

# First Time Imaging Guide

## BioFrontiers Advanced Light Microscopy Core

### Introduction

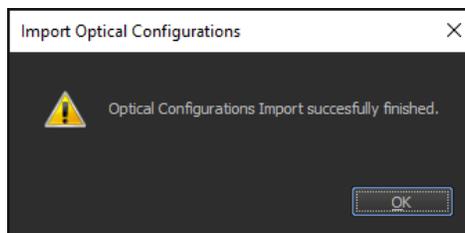
This guide explains the steps to acquire your first image on a Nikon Ti microscope. It assumes that you are using the training slide, which contains fixed and stained cells.

### Acquiring a single image

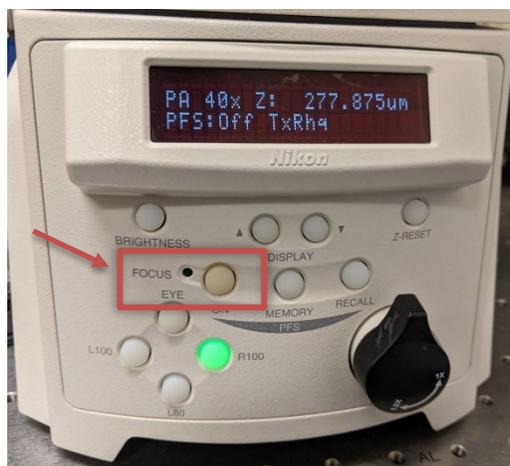
1. If the microscope is off, turn on the microscope following the protocol taped to the wall.
2. Start Nikon Elements:
  - a. Click on the icon on the desktop



- b. Click OK when the dialog box appears saying “Optical Configurations Import successfully finished.”



- c. If you hear a beep and the perfect focus button is flashing, disengage the system by pressing the button on the microscope base.



3. Place the appropriate sample holder with the cutouts into the microscope chamber:



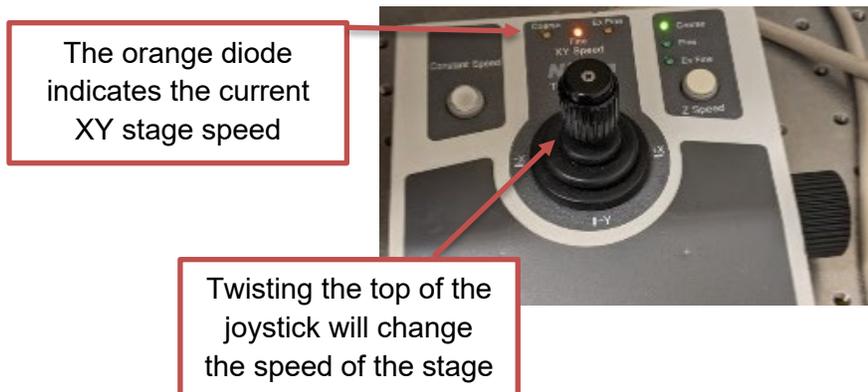
Note: You can turn on the lamp using the footswitch if you need light.



