Bucknell UNIVERSITY

Fluorescence Microscope Nikon Ti-U

Basic guide to using the fluorescence microscope

Written By: Jacob Nickle

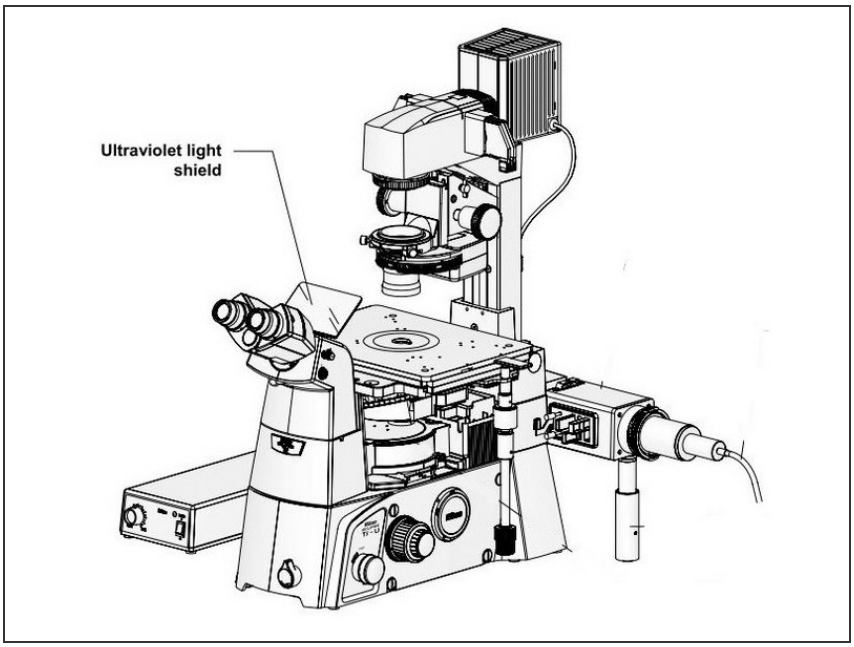


INTRODUCTION

This tutorial only covers basic use and does not include in-depth knowledge or theory of florescence microscopy.

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Step 1 — Warnings and Cautions



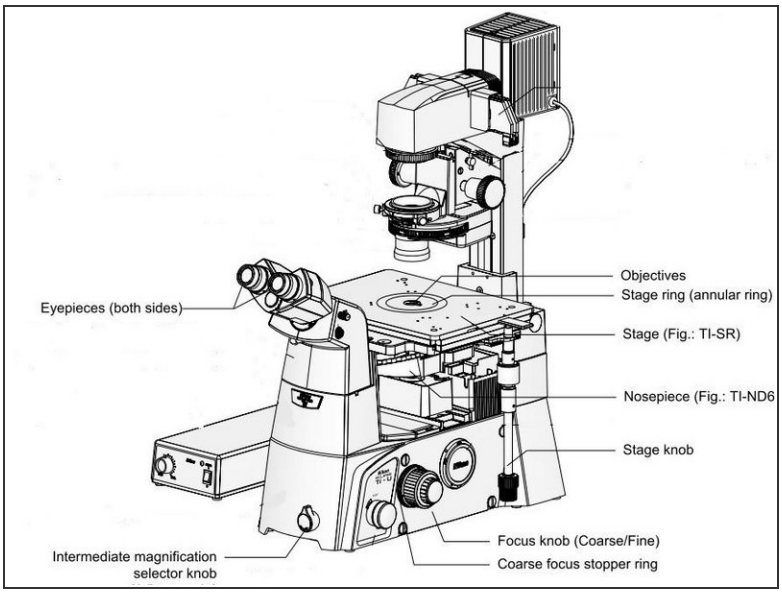
⚠ While the ultraviolet light is passing through the objective, do not view the light directly unless using the ultraviolet light shield.

