

Plan ahead and sign up on the GOOGLE calendar shared with you for when you plan on using the microscope. Include your cell phone number in case you need to be contacted if there is a problem.

Nikon Eclipse E800 Epifluorescent Microscope

Before turning anything on, ask yourself if you will need the epifluorescence from the LED lamp to view your sample.

We have the capability to view blue, green, and red fluorophores with the epifluorescent filters.

SETTING UP

- **FIRST, SIGN INTO THE LOG BOOK**

- If you will use the LED lamp, turn on this power supply switch in the front of box (indicator light will already be on – this switch is in the back and should always be on).
- If you will be using transmitted light from below the stage, turn on the microscope power box (below the LED lamp power supply box)
- Turn on the fine focus box to the left of the microscope.
- If you are using the camera (Zeiss 503 color) attached to the microscope to take pictures of epifluorescence
 - turn on the mini PC.
 - Open ZEN (blue edition)

VIEWING AN IMAGE FROM A SLIDE

- Ensure that the optical path is open to the binocular eyepieces using the manual slider bar located at the top right of the microscope. (in to eyes, out to camera – diagram reads IN OUT/VTF) – left and right levers must be out.
- Place a slide on the stage under 10X and use the course adjustment knob to focus.
- View under white light DIA.
- Adjust with coarse adjustment knob – thumbs up, stage down.
- Move the objective to 20X and focus with the fine focus box.
- If you are using the filter cubes for the LED lamp, slide the lever on the front of the microscope into the appropriate position. From left to right – position 1 (white light), position 2 (DAPI), position 3 (FITC), position 4 (TRITC), and position 5 (DAPI/FITC/TRITC)*.
- Turn the knob on the front base of the microscope from DIA to EPI.
- If you do not see excitatory light illuminating the stage, try readjusting the filter cube lever and ensure that the LED power supply ignition light is on. Ensure the shutter is in the proper position.
- Once you have your image in focus with your eyes, move the manual slider bar at the top right of the microscope to the out position to open the optical path to the camera.
- Click the “LIVE” button on the ZEN software and your image should appear on the screen.

- Refer to Introduction to Zen Blue instructions to navigate the software and add graphics to your image.

SHUTTING DOWN

You will do steps in reverse from setting up.

- Close any software that is open.
- Turn off the remote fine focus.
- Turn off the microscope.
- Turn off the transmitted light power supply.
- Turn off the mini PC
- Lastly, turn off the LED power supply (First on-last off)
- Lower stage from objective to remove slide.
- Return to lowest objective position (10X).
- Clean objective lenses:
 - Use **ONLY** lens paper and dab
 - Clean dry objectives first.
 - For oil, gently dab the objective with lens paper. Without using any pressure, swipe the lens surface clean with the paper.

- **SIGN OUT OF THE LOG BOOK**

NOTES

- You can attenuate the laser intensity using the ND (Neutral Density) filters ND4 and ND8.
- The system cost \$110,000. Please contact your faculty mentor if you are unsure how to use the instrument.
- Keep the LED lamp on at least 1 hour (even if you use it for 10 minutes). This extends the lifespan of the lamp. If the lamp has been recently turned off, make sure it has been off at least 15 minutes before turning it back on.
- If you are using the LED lamp, do not place anything over the back of the microscope (including cables from the camera or a microscope dust cover), as the unit needs to cool properly.
- The objectives cost \$4000 each. Always begin with the 10X objective and with the stage lowered. After using Oil Immersion objectives, clean lens with lens cleaner.
- **CLEAN UP** by carrying out anything you carried in so the microscope suite does not get cluttered.

Objectives with NA (numerical aperture):

10x 0.45 dry

20x 0.75 dry

40x 1.30 oil

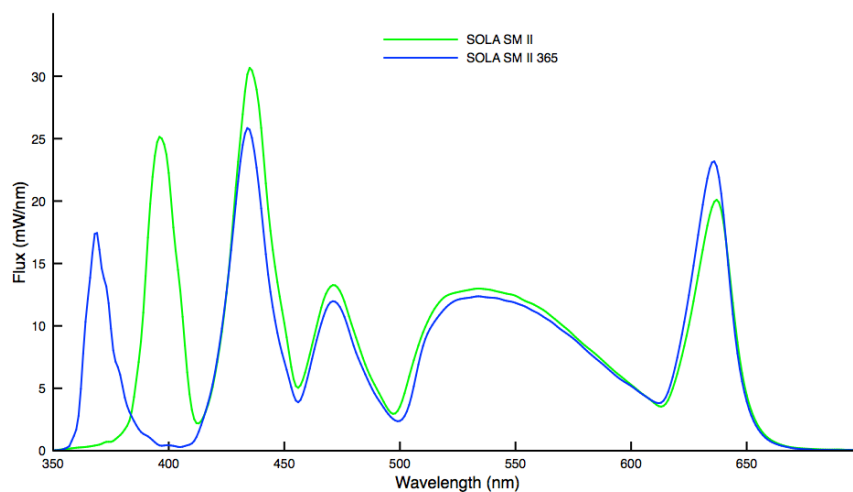
60x 1.40 oil

60x 1.0 water – In Jim Dearworth’s Lab

The 60x objective has been replaced. The 40x and 60 are both oil objectives and must use oil to view specimens. Please use only small drop on the slide. When done viewing, please gently dab or blot away oil from the tip of the objective with lens paper (DO NOT use chem wipes). Also avoid applying any pressure on the tip. By doing so, there is risk of knocking off the outer lens of the objective (somehow this is what happened to the prior 60x objective).

