

Nikon Ti-E Microscope Manual

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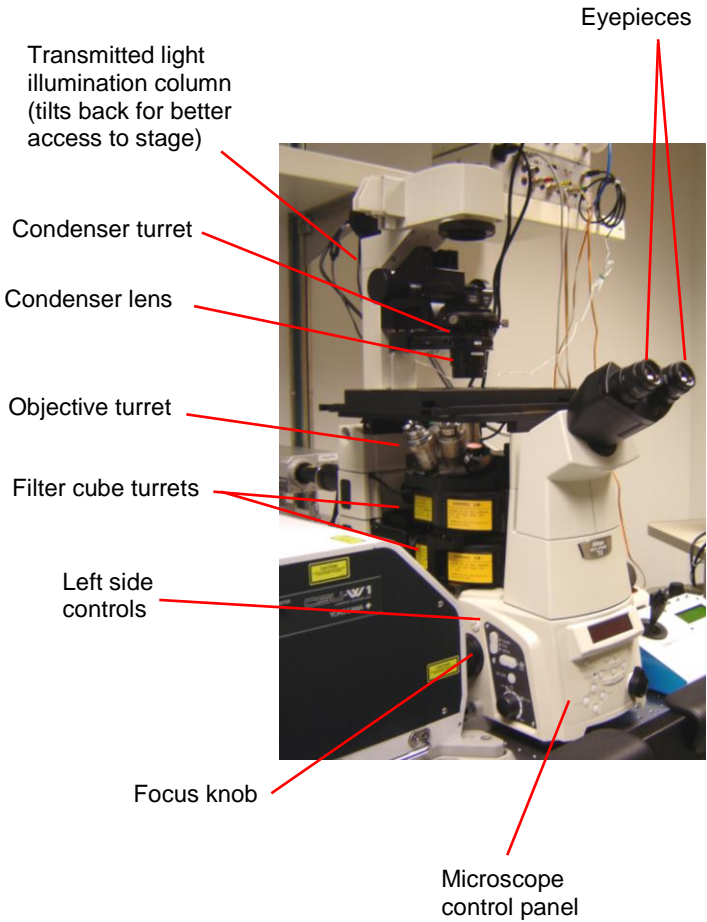
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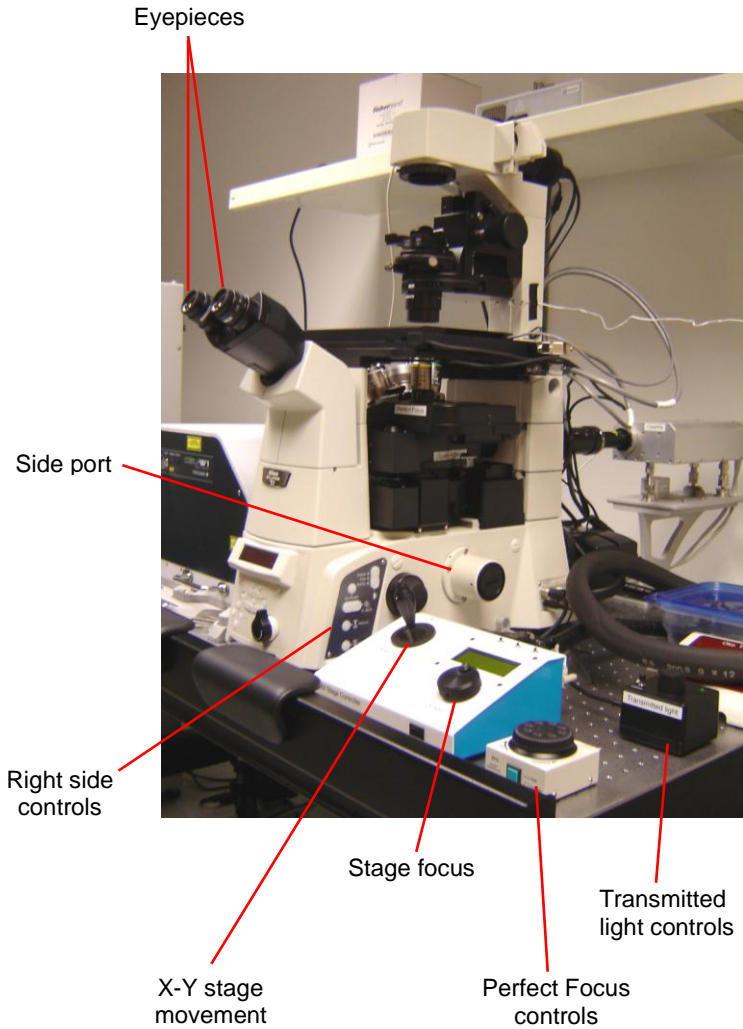


