Author: Chaowei Shang
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LSU Health
SHREVEPORT

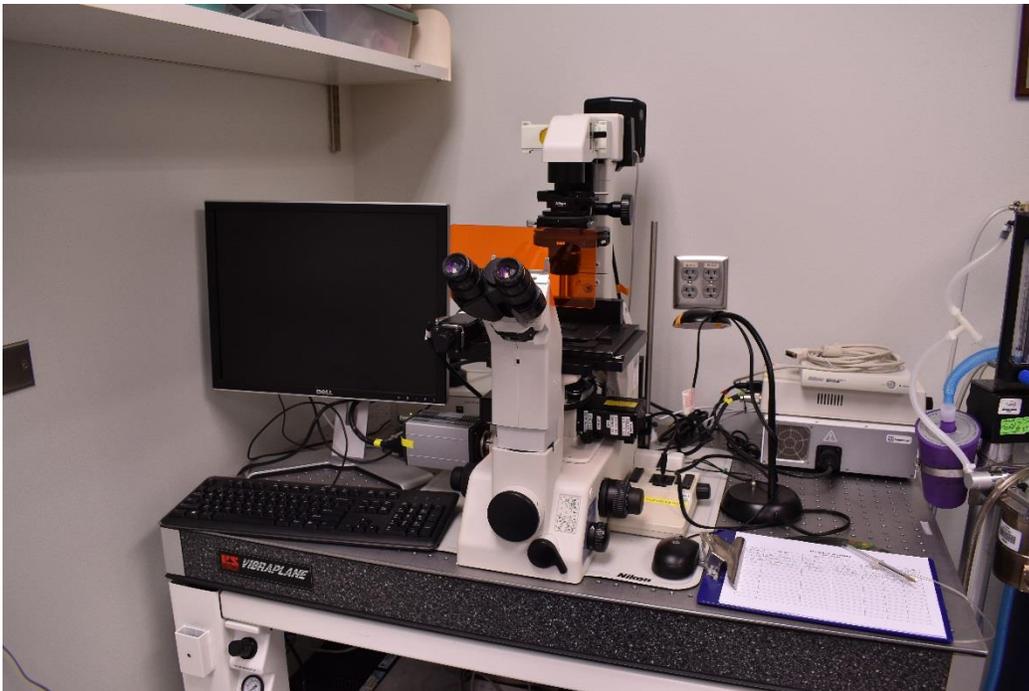# Nikon TE300 Eclipse Wide-Field Microscope

User Guide

LSU Health Science Center-Shreveport

Research Core Facility

Author: Chaowei Shang
Oct 2018

User manual for Nikon Elements software

Equipment: **Nikon TE300 Eclipse microscope**
Photometrics DYNO CoolSNAP camera (For fluorescent images)
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**.

The default camera attached to the Nikon microscope is the Photometrics DYNO CoolSNAP camera, which is the camera used to capture fluorescent images. It will be attached to the microscope at all times unless removed by me or another member of the RCF staff to attach the color camera.

If you need to use the color camera, please let me know ahead of time so I can remove the DYNO CoolSNAP camera and install the DS Fi2 color camera. The current policy is that the color camera will only be available for use during regular business hours (M-F, 8:30 am-5:00 pm); any after hours or weekend use must be arranged ahead of time with me.

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.

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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

Before you open the software, decide which camera you want to use and **turn on the camera**.

- The black and white DYNO CoolSNAP camera is used to take fluorescent images, turn on the DYNO CoolSNAP camera by gently pressing the button on the cord connected to the camera (see photo below).

