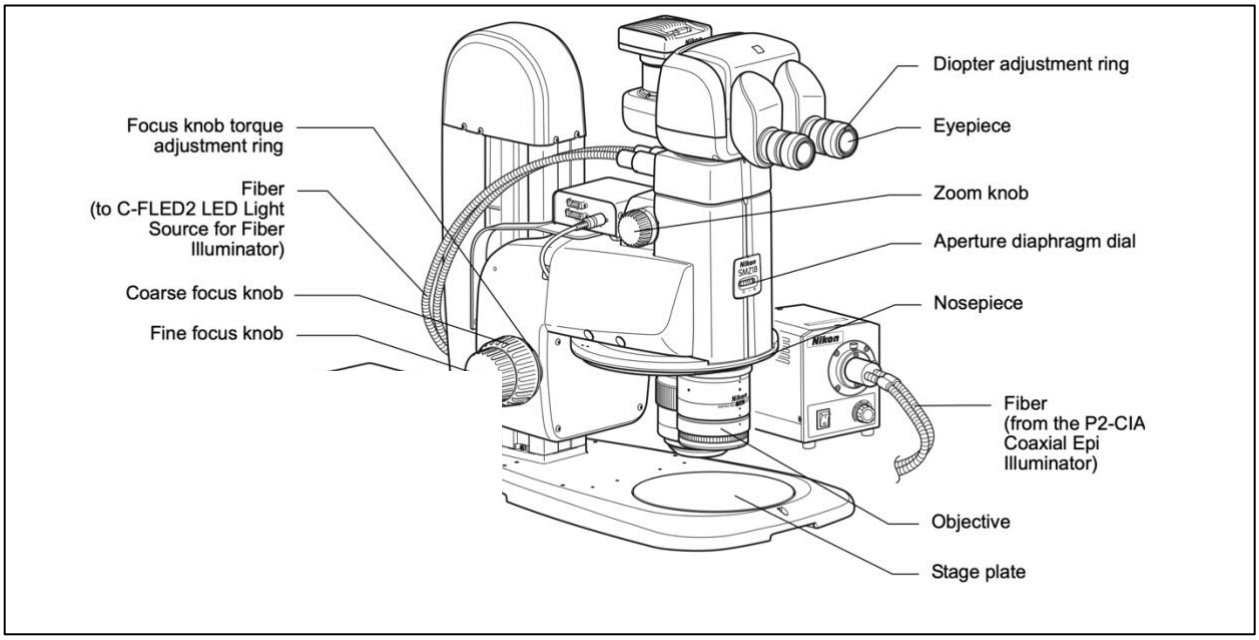
Oblique Coherent Contrast (OCC) illumination

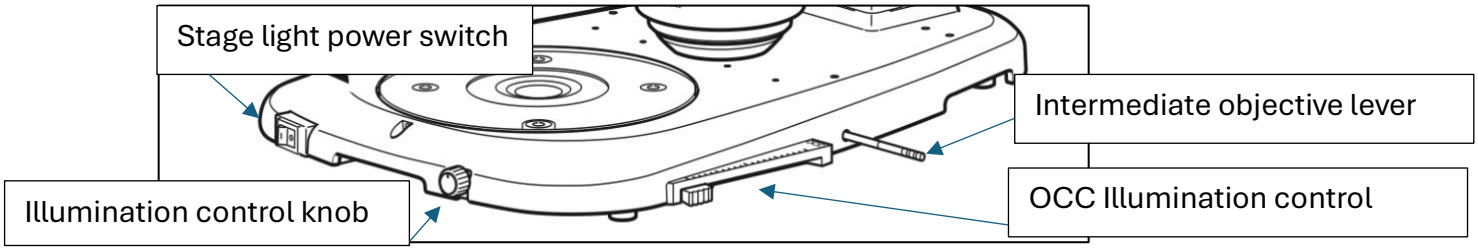
The OCC illuminator utilizes a sliding diaphragm to optimize contrast in bright-field to dark-field illumination configurations. Utilizing the axial light (ring light) the diaphragm position can be moved to operate within Bright-field through various oblique settings to Dark-field microscopy. This can allow viewing of changes in the specimen appearance to highlight varying features.