Getting to know the microscope

View from left



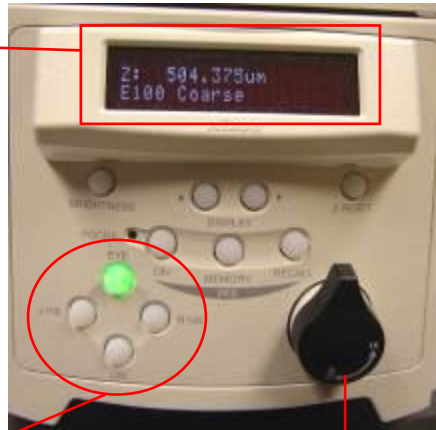
View from right



Microscope controls

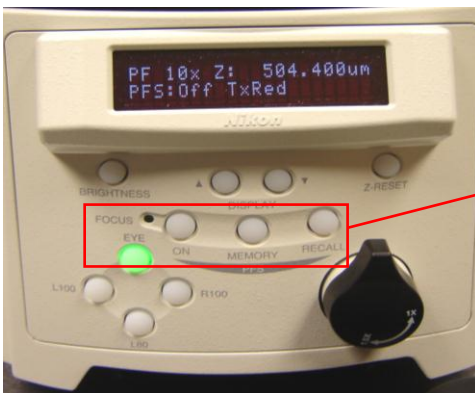
Microscope control panel

LCD display panel



PORTS – directs the light
Eye – 100% to the eyepieces
L100 – 100% to the left port
R100 – 100% to the right port
L80 – 80% to the left port,
20% to the eyepieces