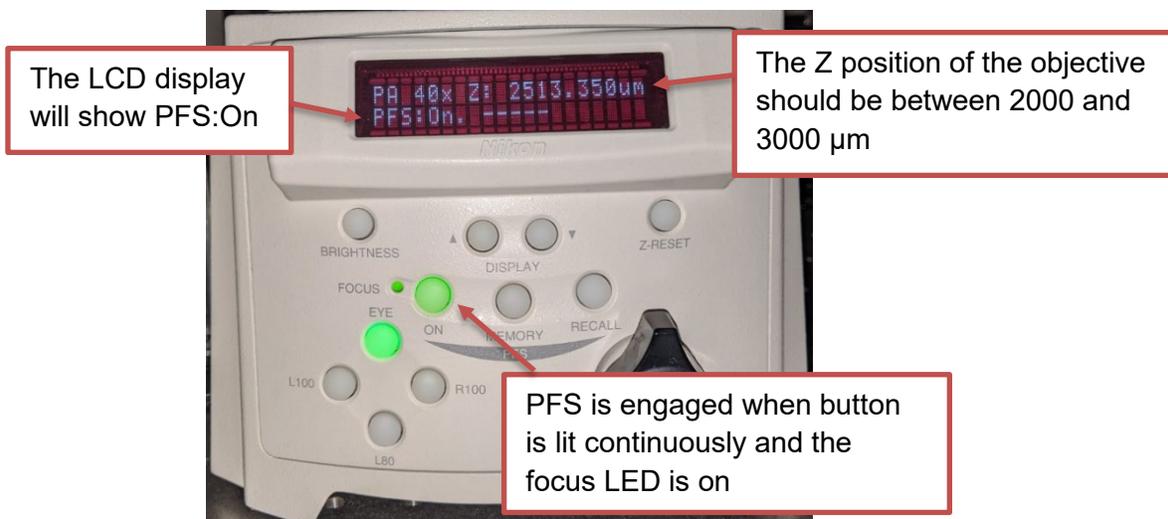
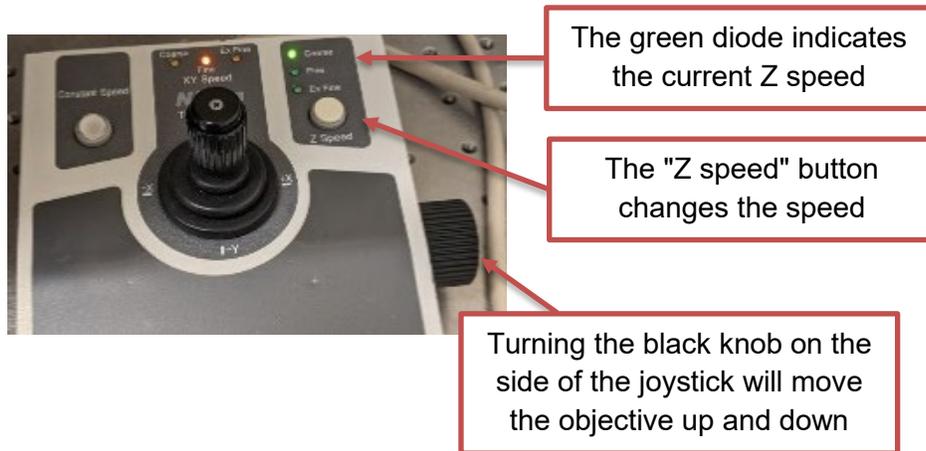
4. Select either the 20X or 40X objective in Elements by clicking on the corresponding button on the Macro Panel (left of the screen by default). These are air objectives. Wait for the microscope to finish changing objective before moving on.



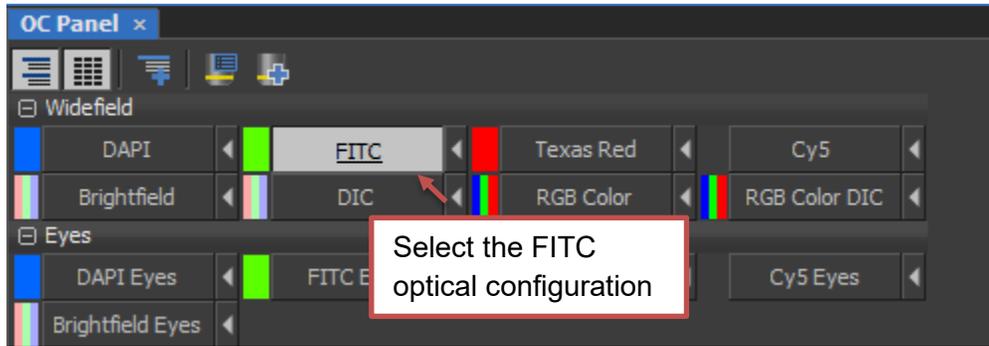
5. Move the lens to be roughly at the center of the sample holder using the joystick. The speed of the XY stage movement can be changed by:
- Twisting the top of the joystick to go from Coarse (Fastest) → Fine → Extra Fine (Slowest)
  - The angle of the joystick (pushing harder moves the stage faster)