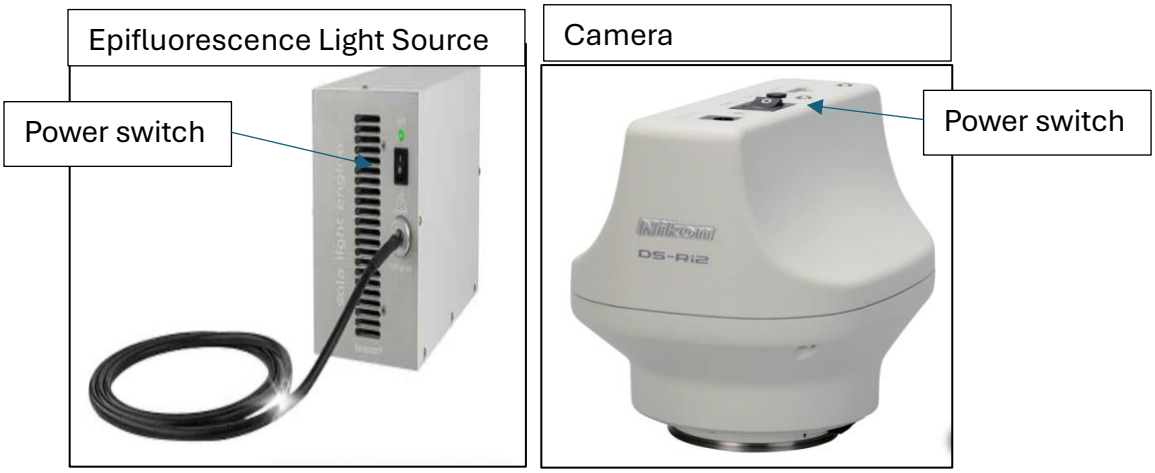
Epi-fluorescence

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-fluoresecence) 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.

