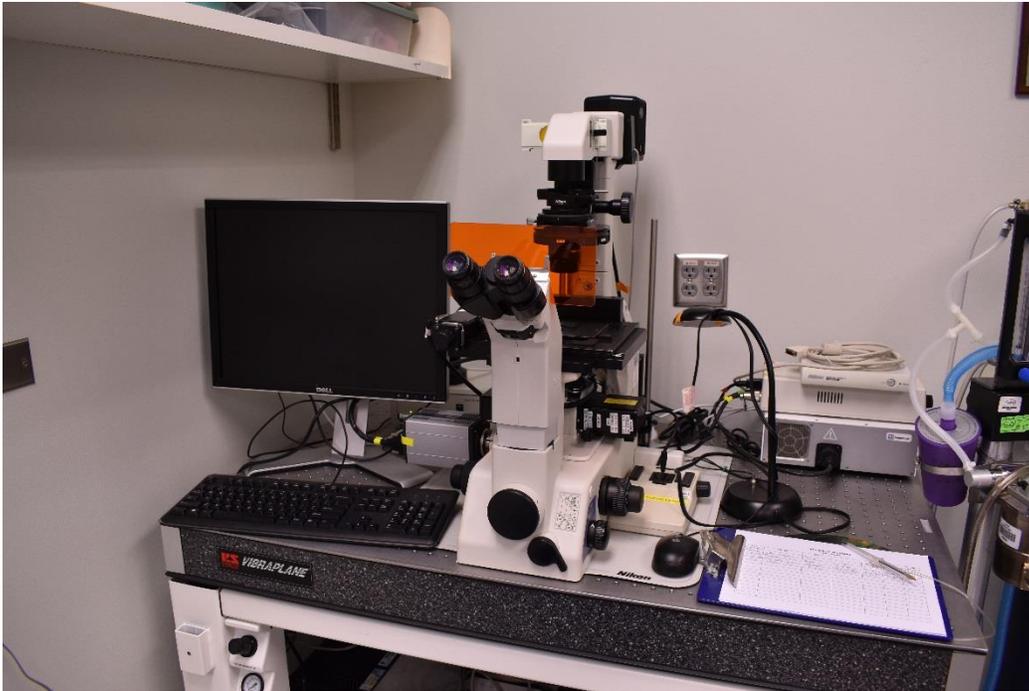


# Nikon TE300 Eclipse Brightfield Microscope



## User Guide

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# User manual for Nikon Elements software

**Equipment:** Nikon TE300 Eclipse microscope

DS Fi2 color camera (For colored images)

If the room lights are not on when you arrive to use the Nikon, **check and confirm that no one is using the Zeiss two-photon microscope before you turn on the light.**

Check whether the floating table and the microscope is clean, if not, clean it.

Sign in on the sign in sheet; please use both your given name and your surname, your department, your PI, and provide a telephone number at which you can be reached. If you do not use your lab telephone as your main contact, please provide your mobile number.

## Turning on/preparing microscope

1. Turn on the surge protector by pressing its power button
2. Turn on the computer



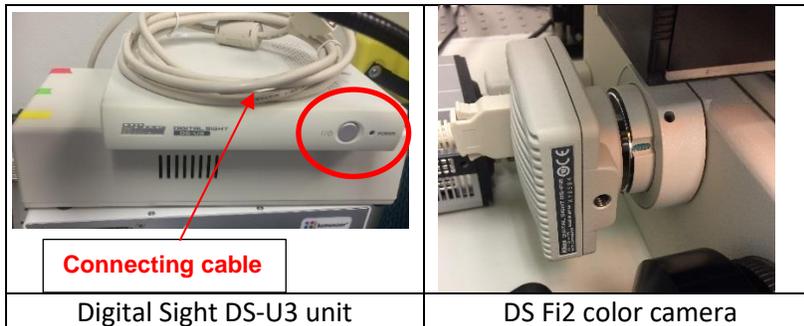
Once the computer is on, please login to your own user account with the following user ID and password:

**User ID:** `lsumc-master\username`

The password is your personal school email password

Turn on the camera **before** you start the software.