- For the DS Fi2 color camera, install the camera and connect it to the power DS-U3 unit via cable. 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.
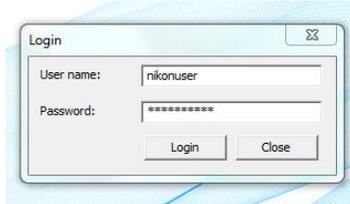
camera power switch		
Photometrics DYNO CoolSNAP camera	Digital Sight DS-U3 unit	DS Fi2 color camera

Connecting cable
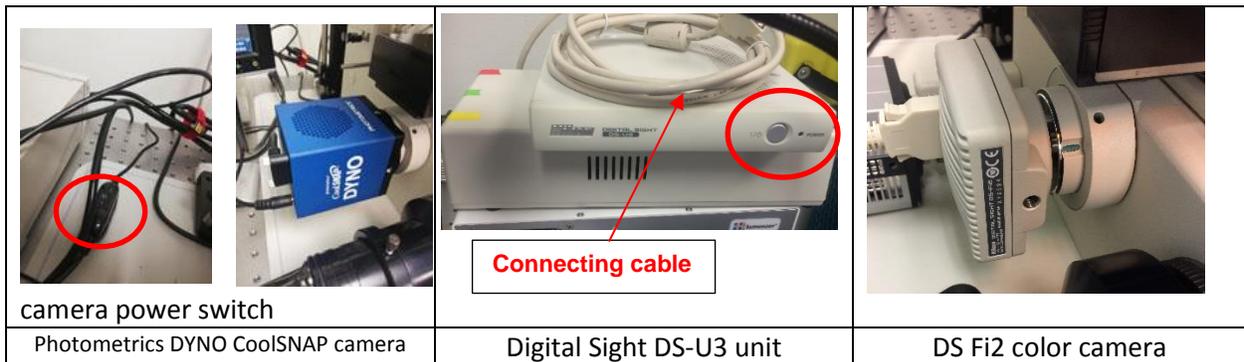
Open the **NIS Elements software** by double clicking on the icon .

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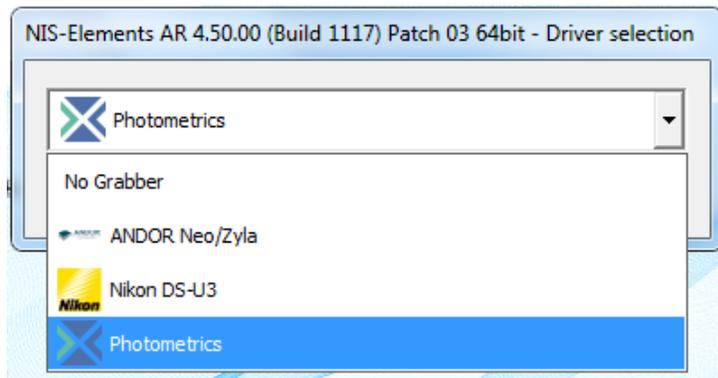Oct 2018

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 either the Photometrics or the DS-U3 color camera (Neglect the ANDOR option).

1. The Nikon lamp switch on the left side of the scope should be pushed in (**ON**). If not, push it in.
2. Check to see that the ND filter sliders and the fluorescent manual shutter are pushed in on the left side of the microscope.
3. 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".
4. 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.
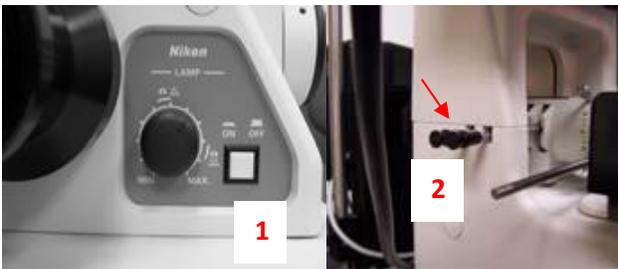
Nikon lamp switch   Filter sliders   Screws and screw driver   Motorized stage joystick
                      Manual shutter   for changing stage