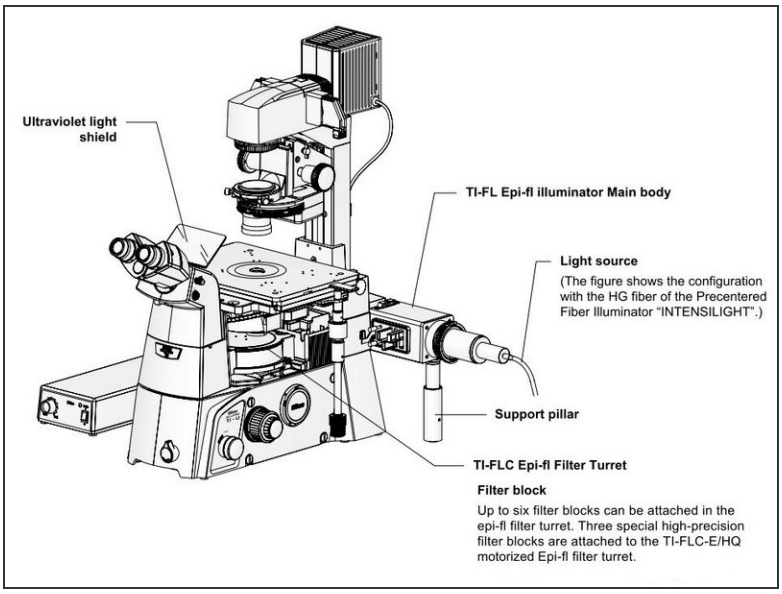
⚠ Be aware and adhere to all other warnings and cautions as stated in the product manual.

ⓘ Images used in this tutorial have been taken from the Nikon Ti-U and Nikon TI-FL Epi-fl product manuals.

ⓘ This microscope is capable of various imaging configurations including bright-field and phase contrast which will not be used here.

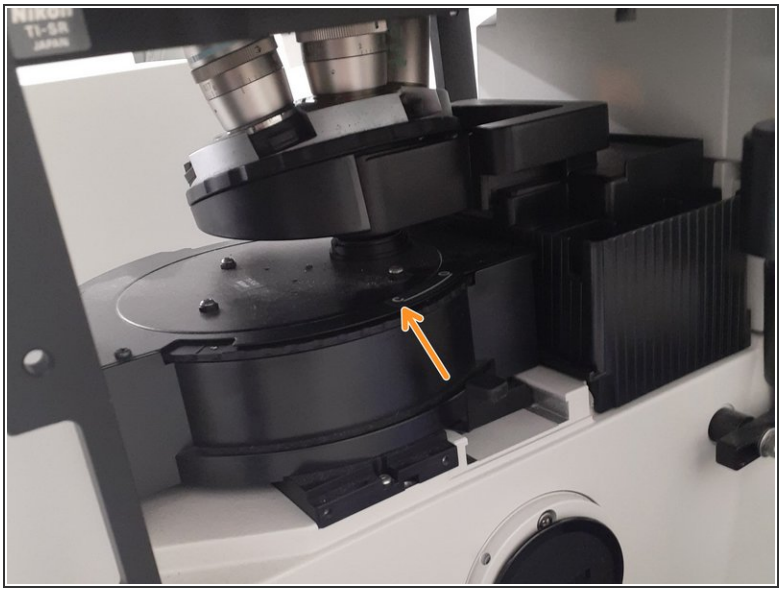
Step 2 — Definitions





- Stage - Platform where prepared microscope slides are placed and controlled.
- Objective - Used to magnify image.
- Focus - Adjusting distance between the lenses and the stage which holds the sample in order to make image look clear.
- Shutter - A mechanical cover which blocks light from passing through.
- Filter Block - Directs and filters light allowing only a particular wavelength section to be seen.