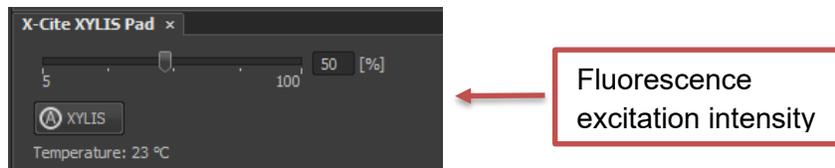
6. Place your sample on the holder with the **cover slip facing down towards the objective**.
7. Start the perfect focus:
  - a. Press the PFS button on the microscope base. You will hear several beeps and the light will start blinking, indicating that the microscope is searching for the focal plane.
  - b. Raise the objective *slowly* using the black knob on the side of the joystick until you hear a beep. The PFS button will be continuously lit when perfect focus is engaged correctly and the LED next to the FOCUS display will be lit. Be careful not to push the objective into your sample - this will damage the objective and lead to costly repairs.
    - i. The display on the LCD screen on the microscope base indicates the Z position of the objective. The typical Z-position is between 2000-3000  $\mu\text{m}$ .
    - ii. If you turn the knob the wrong way (i.e., lowering the objective), you might hear a continuous tone. This is fine - you just have to reengage the PFS system by pressing the button.



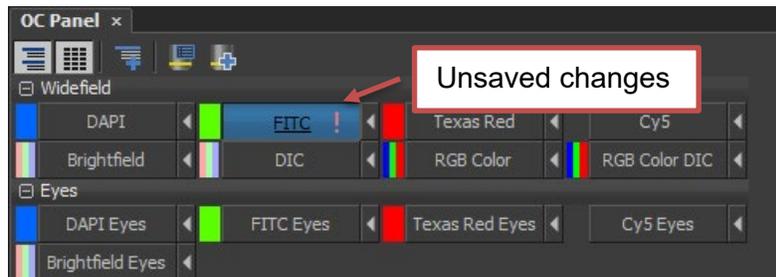
- Select the FITC channel by clicking on the button in the OC Panel. The button will appear depressed when selected. You will hear the microscope change filter cubes. Wait until the sound stops before proceeding.



- Change the illumination intensity by moving the slider under the X-Cite XYLIS pad. Set this to 20%.

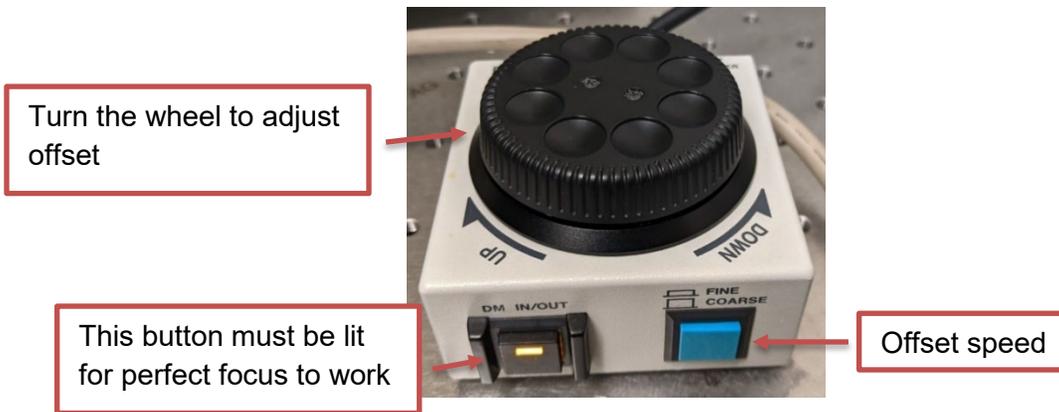


- If you changed the illumination intensity, save the settings by clicking on the  button next to the optical configuration.



- Click on the  icon on the main toolbar (under the menu) to start live view.
- If the image appears black, click on the  button in the toolbar above the image to automatically adjust the lookup table.
- Adjust the perfect focus offset using the perfect focus wheel:
  - The blue button can be used to change the offset change speed. When the button is pressed in, turning the wheel changes the offset a little bit. When the button is out, the turning the wheel changes the offset a lot. You should start with coarse adjustment until you start to see some cells, then switch to fine.