- Turn on the Nikon Digital Sight DS-U3 unit, which is connected to the camera via a cable. The green light will come on; once it stops blinking and remains a solid green, the camera is ready to use.



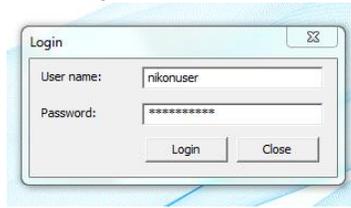
Open the **NIS Elements software** by double clicking on the icon

A log-in dialogue box will appear after clicking on the software icon. Log in use:

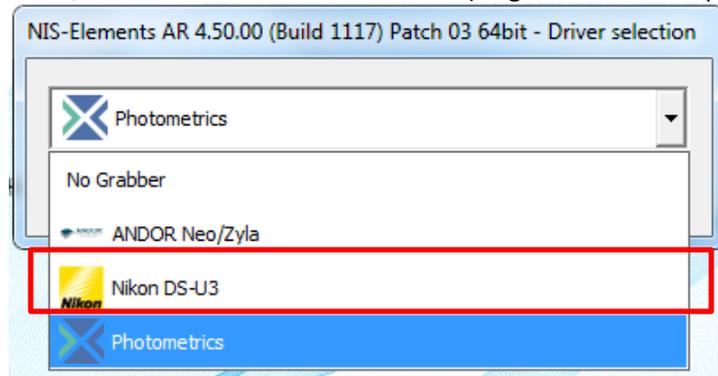
**User ID:** `nikonuser`

**Password:**

**corescopes**



In the camera dialogue box, choose the DS-U3 color camera (Neglect the other options).



1. The Nikon lamp switch on the left side of the scope should be pushed in (**ON**). If not, push it in.
2. Choose the correct **stage insert** and use the nylon screws to fasten it in place. Extra screws are in a white plastic box and the yellow screwdriver is for changing stage. You can find stage inserts in the box labeled "Nikon Wide-field stage".
3. The motorized stage joystick located to the right of the microscope has three speeds: 100%, 50% and 25%, which can be changed by pressing the switch to the left of the joystick. The switch continuously cycles through the speeds, so try different speeds to find the one you prefer.



Nikon lamp switch



Screws and screw driver for changing stage



Motorized stage joystick and focus knob

### **Setting up for imaging (objective, plates, slides)**

1. Lower the objective positions with the focus wheel. The lengths of the objectives are different, and the taller objectives can easily hit the stage while you turn the objective turret.

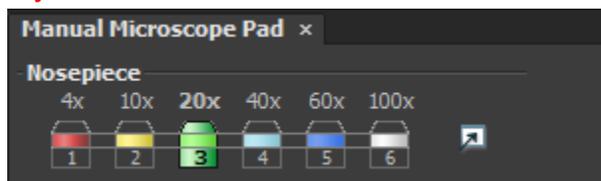
2. Place your sample securely in the stage tray. Remember to invert slides, **coverslip side down**.
3. **The microscope objectives are engineered to work best with coverslips 0.17 mm in thickness, usually referred to as number 1.5 or 1 ½ when ordering.**
4. Plates and dishes should not be flipped. If possible, you may want to remove plastic lids, since plastic can distort imaging.

### **Observing images through the eyepieces (light path: A)**

1. When viewing your sample through the eyepieces, set the selector knob on the lower right side of the scope to **A**, which directs 100% of the light to the eyepieces. B & D allow light to go to the eyepieces and the camera at the same time; C directs 100% of the light to the camera.



2. Rotate the objective turret manually to select an objective; please do not touch the objectives as you rotate the turret, since those with correction collars can have the collars inadvertently changed.
3. In the **OC Panel** of the software, click on the objective name corresponding to the one that you have manually selected. **Note:** Clicking on objectives in the OC panel does not control the actual turning of objective turret, but it tells the software which objective is currently in place and allows the software to adjust the acquisition settings best for the selected objective. Although you have to manually choose objectives, you still need to click on the corresponding objective button in the software to ensure best imaging result. Scale bars won't be in the correct um format if no or wrong objective is selected.



