Use of the Binocular Microscope

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Required Materials

Make sure you have the following materials:

- Microscope
- Glass slide of a biological specimen
- Labeled diagram of a binocular microscope

Module Goals

The goals of this learning module are to:

1. Identify the parts of a typical microscope and describe their functions
2. Properly use and maintain a microscope

Preview: You will learn how to

1. Get the sample in focus with the lowest power objective
2. Change to a high power objective without lowering the stage
3. Follow clean up procedure

Overview of a compound microscope

Imaging System

Stage with Sample Slide

Illuminating System

Light Source

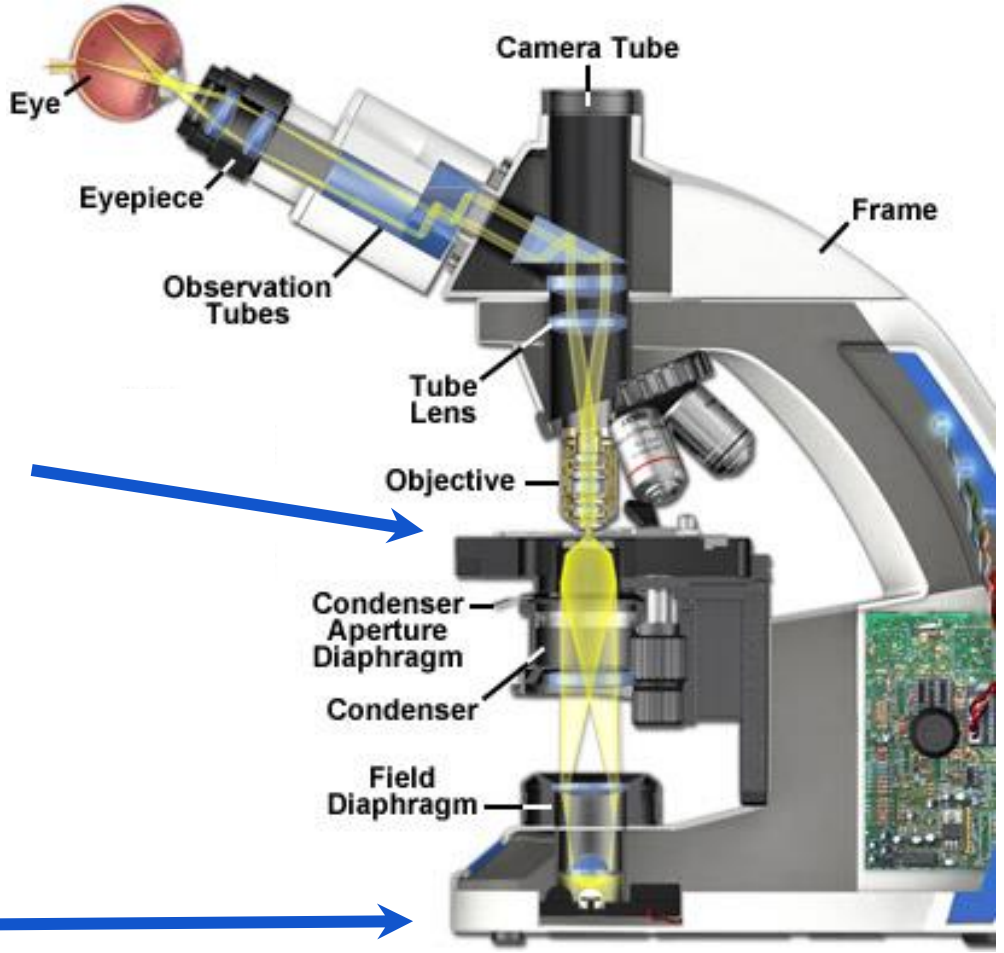


Image from Zeiss Microscopy Online Campus

Imaging System

Eyepiece

Objective Lens

The magnification of a sample is determined by the objective lens and the eyepiece

Imaging: Objective Lenses

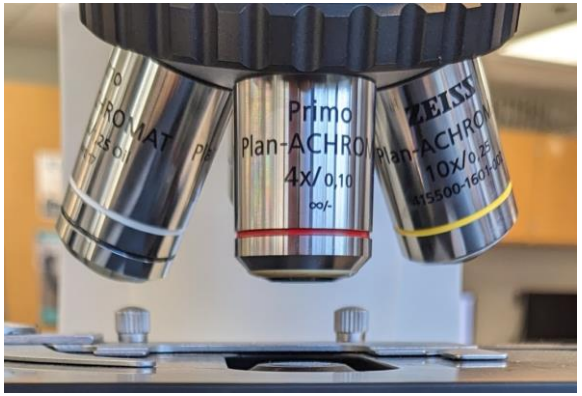
These are typical low power objective lenses from different microscopes

- Notice that each of these magnifies an object by a factor of 10
- 10 and 10X both mean 10 times larger

Imaging: Verifying Magnification

Always double check the power of magnification printed on a lens before using it:

- Determine the magnification of each objective lens on your microscope
- Confirm that the eyepieces on your microscope have a magnification of 10X



Imaging: Calculating Magnification

The total magnification is the magnification of the objective **multiplied** by the magnification of the eyepiece

Objective × Eyepiece = Total Magnification

Using a 10X objective with our 10X eyepieces gives a total magnification of 10 × 10 = 100X.