Step 3 — Controlling light





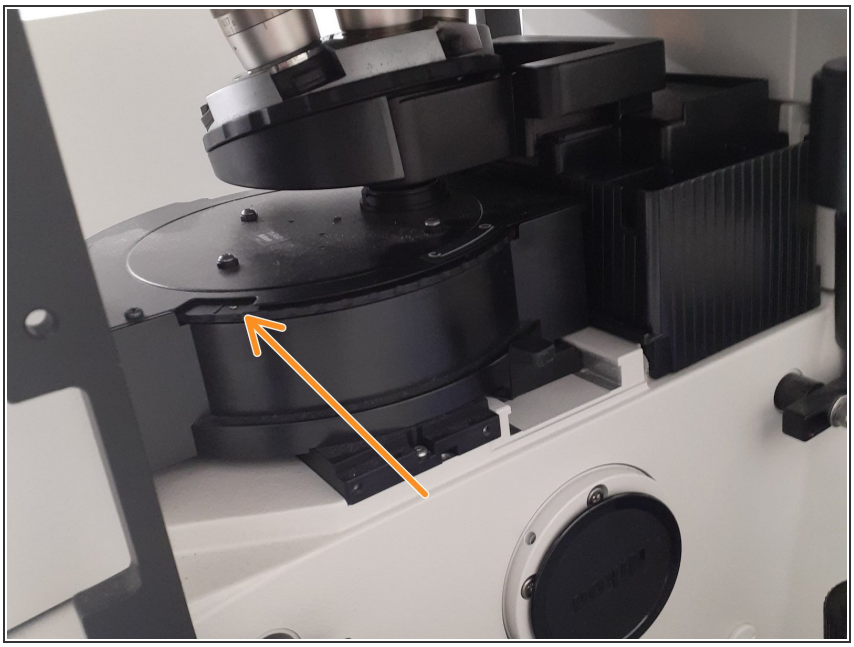
- Make sure that the shutter is closed by positioning the lever on the Epi-fl turret to the "C" position as shown in the picture.

  ⓘ "C" is for closed while "O" is for Open

- To avoid stray light entering the image, shut the curtain and turn off the light.
- Turn on the mercury lamp using switch as shown in the picture.

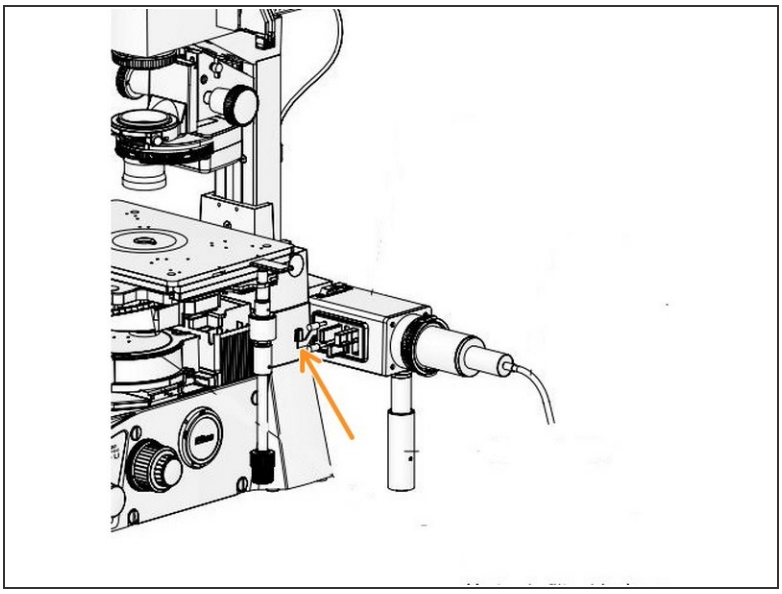
⚠ This lamp must stay on for at least an hour before turning off. Failure to do this may cause the bulb to prematurely fail.