- b. When the cells are in focus, click on the  button next to the selected optical configuration to save the settings.



14. Stop live view by clicking on the  button.

15. Click on the  icon to take an image.

16. Save the image by selecting File > Save As.

**Congratulations!** You have successfully acquired your first image.

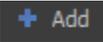
## Acquiring a multi-channel image

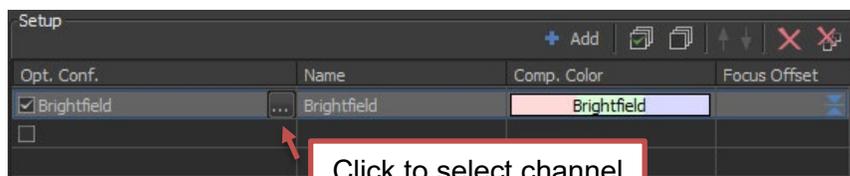
This section of the guide describes how to capture a multi-channel image using the ND Acquisition tool. It assumes that you have completed the first part and successfully acquired your first image.

1. First, repeat steps 8 - 13 to set up the optical configurations for the following channels. You should change the illumination intensity so the cells are visible in the image.
  - a. DAPI - this is the nuclear stain
  - b. Texas Red - this stains the actin filaments in the cell

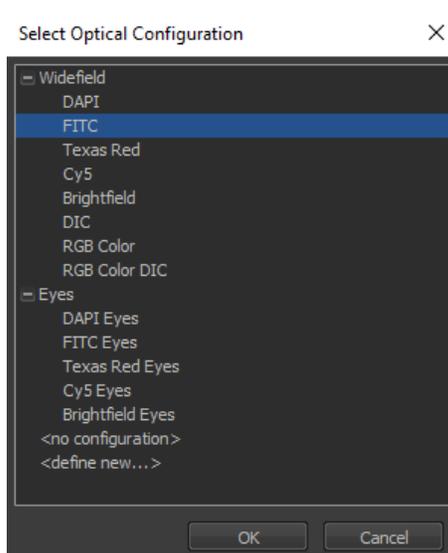
Make sure you save the configuration for each channel by clicking on the  button after you make a change.

2. To acquire a multi-channel image, you will need to use the ND Acquisition tool. This tool should be found on the right side of the interface. Set up the ND Acquisition to acquire multiple channels by:

- a. Click on the checkbox next to the  $\lambda$  tab .
- b. If there are optical configurations in the list below, click on the  button to clear them
- c. Add each channel to the acquisition by:
  - i. Clicking on the  button
  - ii. Click on the ... button next to the channel name under the "Opt. Conf." column.



- iii. Select the FITC channel under Widefield, then click OK.



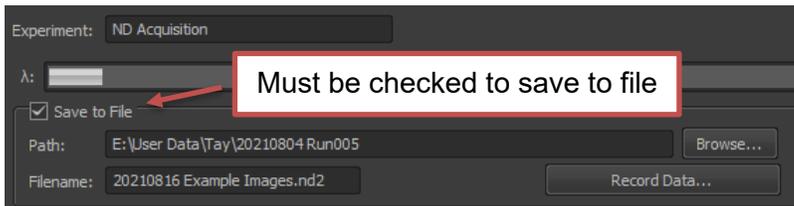
- iv. Repeat steps i and ii to add the DAPI and Texas Red channels.
- v. Check that the channel has a check mark next to it (you can skip a channel by clearing the check mark).
- vi. Channels will be acquired from top to bottom. To change the order, select the channel and use the arrows to move them up or down.

3. Ensure that the Use PFS button is checked.

4. Make sure that the rest of the tabs are unchecked. This will remove them from the acquisition.



5. At the top of the ND Acquisition panel, make sure the box next to “Save to File” is checked.



6. Change the output path and filename as appropriate.

We suggest:

- a. **Output directory:** Save your images under the folder that corresponds to your lab PI. If the folder doesn't already exist, you can create it. Create a new subfolder with your name.
- b. **Filename:** Label images with the acquisition date and a short description, e.g. “20210816 Training”.

7. Acquire the image by clicking on “Run now”. You should see a multi-color image appear in the display.