[The objectives currently mounted on the turret comprise the following: 4x, 10x, 20x, 20x, 40x dry, 40x oil. **Please note that only ONE (40x oil) of these is an oil objective**, requiring the use of immersion fluid; the others are all air objectives, which do not require any immersion fluid, and will in fact be damaged by oil, glycerin, or water. If needed, 60x (oil) or 100x (oil) objectives are available to be checked out.]

PLEASE use ONLY the Nikon oil in the brown plastic container at the microscope station with the 40X oil objective.



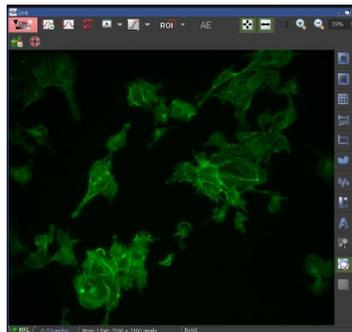
4. Turn on the light switch
5. Look through the eyepiece with transmitted light. Find and focus on your sample.

### **Observing live images on the computer and acquisition (light path: C)**

1. To view samples in the software, manually set the selector knob on the lower right side of the scope to **C**, which directs 100% of the light to the camera.
2. Click on the **live** button in the top tool bar at the top of the software.



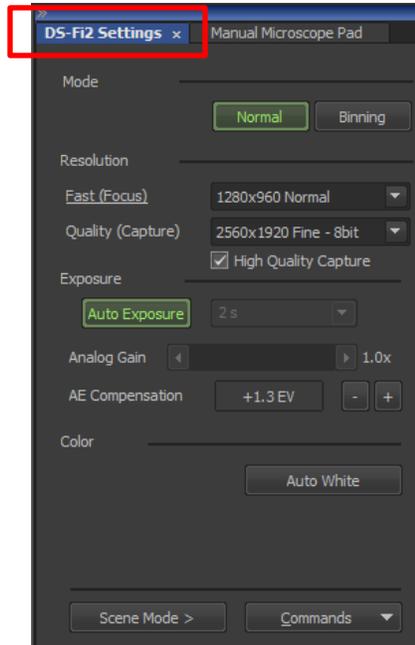
3. A live view window will pop up.



Live view window

4. Use the fine focus knob to readjust the focus on screen, **because the focus through eyepiece is not exactly the same with the focus of the software.**
5. Adjust the white balance of an image using the AWB button at the top tool bar of the live view window.
6. Capture an image by clicking the camera button  .

### **DS Fi2 (color) Camera Settings**



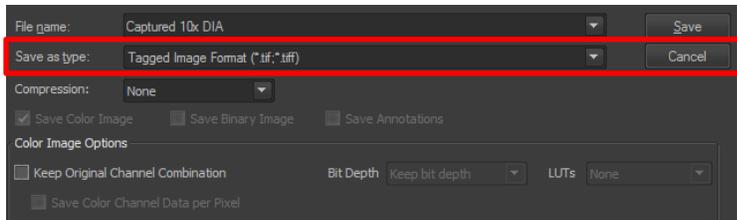
- **Mode:** Normal and Binning. Default Normal.
- **Resolution:** you will see **Fast (Focus)** and a drop down menu, and **Quality (Capture)** and another drop down menu. Below those menus is a box called **High Quality Capture** that can be checked or unchecked.
- **Exposure:** Uncheck auto Exposure to manually change exposure time.
- **Analog Gain:** software sets value
- **AE Compensation:** software sets value
- **Color:** Auto White button will add white balance to your image.
- Scene Mode >: no action
- Commands: no action

### Saving an image (ND, TIFF, JPEG; folders; offline access)

When you are ready to save an image, go to **File** and choose **Save As**. You will have choice in which format to save the image. Each user has a personal folder located in the Users Folder on the desktop. Images can be saved there temporarily, but should be removed in good time. The easiest way is to use a flash drive and the USB ports on computer.