Filter set	Chroma #	Excitation	Emission
DAPI	49000	325-375nm	435-485nm
FITC	39002	465-495nm	515-550nm
TRITC	39004	530-550nm	575-635nm
DAPI*	69000	390-415nm	440-460nm
/FITC		470-490nm	508-532nm
FITC/		545-560nm	574-617nm

*Note that the DAPI channel with the DAPI/FITC/TRITC filter set (69000) does not excite DAPI well. Nikon replaced the mercury lamp with a LED (Sola SM II 365) in spring 2018 that has an excitation at 365 nm below the band the excitation band available for this filter set. Some DAPI stain will be apparent but it will be weak. To compensate a sole DAPI channel is available that is compatible with the LED lamp and therefore allows for nice DAPI staining.



49000 - ET - DAPI

Price: \$625.00

Legend Fluorochromes Sets Parts

Catalog Sets
 49000 [Hide Individual Filters](#) x

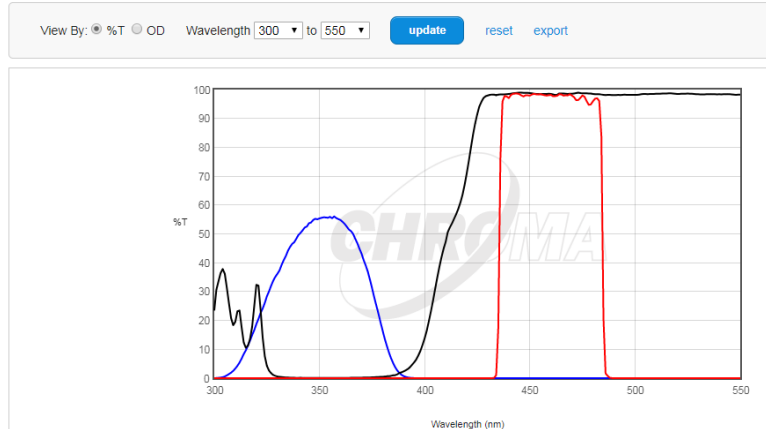
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<input checked="" type="checkbox"/>	AT350/50x	EX		0°	ASCII
<input checked="" type="checkbox"/>	T400lp	BS	79.33 T at 571nm	45°	ASCII
<input checked="" type="checkbox"/>	ET460/50m	EM		0°	ASCII

Add to Cart

Discounted Options

Mounting options are available when adding to cart.

Add to Compare



69000 - ET - DAPI/FITC/TRITC

Price: \$1,100.00

Excitation Intensity Note

Legend Fluorochromes Sets Parts

Catalog Sets
 69000 [Hide Individual Filters](#) x

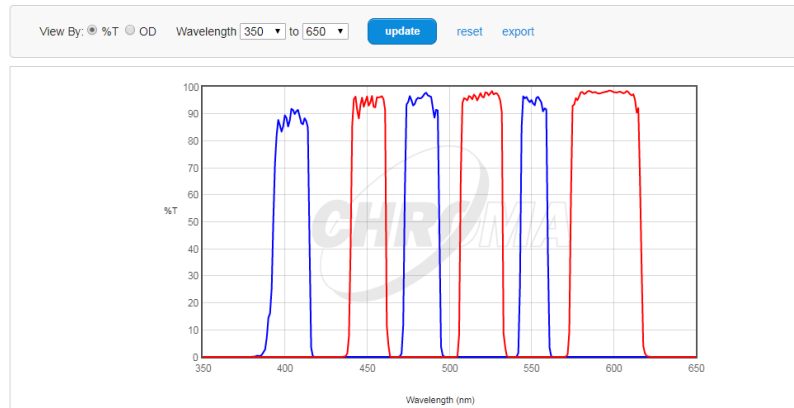
PLOT	FILTERS	TYPE	%T-OD	AOI	DATA
<input checked="" type="checkbox"/>	69000x	EX	0.00 T at 637nm	0°	ASCII
<input type="checkbox"/>	69000bs	BS	79.33 T at 571nm	45°	ASCII
<input checked="" type="checkbox"/>	69000m	EM	0.00 T at 637nm	0°	ASCII

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Other links for epifluorescent filters:

<https://www.microscopyu.com/techniques/fluorescence/nikon-fluorescence-filter-sets/blue-excitation-filter-sets/blue-excitation-b-2e-c-bandpass-emission> "FITC"

<http://www.microscopyu.com/articles/fluorescence/filtercubes/green/g2a/g2aindex.html> "TRITC"

<http://www.microscopyu.com/articles/fluorescence/filtercubes/triple/dapifitctritc/dapifitctritcindex.html> "DAPI/FITC/TRITC"

The confocal lasers and filter parameters for the microscope are:

488, 543, 633 with barrier filters for green (540LP Dichroic beam splitter and a 515/30 band pass emission filter), red (640LP Dichroic beam splitter and a 595/50 band pass emission filter), and far red (650LP long pass emission filter).

Confocal and Epifluorescence Microscopy Training Quiz

1. Immersion oil is often used for high magnification objective lenses. Why?
2. Why must the cover slip be fixed in place, if you are using immersion oil?
3. To calculate the final magnification of a specimen, you take the magnifications of the objective lens and the ocular (eye piece) and _____ them.
 - a. add
 - b. multiply
 - c. divide
 - d. none of the above, you only need the magnification of the objective.
4. Fluorophores emit light wavelengths that are _____ (shorter/longer) than the wavelengths with which they were excited. This is called _____.
5. If you have a blue stain (such as DAPI), what wavelengths of light would you use to excite it?
6. The spectrum of LED lamp is especially useful, however they pose unique dangers. List precautionary behaviors necessary for the microscopist.
7. Key elements of confocal microscopy are listed below. Write a separate sentence using each term that reveals you understand its role.

Pinhole

Scanning head

Photomultiplier Tube (PMT)

Out of focus light

Z dimension

Computer assisted projection

Bleaching