Detailed Microscoped Images



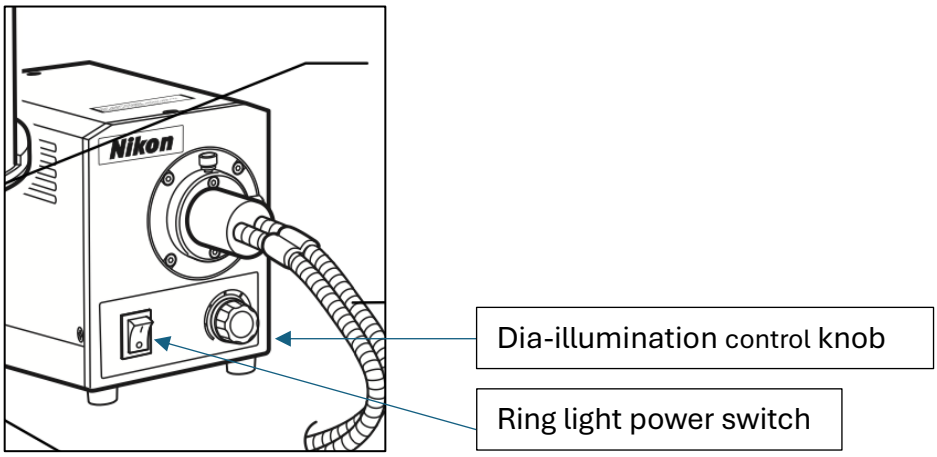




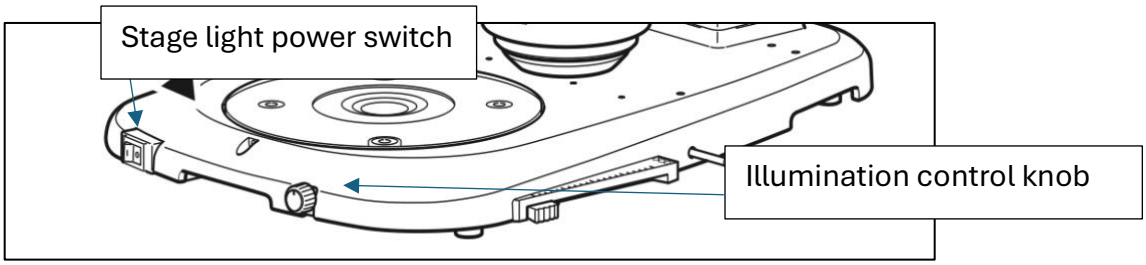
Brightfield Microscopy Adjustment

1. Turn on the power to the light source of your choosing – ring light (episcopic) or stage light (diascopic)

    - Ring light power supply is located to the right of the microscope. Power switch is a rocker switch next to the knob for illumination brightness control.

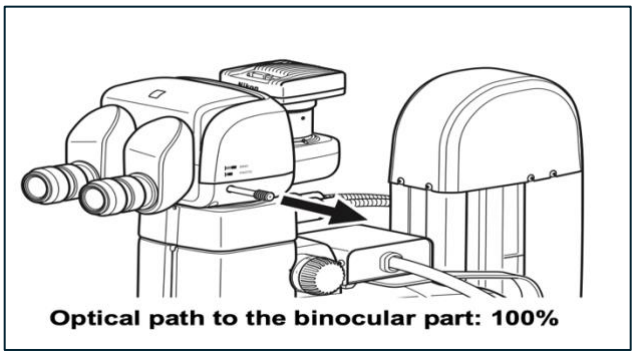


    - Stage light is powered by a rocker switch located on the left-hand side of the base of the stage. The brightness adjustment knob is located to the right of the power switch.



    Note: If no light is present, check the OCC slider (on the right-hand side of the stage) to make certain it is not set to darkfield setting and blocking the light source.

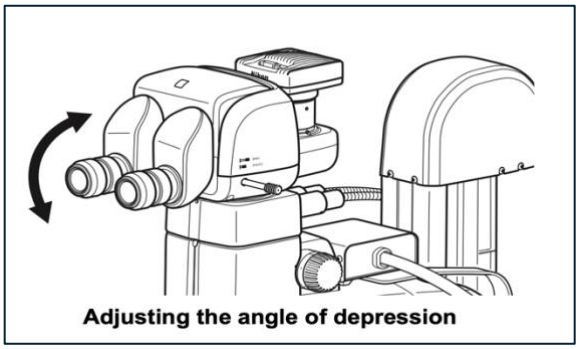
2. Set a sample on the stage
3. Adjust the brightness of the light source
4. Set the optical path to 100% to the binoculars/eyepieces



Optical path to the binocular part: 100%

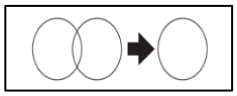
5. Adjust the depression angle of the tilting tube for comfort. The eyepieces have a singular 360 rotation for sitting/standing.

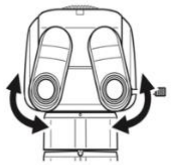
   This mechanism moves freely, if you need to add force – you are rotating it incorrectly or there is an issue with the mechanism.



