MICROSCOPE 3

Nikon TS100 Inverted Compound Microscope

TRAINING GUIDE

Instruction for use of Microscope 3. Detailing the techniques available, location of supplies, and requirements for use of this shared resource

3364G KHS Advanced Microscope Suite

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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, 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 problems

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

Note: This unit does not have a computer for capturing images.

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 (step 10 below)

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.

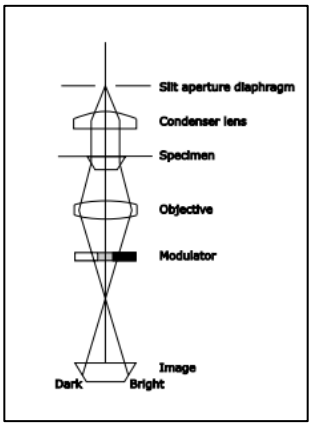
NAMC (Nikon Advanced Modulation Contrast) Microscopy:

NAMC is used to visualize changes in phase across a sample creating a pseudo 3D image. This technique is similar to Differential Interference contrast (DIC), but NAMC does not require polarizing light. NAMC works through plastic and glass (petri dishes/slides with covers) which often depolarizes light creating halo effect and/or shade-off artifacts with large samples features.

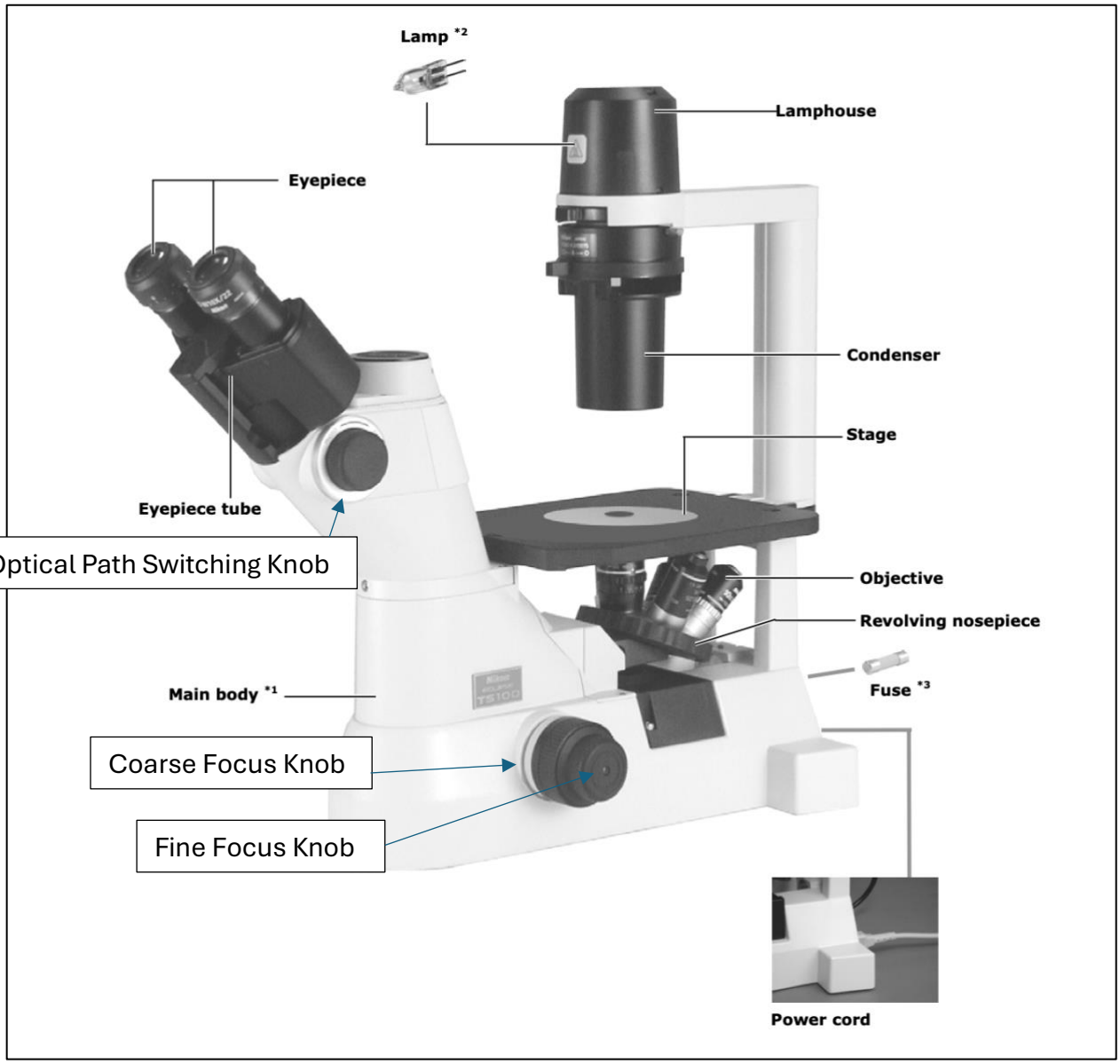
See figure below for the principle of the NAMC technique. There are 2 key components for this technique:

1. Slit diaphragm on the condenser aperture (slide)
2. Modulator inside the NAMC objective.

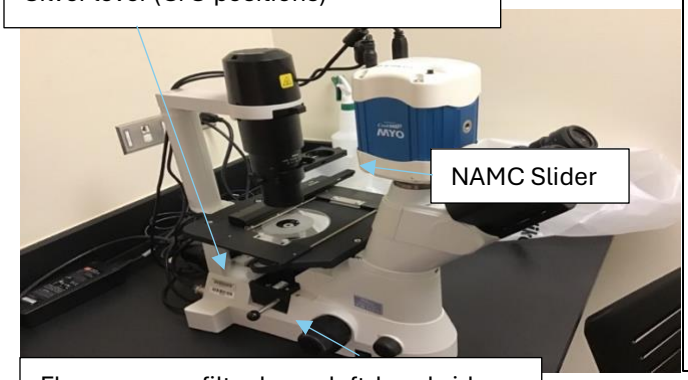
The modulator is a density filter placed at the exit pupil of the NAMC objective, it divides the exit pupil into 3 regions, dark, half-dark and transparent. In NAMC the image appears in relief just like in DIC (differential interference contrast). There is no influence of double refraction, thus enabling viewing of specimens without the need of a polarizer.



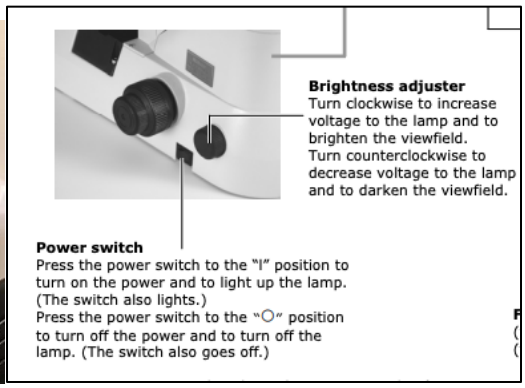
Microscope Detailed Images



Fluorescence shutter
Silver lever (C/O positions)

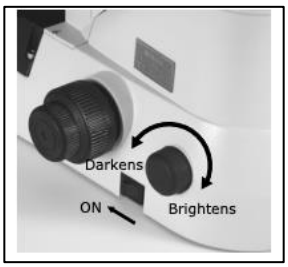


Fluorescence filter lever- left-hand side
Right-hand filter lever not pictured



Bright-field Adjustment

1. Turn on the power
    a. Power switch is at the left-hand side base of the instrument. When powered on the rocker switch will light up green.

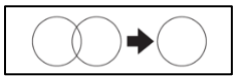


2. Adjust the light intensity
    a. Dia-illumination knob is located above and slightly forward from power switch. Rotating away from you will decrease light intensity, towards you will increase light intensity.