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.
5. Choose a filter position on the filter box.
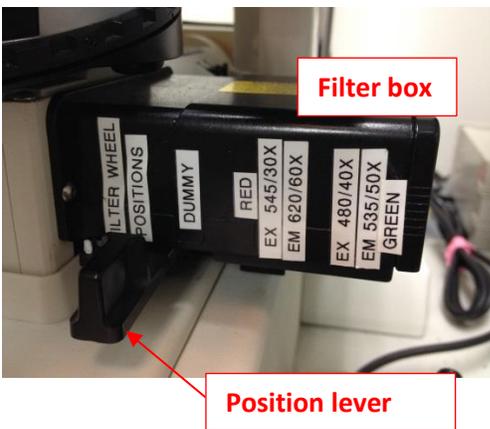
Filter wheel positions (L to R):

- Use filter wheel located inside microscope

- NO filters (color camera, B&W + phase)

- Red filter cube

- Green filter cube

The **Filter Wheel Positions Lever** controls which filters are in the light path during observation and imaging. It slides along the bottom of the filter box located on the right side of the microscope stand. There are four positions: all the way to the LEFT (Filter Wheel Positions) tells the software to use filters from the filter wheel located in the inner back portion of the microscope (DAPI, FITC, TRITC). As the lever is moved to the right through the next three positions, the software is instructed to use NO filters (Dummy, for bright field/phase contrast), the Red filter cube (excitation 545/30x, emission 620/60x), or the Green filter cube (excitation 480/40X, emission 535/50x).

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.
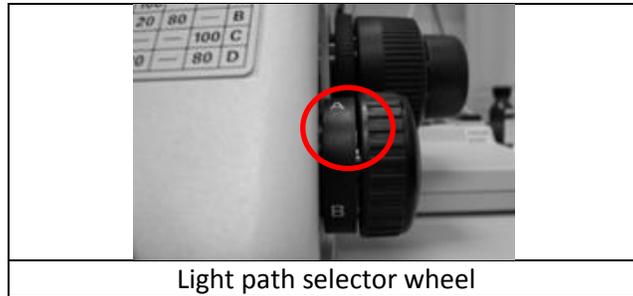
Light path selector wheel

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.**
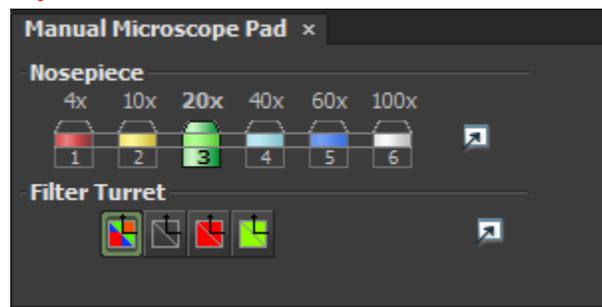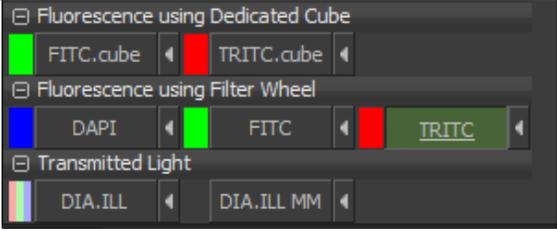

[The objectives currently mounted on the turret comprise the following: 4x, 10x, 20x, 20x, 40x dry, 40x oil. **Please note that only ONE (40x oil) of these is an oil objective**, requiring the use of immersion fluid; the others are all air objectives, which do not require any immersion fluid, and will 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. **OC Panel Tab: Filters**. Click on the Filter Block graphic that allows you to choose either the **Filter wheel** (for Blue/Green/Red), the **Filter cube**, red or green, and the **DIC.ILL** under transmitted light (for bright field, phase, and/or the color camera),. Manually slide the lever to corresponding positions listed below for different filter choices.

Filter selection	Position
Filter wheel (DAPI, FITC, TRITC)	Filter wheel position

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| FITC cube	GREEN
TRITC cube	RED
DIA.ILL & DIA.ILL MM (transmitted light)	DUMMY or Filter wheel position

5. To use bright field illumination, after selecting the **DIA.ILL** button, go to the tool bar at the top of the software and click on the **DIA** button ⬤ DIA. This opens the transmitted light shutter, and the light comes through.