Adjusting the angle of depression

6. Adjust the interpupillary distance



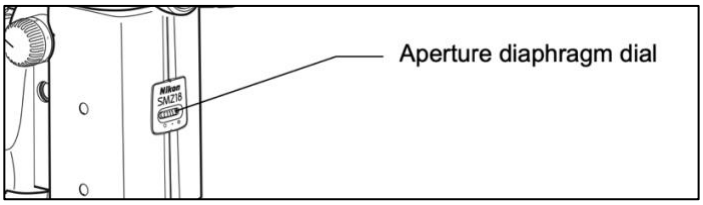


Adjusting the interpupillary distance

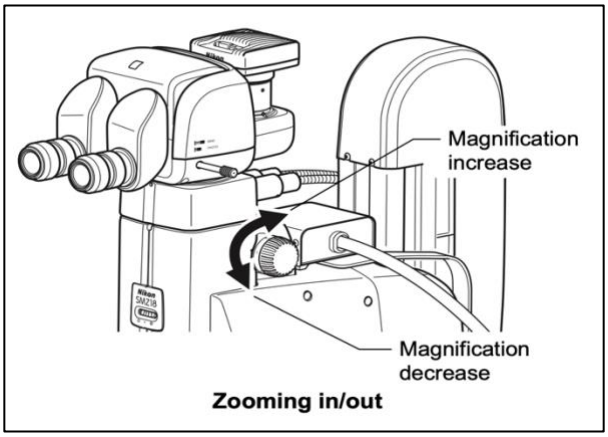
   When you have the specimen in focus, get the best image possible by looking into the eyepieces using both eyes. Adjust the binocular head such that the distance between your eyes allows the field of view from the left eye and the right eye to coincide. It helps to pretend you are looking into the distance.

7. Adjust the aperture diaphragm dial – this allows users to adjust image contrast and depth of field.

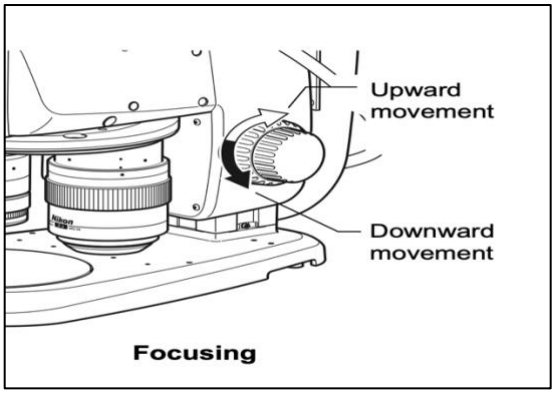
The smaller the zoom magnification the smaller the aperture diaphragm should ideally be. For example, if you are using setting 13.5x on the zoom body, you would likely begin with the aperture fully open. If you are beginning at



8. Change the magnification using the zooming knobs



9. Adjust the focus using the coarse and fine focus knobs



10. Adjust the diopter of the eyepieces once your specimen is in focus.

    Bring the highest zoom body magnification into position and refocus, then return to the lowest magnification position. Without adjusting focus with the fine and coarse focus knobs – utilize the eyepiece diopters.

One eye at a time, start with your left eye (cover the right eyepiece with a piece of paper to prevent squinting) rotate the collar on the eyepiece until you can clearly view the specimen.

Repeat by covering the left eyepiece and viewing through the right eyepiece and adjusting the eyepiece until that eye focuses.

Then look through with both eyes.

11. View your specimen

Darkfield/OCC Microscopy Adjustment

It is generally recommended to set up the microscope in Brightfield to reduce eyestrain and find your specimen.

1. Power off the Episcopic light source (ring light) via the power rocker switch located on the power supply to the right of the microscope.

2. Power on the Diascopic light source (stage light) via the rocker switch at the front left of the stage of microscope

3. Adjust the Illumination intensity via the knob at the front right of the stage of the microscope

4. If no illumination is present – verify that the OCC slider is not obstructing the light.

5. To obtain the best image, adjust the location of the slider to highlight the areas of the specimen you are looking at.

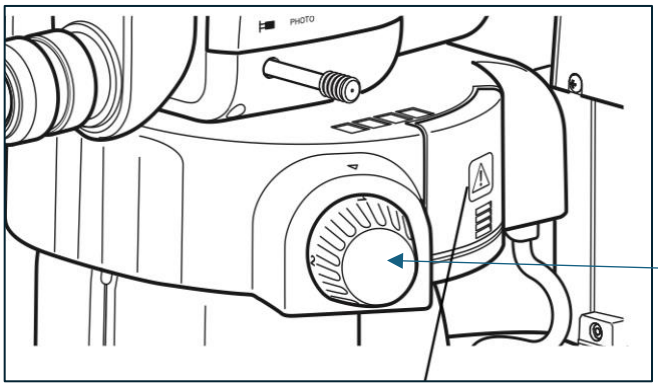
Epi-fluorescence microscopy

It is strongly recommended to start with Bright-Field setup before advancing to Epi-fluorescence as these steps optimize settings for viewing.