Save your image as a .tif file from your original image. Keep in mind that TIFF format is required for publication. JPEG images is not always accepted.

To save into a TIFF file. Go to File->Save as, then set the options as below:



## Shutting down the equipment

1. **If you have used oil, make sure to clean the objective with the lens paper and cleaner provided.** Currently, this only applies to the 40x oil objective used for fluorescent imaging.
2. Clean your slides with cleaner and kimwipes.
3. Lower the objectives and turn the objective to 4X.
4. Log off and Shut Down the computer.
5. Turn off the DS Fi2 color camera by turning off the Nikon Digital Sight DS-U3 unit.
6. Turn OFF the surge protector under the table.
7. **Replace the cover** and remember to sign out on the login sheet.

## Miscellaneous

**Binning** averages the grey values in pixels into various sizes of bundles, which **makes the signal brighter but reduces resolution**. The software allows you to set the binning range for both cameras, with None as the default. The 2x2 selection will produce a good size image for focusing. If you change the binning size, you may need to redo the Auto Exposure. Using 2x2 binning, which averages the information in 4 pixels, increases your S/N ratio by a factor of 4. This is advantageous when you have a weak fluorescent signal. The use of 2x2 binning also decreases image file size and time transfer rates for the camera. Choose No Binning or the lowest value of binning for the best resolution.

### **Tips for Better Imaging**

**Coverslips:** It is very important that your coverslip be glass, size 1 ½, 0.17 mm thick. Some of the objectives have correction collars that adjust for differences in thickness, but some do not. The correction collars may help if you are trying to image cells in plates or flasks. However, they may not be in a position ideal for your samples.

**Plastic:** although in general images made through plastic ware are good, it may be worth the extra money to buy optically corrected plastic.

**Immersion medium objectives:** If you are using the 40x, 60x, or 100x oil immersion objectives, please use ONLY the Nikon immersion oil next to the microscope. Residues from different oil types on the lens

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can cause aberrations in the image. Kimwipes are fine for cleaning the oil off of slides (this can be followed with lens cleaner sprayed onto a Kimwipe, then used to remove the last traces of oil). When cleaning the objective lens, which is very sensitive, use the provided lens paper and draw it gently across the objective, which allows it to soak up and remove the oil without scratching or damaging the lens.

For professional quality images, each component of the microscope system should be set to the optimal position for your samples before you begin to collect images. This is especially important for brightfield images that require Kohler illumination.

### Equipment Specifications for Publication

**Microscope:** Nikon Eclipse TE300 inverted microscope with an epi-fluorescence attachment

Objectives: there are 6 objectives mounted on the turret; the rest are available for check out

Objective	Imm	Type	WD (mm)	Use with	Where located?
4x/	0.20 NA	Dry	Plan Apo	WD 20	Microscope turret
10x/	0.30 NA	Dry	Plan Fluor	WD 16.0	Ph1 DLL Microscope turret
20x/	0.45 NA	Dry	Plan Fluor ELWD	WD 7.4	DIC L Microscope turret
20x/	0.50 NA	Dry	Plan Fluor	WD 2.1	Ph1 DLL Microscope turret
40x/	0.60 NA	Dry	Plan Fluor ELWD	WD 3.7-2.7	DIC M Microscope turret
40x/	1.30 NA	Oil	Plan Fluor	WD 0.2	DIC H Microscope turret
60x/	1.40 NA	Oil	Plan Apo	WD 0.21	DIC H Cabinet
100x/	1.40 NA	Oil	Plan Apo	WD 0.13	DIC H Cabinet
Condenser	A	Bright field			
	∞Ph1	Phase contrast			
	∞L	DIC			
	∞M	DIC			
	∞H	DIC			

**Software:** NIS Elements 4.50 (Windows 7, PC)