6. For fluorescence light, after selecting a fluorescent filter or cube, go to the top tool bar and click on the **SOLA** ⬤ Sola button. This opens the fluorescent light shutter, and the light comes through.

   (The **AUX button** ⬤ Aux is an additional shutter operated by the software. It will be automatically turned on when you choose a fluorescent filter/cube or press the DIC.ILL button.

7. Look through the eyepiece with the selected fluorescence light or 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. Click on the **Autoscale** button ⬜ in the top tool bar of the live view window to view the image.
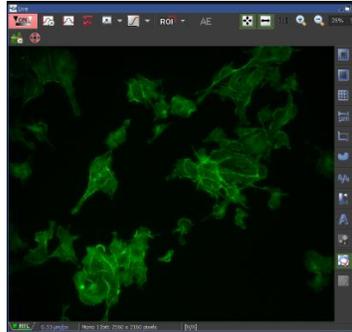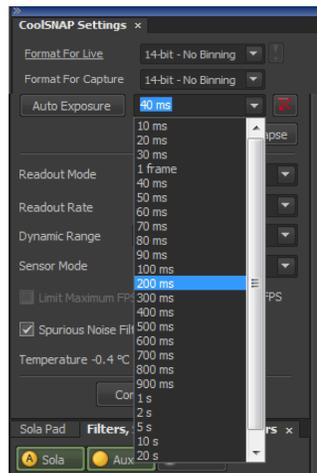
Live view window

4. <u>Use the fine focus knob to readjust the focus on screen</u>, **because the focus through eyepiece is not exactly the same with the focus of the software.**

5. To visualize the pixel intensity, turn on the indicator button. This allows you to visualize the saturation and undersaturation of pixels.

6. To adjust the fluorescent lamp intensity in the **Sola Pad**, drag the white bar to the left (reduce) or the right (increase) to change the intensity of the lamp.
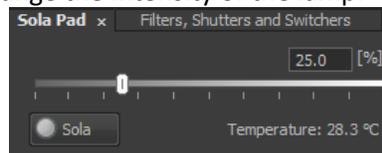
7. In the **CoolSNAP Settings** (Camera settings), adjust the exposure time of the camera for the best results. Avoid overexposed/Saturated signals. You can also chose **Auto Exposure** to let the software adjust the exposure time.


8. Capture an image by clicking the camera button .

9. If you use the color camera, you can also adjust the white balance of an image using the AWB button at the top tool bar of the live view window.

10. To take multi-channel fluorescent images, you need to select the **λ** tab in the ND Acquisition panel at the bottom of the page, and select the channels you would like to use. Press the blue plus sign (**+**) to add or the red X (X) to remove channels. Use the drop down menu to select filters, names of the channel, and the color that you want to use for each channel. Then click on **Run now**. If you select **Save to File** option and set the saving path and name of the image, after a series of images were taken through **Run now**, the image will be automatically saved.
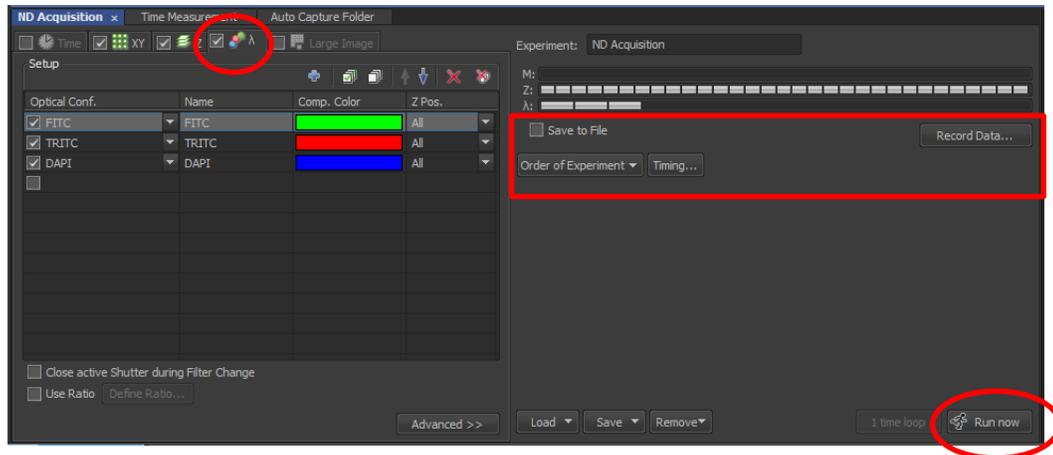
11. An alternative way to generate multichannel images is to take single fluorescent images separately. Then left click on the bottom left button of an acquired image, hold the left click, and drag it to the other image that you want to merge with. Then release the left click on the mouse. Thus two single channel images will be merged.
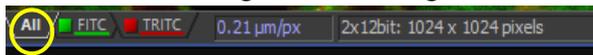
Adjust the Image Properties