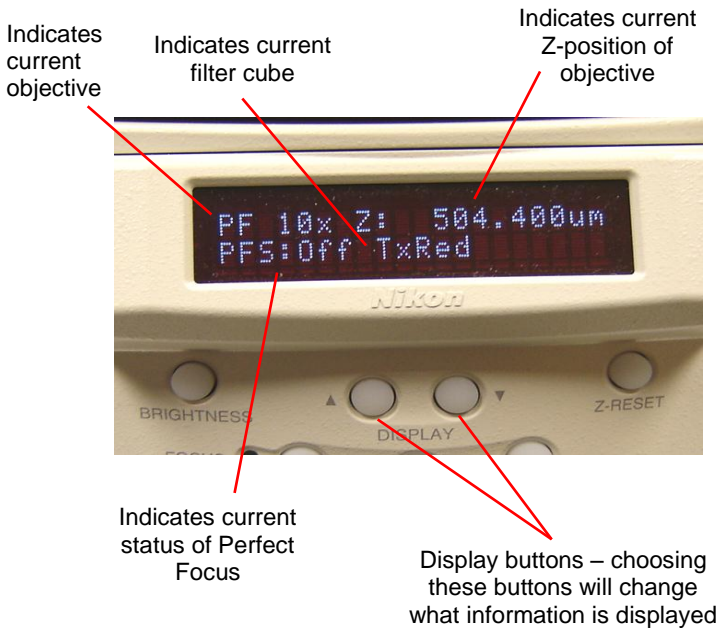
Mag changer



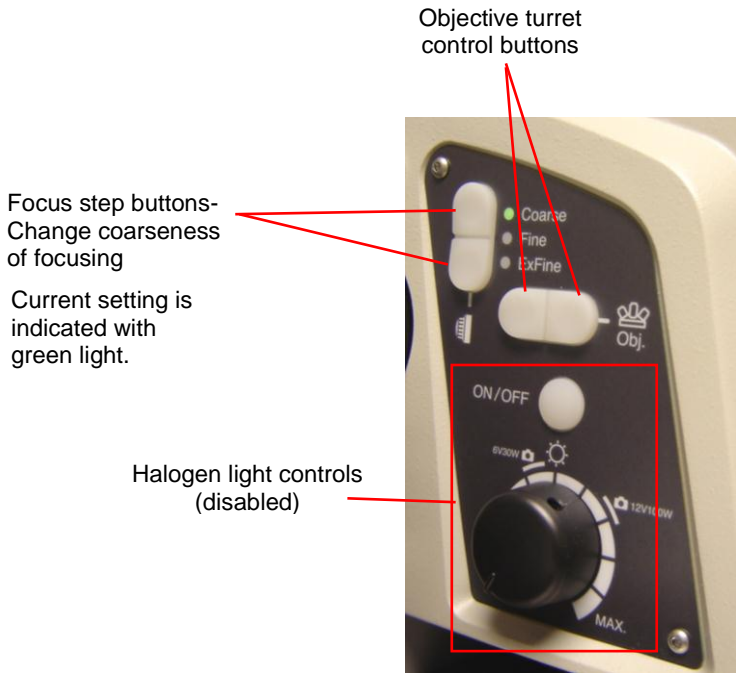
Perfect Focus controls

LCD display panel

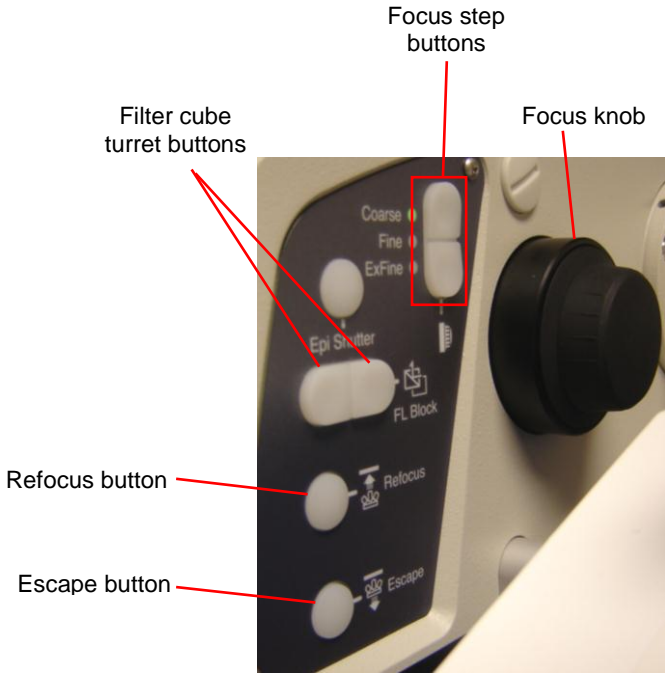
The LCD display panel can display different information depending on which setting is chosen. Pressing the display buttons will change the display setting.



Left side controls



Right side controls

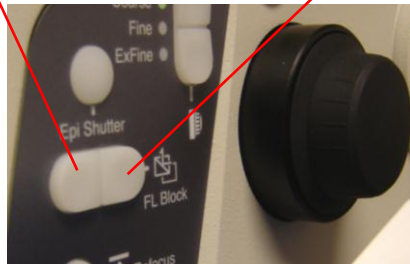


Changing filters

Use the motorized fluorescent filter cube changer on the right side of the microscope:

Press left button
to rotate filter
cube turret left

Press right button
to rotate filter
cube turret right



The current filter will display on the **LCD display panel**.

The filter cube turret contains four filters, the DIC analyzer, plus an empty slot

- **GFP** : cube for green fluorochromes
- **RFP** : cube for red fluorochromes
- **CFP** : cube for cyan fluorochromes
- **YFP** : cube for yellow fluorochromes
- **ANALYZER** : DIC analyzer
- **--** : empty position

Notes:

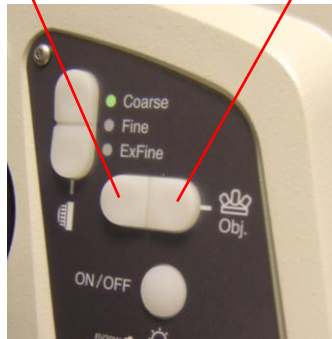
- The **empty** position is used for confocal imaging and for transmitted light observation through the eyepieces