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 look through the orange guard 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

12. Turn off the diascopic and/or episcopic LED illuminmation
13. Bring the desired filter cube into the optical path



- Position 1: Empty
- Position 2: DAPI Exciter: ET395/25x; Dichroic: T425lpxr; Emitter: ET460/50m
- Position 3: EGFP (FITC/Cy2) Exciter: ET470/40x; Dichroic: T495lpxr; Emitter: ET525/50m
- Position 4: DsRED Exciter: ET545/30x; Dichroic: T570lp; Emitter: ET620/60m

14. It is recommended to turn off the overhead (room) light to view the fluorescence of your sample more easily. There are 4 recessed lighting options located under each upper cabinet in the room to facilitate this.

15. There is no “shutter” on this unit, so be mindful of how much exposure your sample is receiving. You can close the aperture diaphragm to decrease the amount of light reaching your sample.

16. Turn on the SOLA illuminator, located to the left of the microscope.

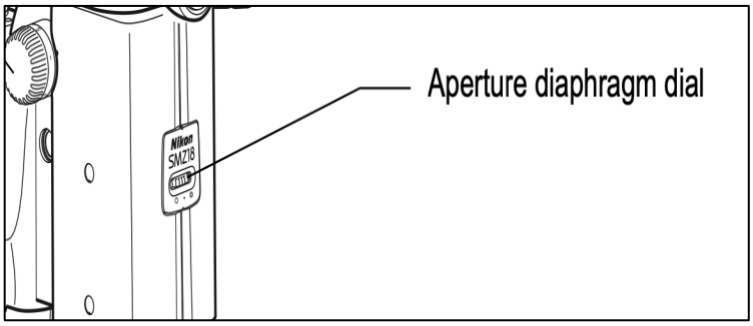
    To turn ON, use the rocker switch on the front of the illuminator.

    It is critical that the LLG be kept safe so that it is not bent, kinked or pinched! 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!

    DO NOT turn off power switch at back of unit, this switch operates the units' cooling fan and will remain on after use to protect the unit from overheating. This switch should always be on.

17. Adjust the aperture diaphragm dial – this allows users to adjust image contrast and depth of field.

    The smaller the zoom magnification the smaller the aperture diaphragm should ideally be. For example, if you are using setting 13.5x on the zoom body, you would likely begin with the aperture fully open. If you are beginning at



18. View your specimen
19. In between analysis, to preserve fluorescence, switch off the fluorescence light on the sola light engine.

Image Acquisition

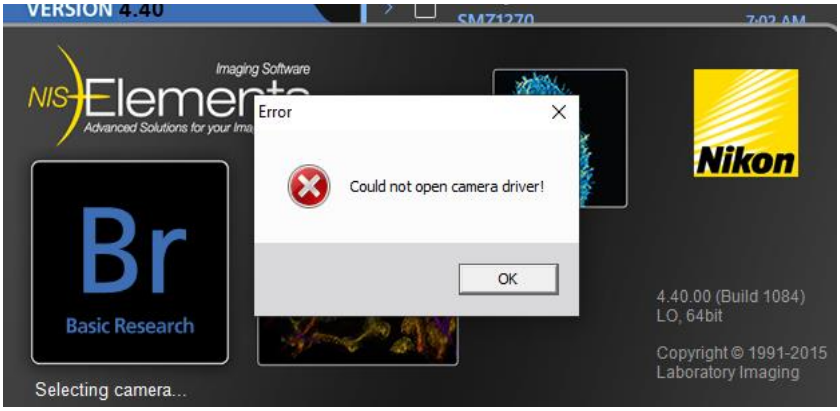
All Microscopes in the Advanced Microscope Suite operate on the same acquisition software: NIS Elements BR (Basic Research)

Acquisition Software:

NIS ELEMENTS - BR

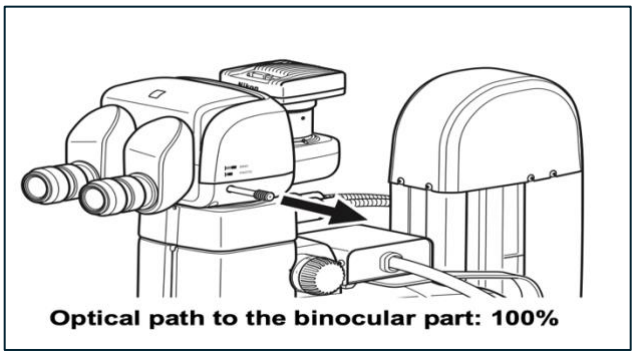
1. To acquire an image power on the computer associated with the microscope
2. Power on the camera (power switch is a rock switch at the top of the camera – located above the head of the microscope)

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



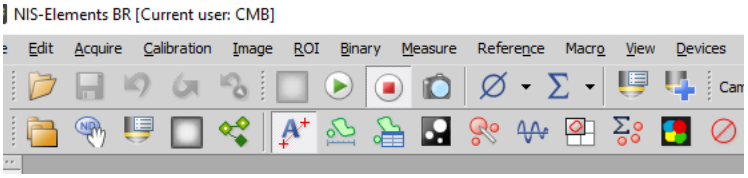
   If this pops up, close NIS-Elements and verify camera(s) are on and then relaunch NIS-Elements

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

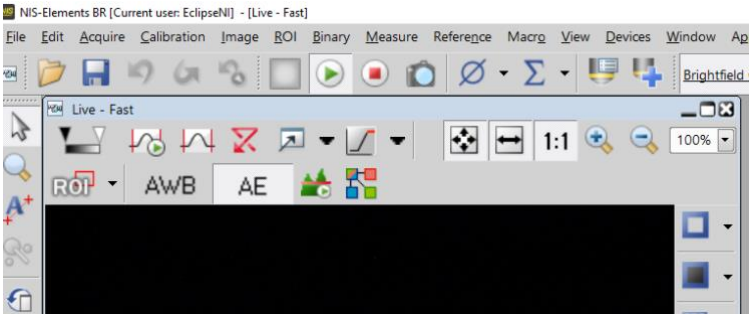


- Lever pulled OUT: 100% light to eyes
- Lever pushed IN: 100% light to camera

4. Open NIS-Elements (found on the desktop of the computer)
5. Once software is initialized, to capture an image, you will select the Play  button located in the top tool bar.

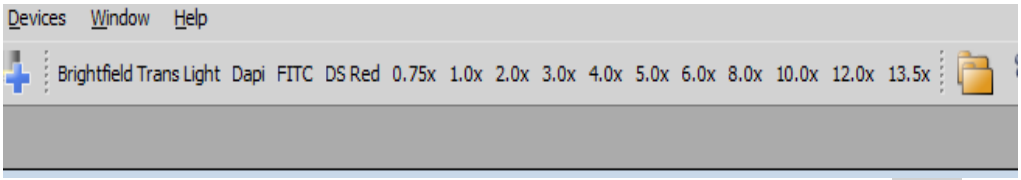


6. This will populate an interior window within the NIS Elements software. The top left of that window will state “Live-Fast” to indicate active camera/live feed of your specimen.

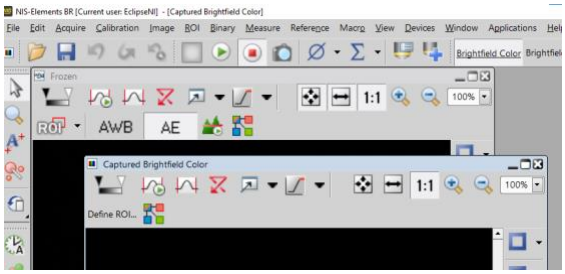


7. Verify settings prior to image capture. There are calibrated options provided at the top of the page for each objective and filter options. Correct selection will make image analysis later (i.e. measurements) easier.