There are two ways to remove background fuzziness from fluorescent photos, either individually or, if required for images in a series, by choosing a set value.

Remove background Method 1 - LUT (look up table): The LUT section is located at the lower right of the entire software layout. To decrease the background, drag the vertical black line from the left to the right, towards the peak of the histogram (to the position of the yellow dotted vertical line in the image). **Do not pass the left border of the peak, this will diminish your actual signal**. To increase the brightness, drag the white vertical line towards the left.

To apply the same LUT to another image. You can Save/Copy the LUT value by clicking [icon] at the top of the LUT window. Then open a new image and Load /Paste LUT Settings from the previously saved one. You can also go to devices->synchronize, to apply the same LUT to all the opened images.
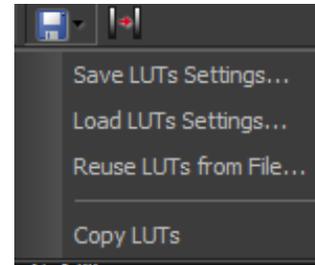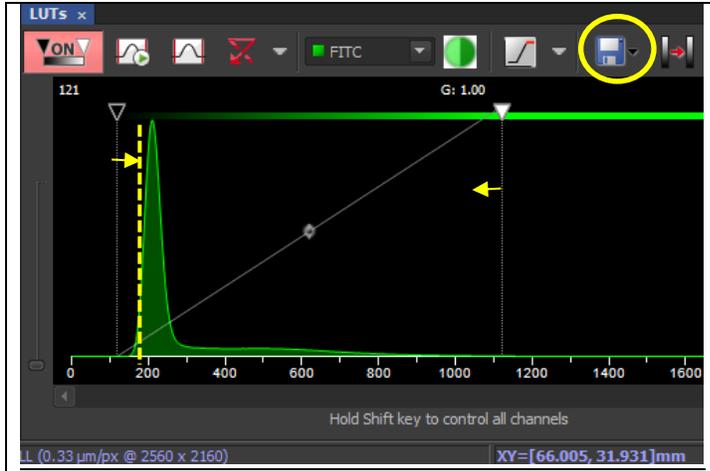
Remove background Method 2: On the right side of an image window, locate and click the 2nd icon, which is the **Background ROI** button [icon]. This will create a rectangular ROI that can be adjusted in size and positioned over an area of background with a uniform color. Once this has been done, go up to the top of the software and click on the **Image** tab. From the drop down menu that appears, click on **Background**, then on **Background using Background ROI**. This will remove scattered light from the background and help clean up your image.

Remove background Method 3: This allows you to choose a constant value that you can apply to images in a series. Follow the same procedure as described above, except when you click on **Background**, choose **Background using Constant**. You will be able to type a value for the constant into the menu; you can determine the constant by looking at the values generated by moving the cursor around the background (they will appear at the bottom of the dialogue window) and deciding upon an average value. This can be applied to images in a series.