Changing objectives

Use the **objective turret** control buttons on the left side of the microscope

Press left key to **increase** magnification

Press right key to **decrease** magnification



Current objective indicated on **LCD display panel**

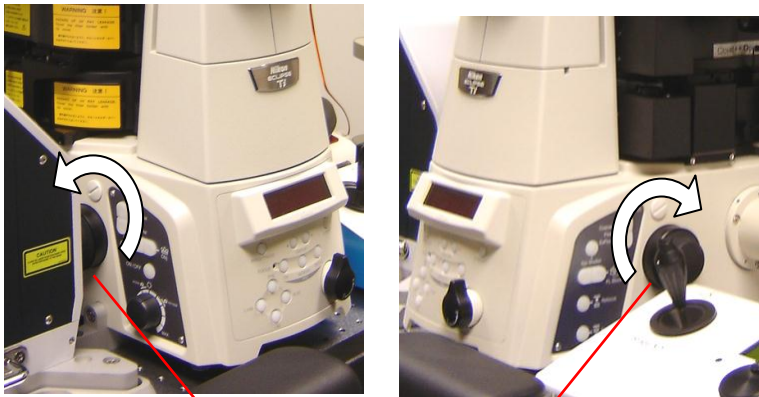
Focus options

To focus using the focusing knobs

One way to focus is using the focusing knobs located on the left and right side of the microscope.

Turning the knob so that your thumb moves **away** from you focuses **down**

Turning the knob so that your thumb moves **toward** from you focuses **up**



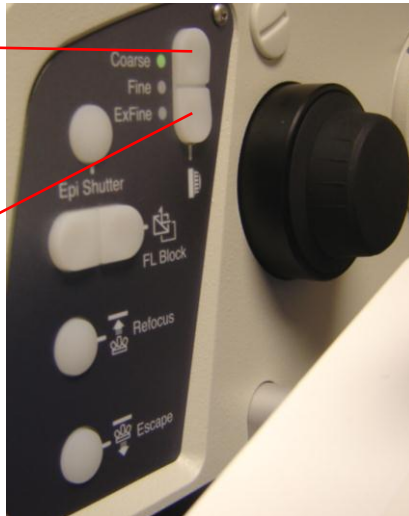
Focusing knobs
(turn in direction of arrows to
focus objective down)

Changing the coarseness of the focusing

The focusing is electronic and has three settings. Use the buttons on either the right or left side of the microscope. Please use the buttons on the right side of the microscope to avoid accidentally changing the objective. A light will display by the current setting.

Press the upper button to change to a coarser focus.

Press the lower button to change to a finer focus.



Escape and Refocus buttons

The Escape and Refocus buttons are located on the Right Side Controls.

Refocus button – pressing this button will raise the objective to the last position before the **Escape button** was pressed. Improper use could result in objective damage. Be very careful when using this button!

Escape button – pressing this button will lower the objective to the “escape” position. To raise the objective after this, press the **Refocus button**.



Eyepieces

Adjusting interpupillary distance:

- Adjust eyepieces to match interpupillary distance by moving the eyepieces closer together or further apart

Adjusting parfocality

- Focus on specimen using electronic focusing controls
- Close right eye and adjust the left eyepiece so that the image appears in focus to your left eye
- Close the left eye and adjust the right eyepiece so that the image appears in focus to your right eye
- The eyepieces are now parfocal

Stage holders

The microscope has two stage holders:

- **Oko Lab heated chamber** (maintains a constant temperature, as well as providing humidified air with or without CO₂)



- **Universal stage holder** (holds dishes and slides at room temperature)



Changing stage holders

To remove either stage holder:

- Lower the objective all the way down
- Lift stage holder out of the stage. There is a spring-loaded tab that holds the chamber in place using pressure.



- When removing the Oko Lab chamber, carefully place it in the plastic container to the right of the microscope and place the plastic lid loosely on the plastic container. Pay special attention so that the wires do not crimped.

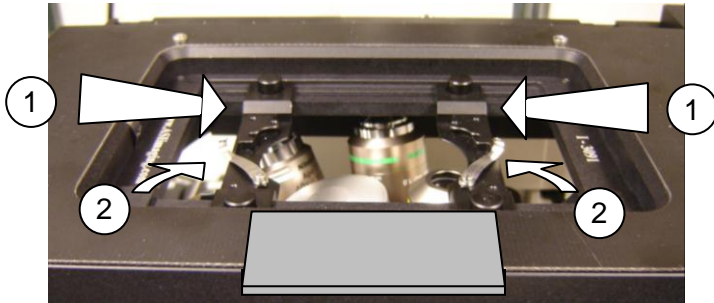


Be careful when placing the wires and tubing here.