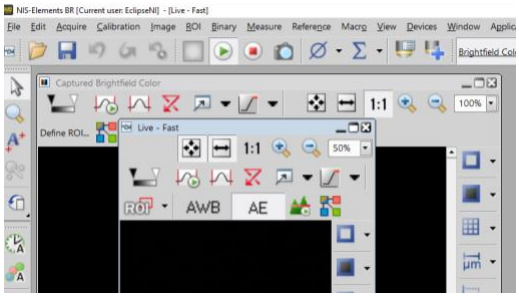
   There are many options to improve image quality – consult the NIS-ELEMENTS guide located at the desktop of each computer for more detailed information.



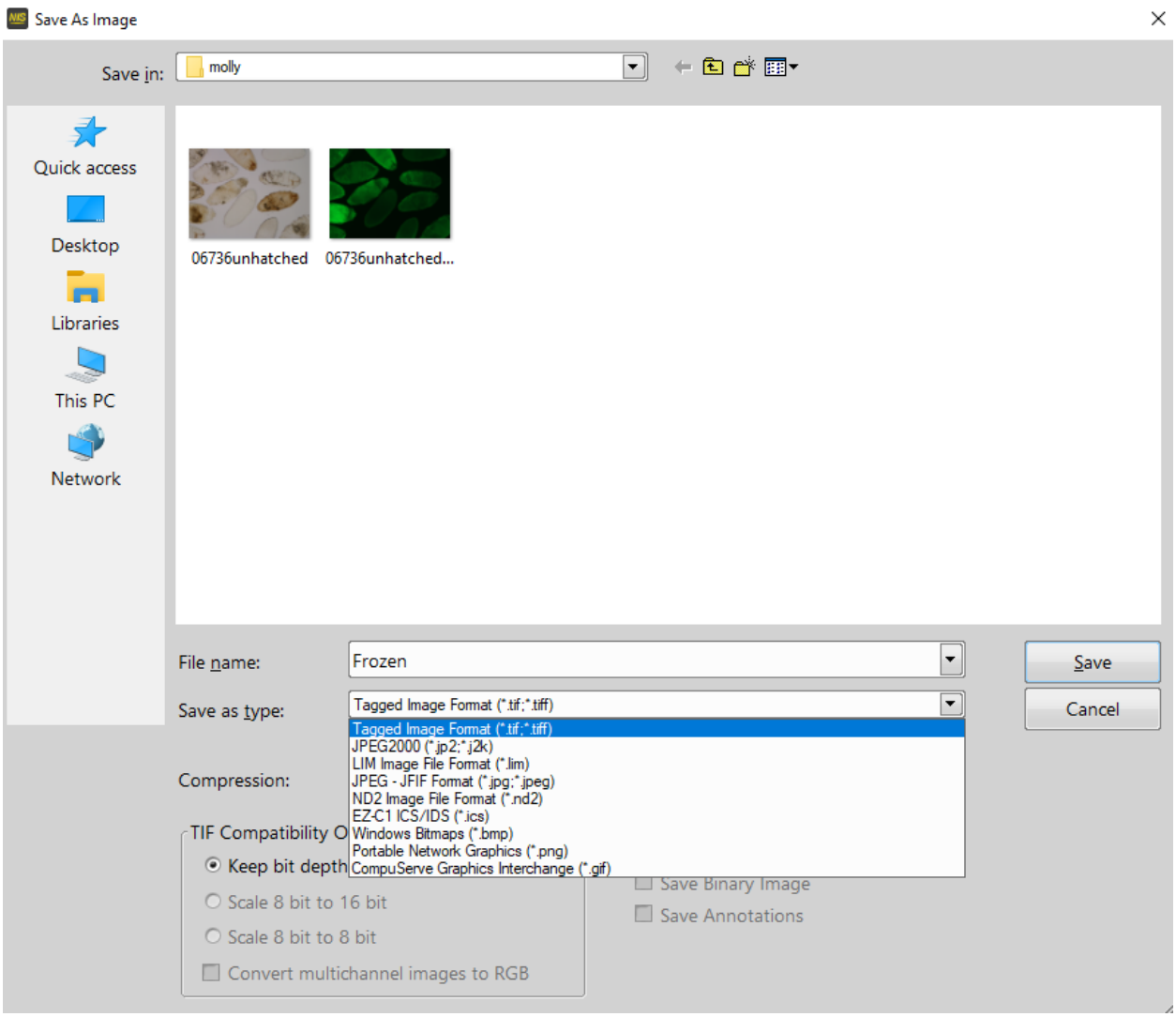
8. To capture an image from “Live-Fast” select the “capture” button  . This action will open a second interior window within NIS – Elements (as pictured below). Viewing the top left corner of each window:
    a. “Frozen” – this is the camera window, the stop icon  is highlighted.
    b. “Captured” –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