3. Verify the optical path switching knob is in the BINO position
    a. Rotate toward wall => BINO
    b. Rotate toward yourself =>PHOTO

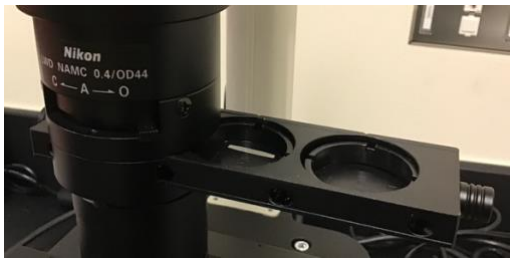
4. Adjust the interpupillary distance

    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.



5. Remove the NAMC slider

    a. Located in the condenser, the NAMC slider is pictured in the image below. To remove, unscrew one of the black thumb screws at the left or right end of the slider and pull out of the condenser.



6. 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 cause cross threading, use the nosepiece as identified in the detailed microscope images page.

7. Focus on the sample

   a. Place a sample on the stage.
   Turn the Coarse Focus knob to raise the objective turret to its upper most limit. Look into the eyepiece and then lower the objective turret using the Coarse Focus knob until you can see your specimen.

   To lower the objective turret, turn the knob away from you (toward the wall). 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 objective turret. Remember that this unit is inverted, so pay attention to the height of the objectives so you do not accidentally damage them by coming into contact with your specimen on the stage.
   - Never rotate the right and left focus knobs in opposite directions.

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

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

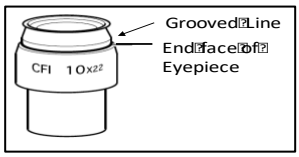
   - Turn the nosepiece to bring the 40x objective in place and focus on your specimen (use the Coarse focus knob if needed then the fine focus knob)

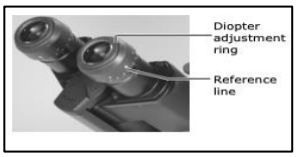
- Return to the 10x objective and look into the right eyepiece with your right eye.

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.





9. Adjust the aperture diaphragm for the objective. Located in the condenser above the slider. (C = closed; O = open)

It is important that you adjust the Aperture diaphragm for each objective used as this affects resolution, contrast, focal depth and brightness of your image. 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.

For example, the 10x objective has a NA of 0.25 and therefore the recommended setting for the aperture diaphragm is 0.175-0.2. Note that the condenser aperture diaphragm has a maximum 0.4 NA, there are no notches on this aperture diaphragm so keep this number in mind.

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.

10. View your specimen

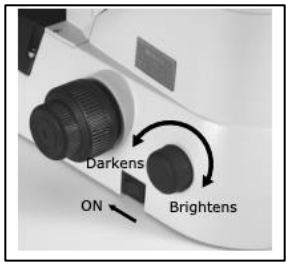
Epi-fluorescence Adjustment

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 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.

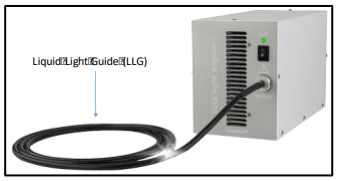
1. After finding specimen using Bright-field. Turn off the Bright-field light source as indicated in the image below.



2. Verify that the path to Epi-fluorescence is closed via the shutter at the back of the microscope.

3. Power on the sola light engine.

   To turn ON, use the toggle 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.

Do NOT turn off the fan power switch at the back of the unit – this will continue to operate after power at the front is powered off. The unit will not power on if this switch is powered off.

4. Bring the desired filter cube into the optical path by pushing in the fluorescence lever for that filter cube. These levers are located on the left and right side of the microscope under the stage.

    a. Left hand side – FITc/Cy2
        i. EX 465-495
        ii. DM 505
        iii. BA 515-555

    b. Right hand side – TR/mCherry
        i. EX 540-580
        ii. DM 600
        iii. BA 605-695

5. It is recommended to turn off overhead lights and utilize the light sources under the cabinets to obtain the best view of the specimen.