Take care not to touch condenser lens surface when removing and replacing the stages!

Placing a slide on the universal stage

- To avoid touching or damaging the objective, lower the objective turret all the way using the **focus knob**



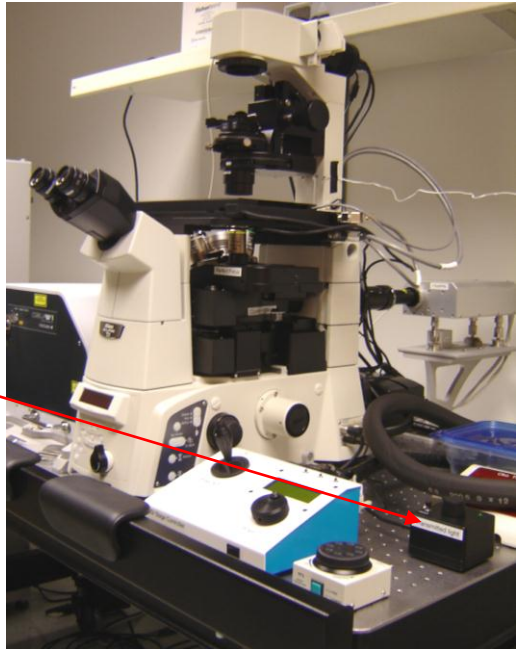
- Position the slide holder clips as shown above
- Insert slide into the holder, pushing in the side supports so that the slide is supported on either side (1)
- Slide clips inward onto slide to secure the slide (2)

Transmitted light imaging

Adjust transmitted light brightness

Use the dial on the **Prior LED** to the right of the microscope.

Prior LED



This light will be on if the Prior LED is on.

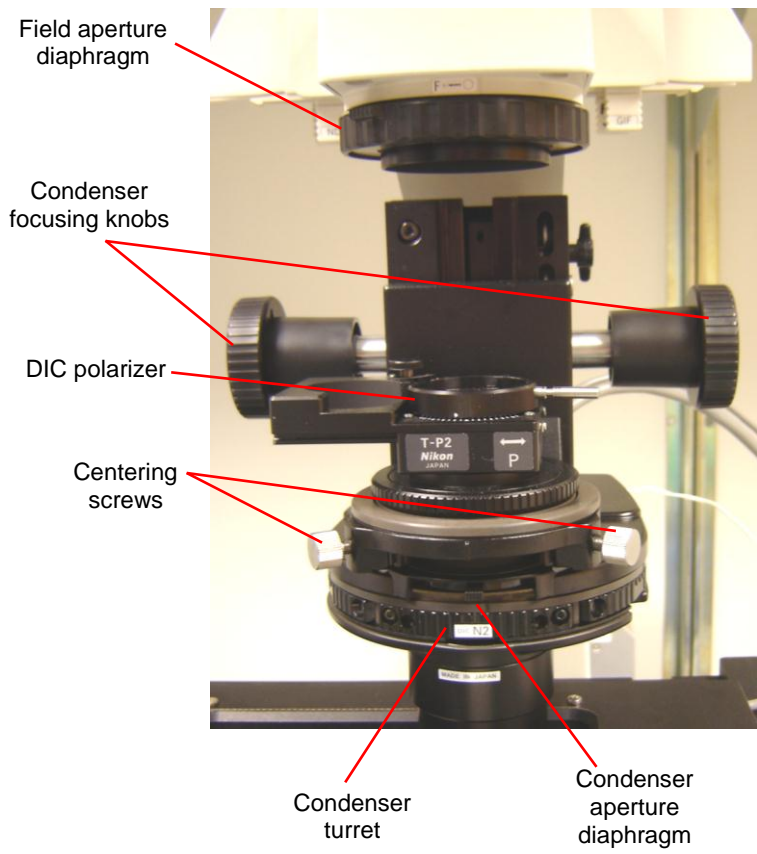


Press this button to turn the Prior LED on or off.