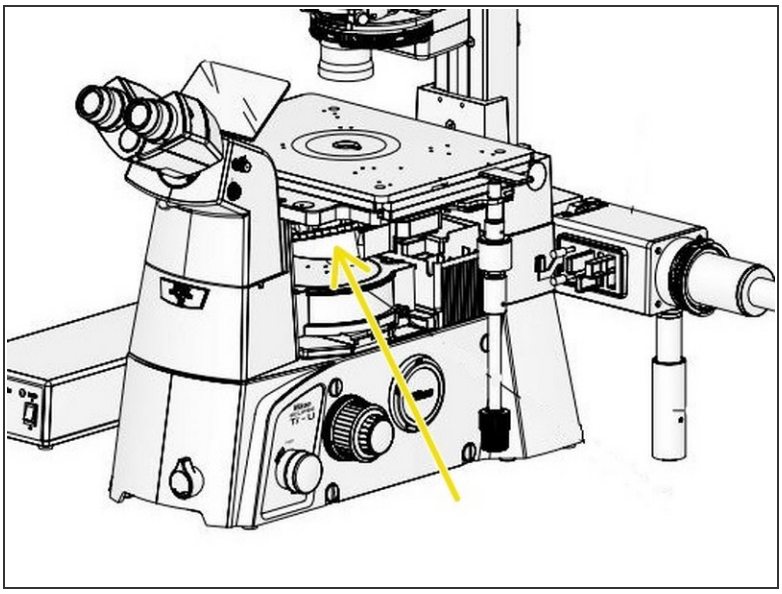
Step 4 — Excitation Method



- There are three commonly used wavelengths in florescent microscopy which this microscope is capable of producing including green(FITC), blue(DAPI) and red.
- Producing these wavelengths is done using filter cubes which are located within the Epi-fl Turret.
- Only 3 of the available 6 slots are filled with a filter cube.
  - Position 3 contains blue (DAPI)
  - Position 4 contains green (FITC)
  - Position 5 contains red
- Place filter cube of choice within the optical path by rotating turret into position as shown in the picture.

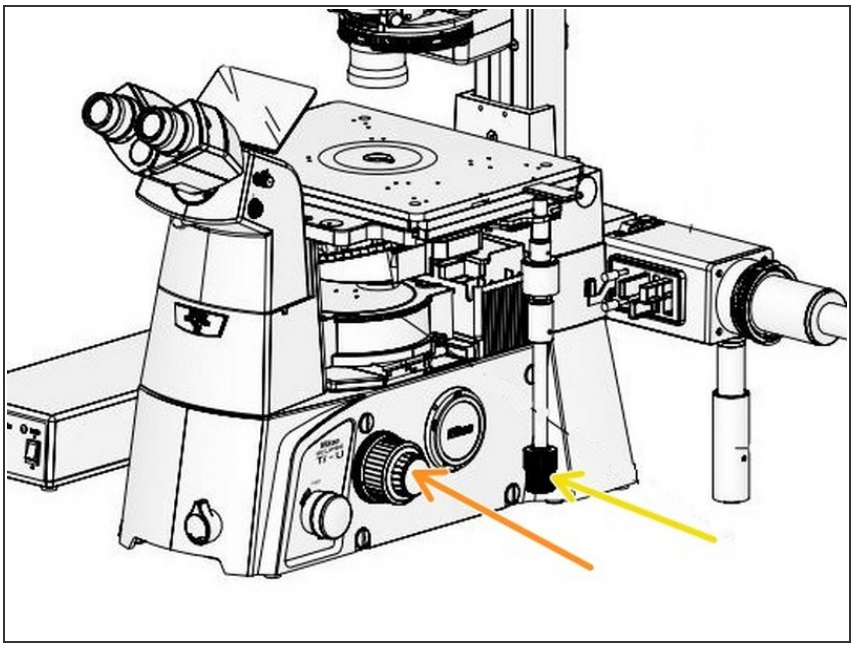
Step 5 — Preparation





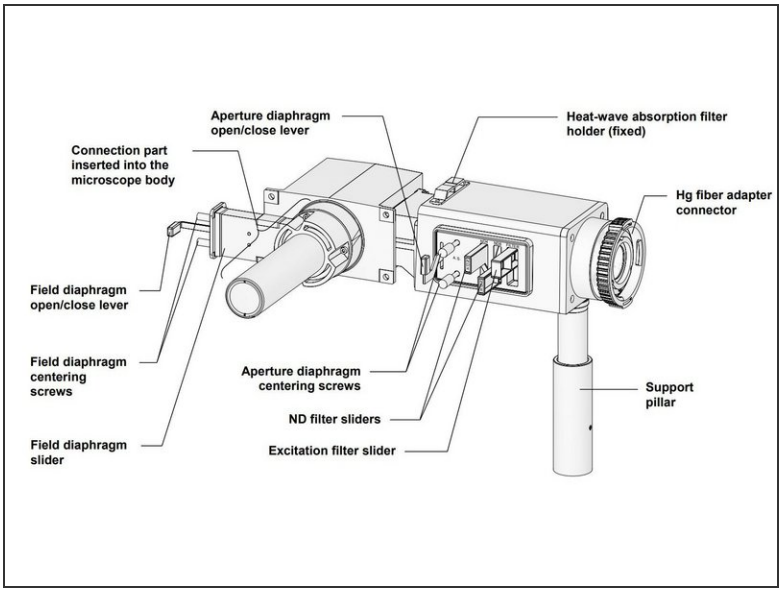
- Place sample onto stage within optical path.
- Insert aperture diaphragm by pulling slider as shown in the picture.
- Make sure light filters are not in the optical path.
  - ⓘ The filters are located next to the aperture diaphragm and should be in the "pulled out" position.
  - ⓘ These filters are used to create repeatable brightness.
- Rotate nosepiece until the 10x objective is in the optical path. Nosepiece location is shown in picture.
  - ⓘ It is best to start with a low magnification objective in order to more easily resolve the image and verify the microscope is working as intended.
  - ⚠ Be mindful that the objective should not touch the sample while changing objectives or while focusing.