6. Open the shutter and view the specimen. The shutter lever is located at the rear base of the microscope (silver lever)

    Photo-bleaching – fluorescence microscopy requires image setup and analysis quickly as photo-bleaching can occur if specimen is exposed for extended periods.

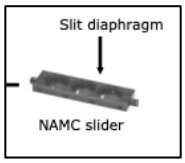
Be mindful of this and remember to shutter the light source when not viewing/capturing images.

NAMC Adjustment

1. Verify aperture diaphragm is fully open. Always fully open the aperture diaphragm, if the aperture diaphragm is closed it will obstruct the slit diaphragm and the proper image cannot be obtained

2. Place the 10x NAMC objective (NAMC1) in the optical path
    a. Turn the revolving nosepiece by gripping the black base plate and not by gripping the objectives as this can lead to cross-threading of the objectives.

3. Slide the NAMC slider to place the NAMC1 slit diaphragm in the optical path



4. Turn the modulator ring on the objective to orient the modulator pattern in the direction in which you wish to apply contrast.

5. Select desired objective (10x, 20x, 40x). If the objective has a correction ring, verify setting matches container in use.
    a. A specified cover glass thickness is indicated on each objective. ("∞ /0.17" indicates a cover glass thickness of 0.17 mm.)
    b. An objective with a 1.2 mark is for a slide glass with thickness of 1.2 mm
    c. Correction collar (manual ring) that compensates for the thickness of the petri dish bottom to eliminate spherical aberration.

6. Select the corresponding NAMC slider position to the selected objective
    a. NAMC1 (10x)
    b. NAMC2 (20x)
    c. NAMC3 (40x)

7. Careful to not alter the position of the correction ring on the objective (if any), turn the modulator ring on the objective to orient the modulator pattern in the direction in which you wish to apply contrast.

    It is easiest to adjust the modulator if you grip the nosepiece with one hand and the modulator ring with the other hand. Adjust while you view the changing contrast.

8. View specimen

Image Acquisition

There is no computer for the microscope currently.

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 TS100 microscope to its default settings

1. Turn off accessory equipment (camera & computer) – this unit currently does not have a computer.
2. Rotate the optical path switching lever so that 100% of the light is directed to the eyepiece
3. The microscope stage should be in its most centered position
4. Remove all filters (Fluorescence levers) from the optical path
5. Power off the Epifluorescence light source (sola light engine)
    a. Front Light switch OFF
    b. Back rocker switch ON at all times (operates cooling fan which will automatically shut off when unit is cooled)
6. Return the lowest objective to the optical path
7. Return the diopter adjustment rings to their reference position
8. Return NAMC slider if removed from optical path
9. Turn the Dia-illumination brightness control knob clockwise until it is at its lowest illumination setting.
10. Turn off Dia-illumination. Accomplished by switching off the rocker switch.
11. Cover the microscope

Technical Specifications

(1) Make: Nikon
(2) Model Name: TS100
(3) Optical System: CF160 (Infinity-corrected CF optical system)
(4) Eyepieces: 10x Field number: 22 (C-W10XB/22) with 0 setting
(5) Microscope magnification:
    a. 10x/0.25 ∞/1.2 WD 6/2 NAMC1
    b. LWD 20X/0.40 ∞/1.2 WD 3.1 NAMC2
    c. LWD 40x/0.55 ∞/0-2WD 2.7-1.7 NAMC3 (correction collar)
(6) Condenser: LWD NAMC 0.4/ OD 44
(7) Epi attachment: T1-FM
(8) Fluorescence filters
    a. Right lever – TR/mCherry
        i. Ex. 540-580 DM 505 BA 605-695
    b. Left lever – FITC/Cy2
        i. Ex. 465-495 DM 505 BA 515-555
(9) NAMC Slider – 3 positions (no empty/open for brightfield will need to remove)
(10) Camera: Photometrics CoolSNAP – MYO
    a. 1940 x 1460 imaging array 4.54 x 4.54 μm pixels
    b. High resolution, high sensitivity camera for moderate to low-light life science applications