## 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 into .ND2 (the NIS-Element software file format), which can only be opened with the NIS Elements software and Image J/FIJI. **It is important to save your image in the .ND2 format, because this format saves all information (exposure time, LUT of the image) and many Journals ask for the original format images.**

Then, you can make a .tif file from your original image, but this process cannot be reversely done. 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:
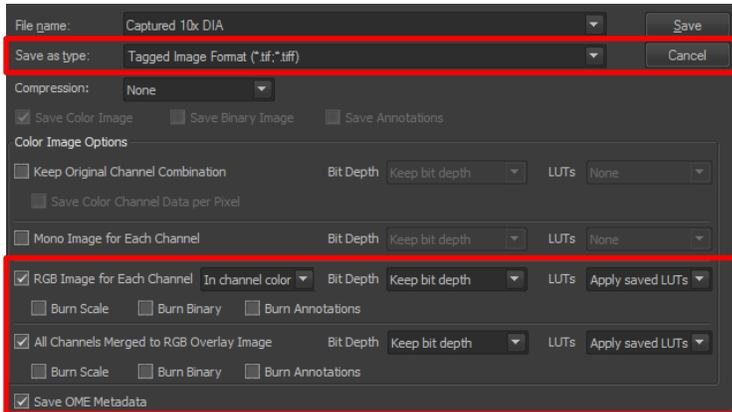
Shutting down the equipment

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 Photometrics DYNO CooSNAP camera by pressing the switch on the cord, or 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.

DS Fi2 (color) Camera Settings

The ANDOR Neo/Zyla camera is used for fluorescent imaging. Its settings are listed on page 8 (Step7 and 8). If you use the color camera (DS Fi2), the camera settings are different.
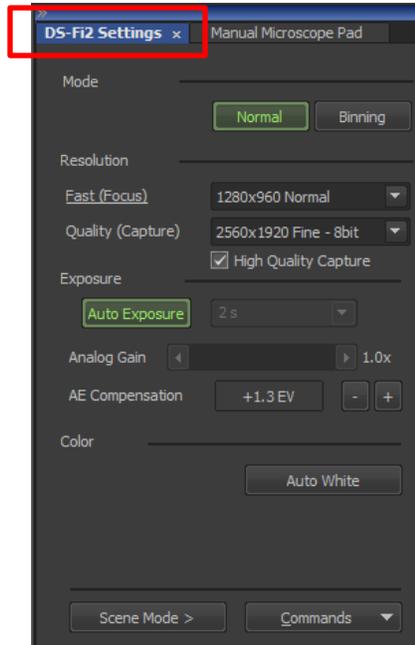
- **Mode**: **Normal** and **Binning**. Default **Normal**.
- **Resolution**: you will see **Fast (Focus)** and a drop down menu, and **Quality (Capture)** and another drop down menu. Below those menus is a box called **High Quality Capture** that can be checked or unchecked.
- **Exposure**: Pressing Auto Exposure button => 184 ms in drop down menu (can be changed)**.**
- **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

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

<u>Coverslips</u>: 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.

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

<u>Immersion medium objectives</u>: 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 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

Filters: There are Chroma filters in the filter wheel inside the microscope stand, and additional filter cubes mounted separately on the outside of the microscope stand

Chroma 8300 filter set with single band exciters

- DAPI (360/40x)
- FITC (492/18x)
- Texas Red (572/23x)
- Dual band exciter for FITC and Texas Red
- Triple band exciter for DAPI, FITC and Texas Red in the Prior filter wheel with a stationary multiband beamsplitter and emission filter

Additional filter cubes

- EF-4 FITC HYQ (Ex 480/40x; Em 535/50x)
- EF-4 TRITC HYQ (Ex 545/30x; Em 620/60x)

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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)