Koehler illumination

Koehler illumination is essential to obtain good transmitted light images

- Select objective
- Open the **condenser aperture diaphragm** (move lever to the right)
- Focus on specimen
- Close the **field aperture diaphragm** (twist ring to the left)
- Focus condenser using **condenser focusing knobs** until the image of the aperture is sharp
- If necessary center the field diaphragm in the field of view using the two **centering screws** located on the front of the condenser
- Open **field aperture diaphragm** until it just disappears from the field of view
- Close down the **condenser aperture diaphragm** until the desired contrast is achieved



Bright field observation

Bright field observation means observation with transmitted light using no contrast enhancement method (e.g. no phase contrast or DIC)

For bright field observation:

- rotate **condenser turret** to empty position
- switch **filter cube turret** to empty position



Condenser turret in
empty position

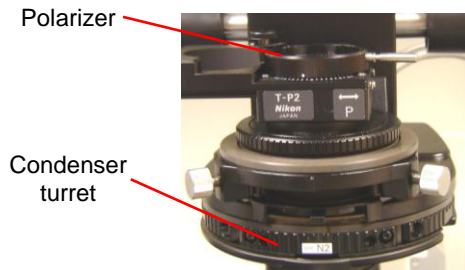
Differential interference contrast (DIC)

For DIC, you need:

- A **DIC objective** (i.e. any objective with a magnification greater than 10x)
- A **condenser prism** in the **condenser turret**
- A **polarizer** above the condenser
- An **analyzer** in the filter cube position
- An **objective prism** beneath the objective (always in position)

To set up DIC:

- Select a DIC objective (any objective except the 10x)
- The objective DIC prism required for that objective is already installed underneath the objective
- Manually rotate **condenser turret** to the N2 position (all of the DIC objectives use the N2 condenser prism)
- Insert **polarizer** into light path
- Change to the **analyzer** position in the filter cube turret

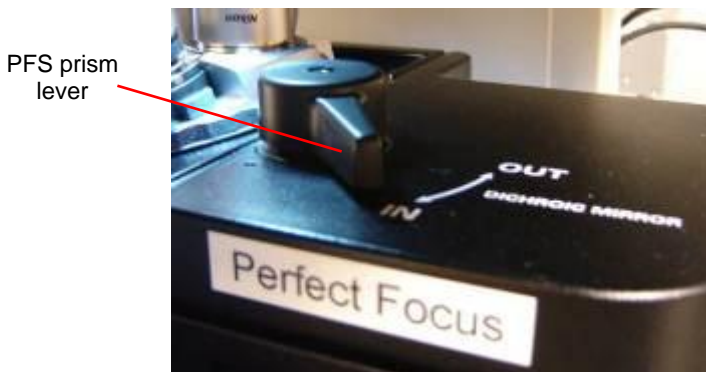


Using Perfect Focus System (“PFS”)

The microscope is equipped with a **Perfect Focus System**. When this system is engaged, the microscope will maintain a specific focus by utilizing a very long wavelength beam of light to determine what the offset from the coverslip should be. The **PFS** will only work within a small distance of the coverslip, so it will not be usable for every sample.

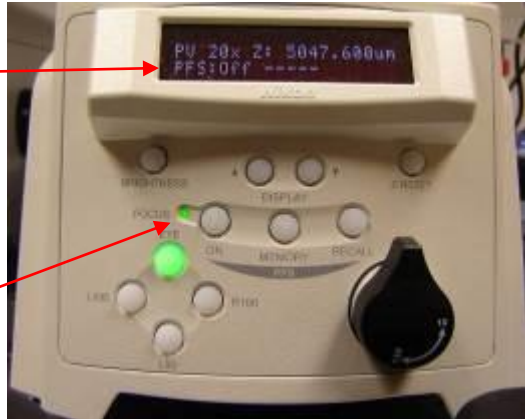
To use **PFS**, there are several things that need to happen.

- The **PFS prism** needs to be in the “in” position



- The sample needs to be in the right focal range. The **Focus** green light will be on when the sample is in the PFS range.

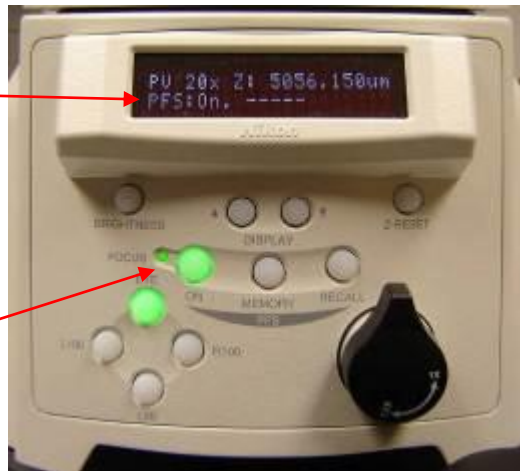
The display indicates that PFS is off.