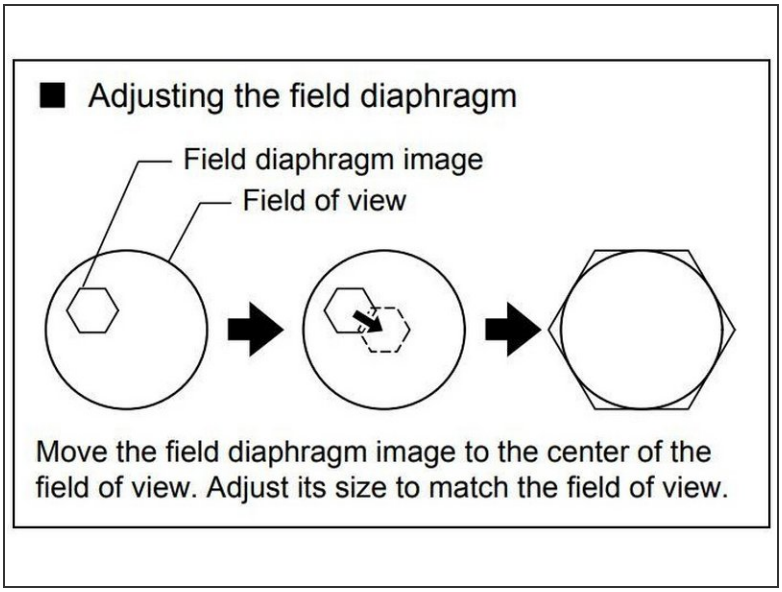
Step 6 — Imaging



- Open the shutter by positioning the lever to the "O" position.
  - ⓘ If all of the steps were correctly followed prior to this point, looking through the eyepiece should result in some sort of image.
- Adjust the focus using knobs as shown in the picture.
  - ⓘ The larger knob is coarse while the inner knob is fine.
  - ⚠ Avoid colliding objective with the sample while focusing.
- In conjunction with the previous step, adjust sample position by using the stage positioning knobs.

Step 7 — Optimizing Imaging





ⓘ Stray light can be further mitigated by centering and circumscribing the field diaphragm.

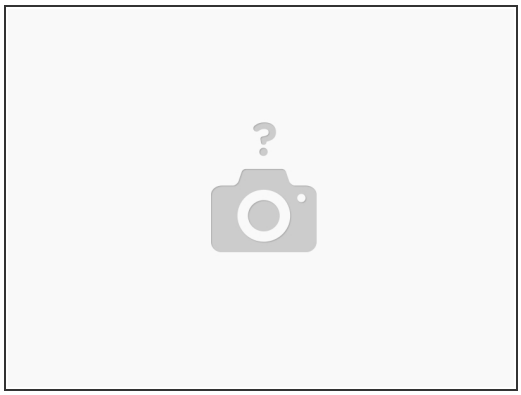
ⓘ The field diaphragm lever is located on the left side of the microscope. Picture shows location.

ⓘ Too much light exposure can result in a bleached sample. Try and minimize the light exposure by utilizing the shutter lever.

- Change objectives and refocus for greater magnification.

⚠ Again, avoid touching the sample with objective.

Step 8 — Conclusion



- If there are any other questions or concerns, refer to the product manual or ask the Lab Director.

⚠ Once imaging is complete, remember that the lamp can't be turned off unless it has been on for at least an hour. Please turn off before leaving.