9. 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



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



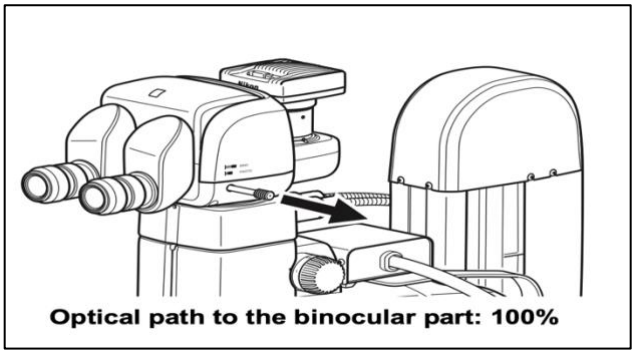
Remember this is a shared resource, so be mindful of where/how you store your data so you can easily find your data again later. There are multiple login options, make sure you know which profile you are logged in under.

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

11. 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

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



3. Return the diopter adjustment rings to their reference/zero position.
4. Turn of Bright-field light sources
    a. Check that both the ring light and stage light are powered off
5. If using epi-fluorescence accessories
    a. Switch off SOLA illuminator, only the front power switch. Verify that the secondary fan power is still on at the back of the unit.
    b. Rotate fluorescence turret to empty position (position 1) to remove fluorescence cube from light path.
6. Cover the microscope

Technical Specifications

1. Microscope Make: Nikon
2. Microscope Model: SMZ18 (18:1 zoom ratio)
3. Microscope Objective – SHR Plan APO [1x] WD: 60
4. Intermediate selector lever objective options (stage right hand side)
    a. IN: 1.6-2x
    b. OUT: 0.5-1x
5. OCC Illuminator Slider
6. Zoom body range: 0.75x – 13.5x
    a. 1
    b. 2
    c. 3
    d. 4
    e. 5
    f. 6
    g. 8
    h. 10
    i. 12
    j. 13.5
7. Light source
    a. Ring light
        i. Nikon
        ii. C-FLED2 power supply
        iii. Ring light P2-F1R
    b. Epifluorescence
        i. SOLA Light Engine
        ii. SOLA SM5-LCR-VA
8. Eyepieces: C-W 10xB/22 Nikon
9. Fluorescence cubes
    a. Position 1: Empty
    b. Position 2: DAPI
        i. Exciter: ET395/25x
        ii. Dichroic: T425lpxr
        iii. Emitter: ET460/50m
    c. Position 3: EGFP (FITC/Cy2)
        i. Exciter: ET470/40x
        ii. Dichroic: T495lpxr
        iii. Emitter: ET525/50m
    d. Position 4: DsRED
        i. Exciter: ET545/30x
        ii. Dichroic: T570lp
        iii. Emitter: ET620/60m

10. Camera
    a. Make: Nikon
    b. Model: DSRi2
    c. Color camera
    d. Frame rate: 6 fps at full 4908x3264 resolution (up to 45 fps at 1636x1088) with ISO sensitivities from 200 – 12800
    e. Pixel size: 7.3 μm x 7.3 μm
    f. Exposure time: 0.1 ms to 120 sec
    g. Sensor: 16.25 megapixel FX-format (full0frame) CMOS