The **FOCUS** green light indicates that the sample is in the PFS range.

- When the **Focus** green light is on, the PFS button can be pressed, turning PFS on.

The display indicates that PFS is on.



The **ON** button lights up when pressed.

- Use the **PFS focus** controls to change focus. The focusing knobs on the microscope and the ASI stage will not do anything when the PFS is on.



PFS focus



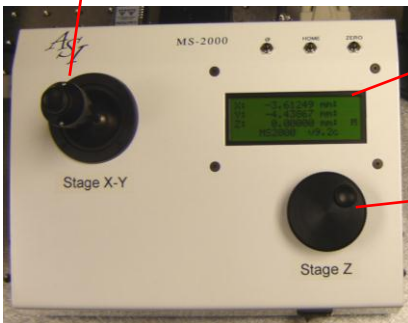
Using the ASI Stage

The microscope is equipped with an ASI motorized stage. The stage can be used to move the sample in the X-Y direction, as well as changing the focus.

ASI Stage Controls



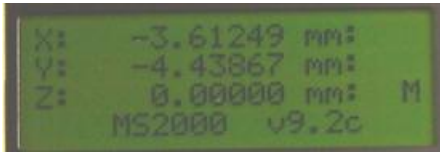
Stage X-Y Joystick - Move the joystick to move the sample. Push the button on top of the joystick to change from fast to slow



Stage Information Panel

Stage Focus

The **Stage Information Panel** displays the current position of the stage. The coordinates of the stage position can be recorded, allowing a user to easily return to a specific position on a slide.



Objectives

OIL immersion:

- Clean coverslips before using immersion objectives
- Apply oil to the objective or to the coverslip before placing your slide on the stage
- Use only NIKON IMMERSION OIL
- Use the minimum amount of oil necessary
- If you use too much oil it may run down the side of the objective and damage the optics!

To apply oil to an objective:

- tilt the oil bottle so that oil gathers in the dropper
- squeeze gently so that oil reaches the end of the dropper (hold against a piece of lens paper to avoid a mess)
- apply oil to objective by gently squeezing over the lens without introducing bubbles
- **DO NOT TOUCH THE LENS DIRECTLY!**

WATER immersion:

The 40x and 60x water immersion objectives use water rather than oil as the immersion medium.

Contact Paula for instructions on using these objectives.

Cleaning objectives

This microscope is equipped with the highest quality objectives, with a total value exceeding \$30,000!

You must exercise great care to preserve these objectives

To clean oil off immersion objectives:

- Remove specimen from stage
- Blot (not wipe!) off excess oil using lens tissue
- Use a fresh area of the lens tissue for each blot
- Repeat blotting until no more oil comes off onto the lens tissue
- Wipe the metal housing around the lens using lens tissue
- It typically takes 2 or 3 sheets of lens tissue to properly clean a single objective

What to do if you accidentally get oil on a dry objective:

- Blot off excess with lens tissue
- Then contact Paula, who will clean the lens with special cleaning fluid (do not attempt this yourself!)
- Lens tissue alone will not get all the oil off the lens and this will interfere with the optics

Specimen preparation

Some general tips:

- Select fluorochromes that are optimally excited by the confocal laser lines
- For multiple labeling, the less overlap between the excitation and emission spectra the better
- Always use #1.5 coverslips
- Mount coverslip to slide securely and seal with nail enamel or mounting medium that solidifies
- Do not observe on the microscope until the nail enamel or mounting medium has hardened!
- Secure the slide with the clips when acquiring Z series

DOs and DON'Ts

DOs

- Use only lens tissue to clean objectives
- Use Kimwipes to clean coverslips and slides

DON'Ts

- Never touch any optical surface with anything other than lens tissue
- Never clean the objectives with any kind of solvent
- Never use Kimwipes to clean objectives or any other optics
- Never use any liquid cleaners or solvents to clean the microscope optics or any part of the microscope!

When to ask for Paula's assistance

- Adding or removing objectives
- Adding or removing filter cubes
- Cleaning oil off 10x or 20x dry objectives
- Cleaning oil off the 40x or 60x water objectives
- Using the objective warmer
- Using the Okolab heated chamber
- Any other time you are unsure what you are doing!