For the 40X objective, 40 × 10 = 400X

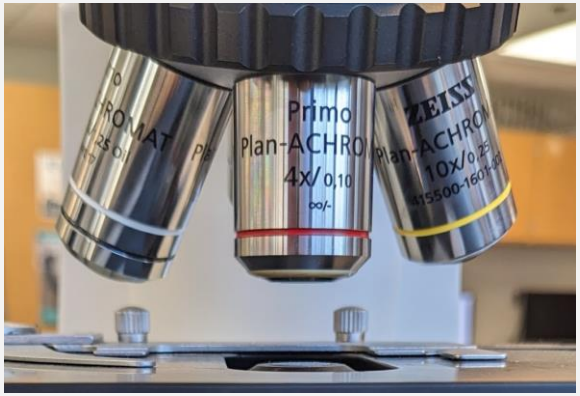
A total magnification of 400X means the image looks 400 times larger than the original sample

Use the slide provided to practice these steps

1. Start with the lowest-power objective

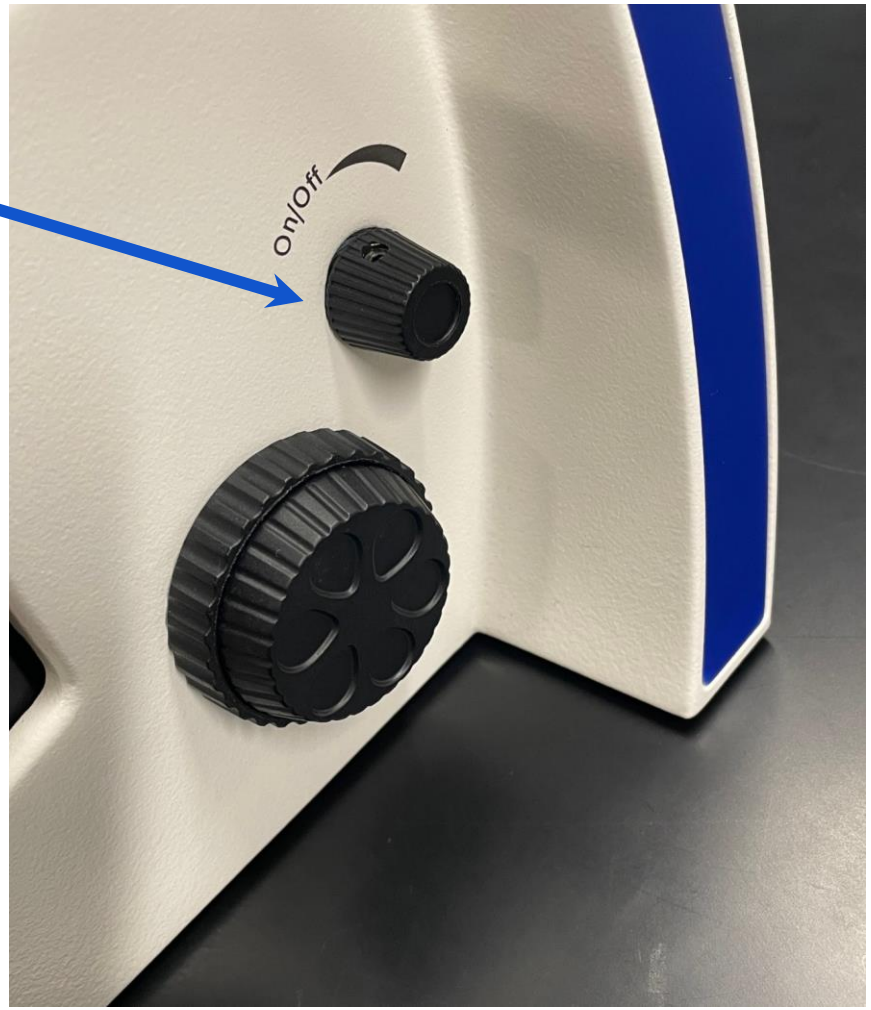
Turn the lowest power objective into place above the stage:

- Notice that all the objective lenses turn on a turret and that they click into place
- As a general rule, always use the lowest power objective lens to find your specimen
- On the microscope in front of you, this is the 4X lens and is the shortest



Light Source

The on / off rotary dial is also used to adjust the brightness of the substage lamp.



Turn on the lamp and adjust the brightness

- one or two blue LEDs should be lit up

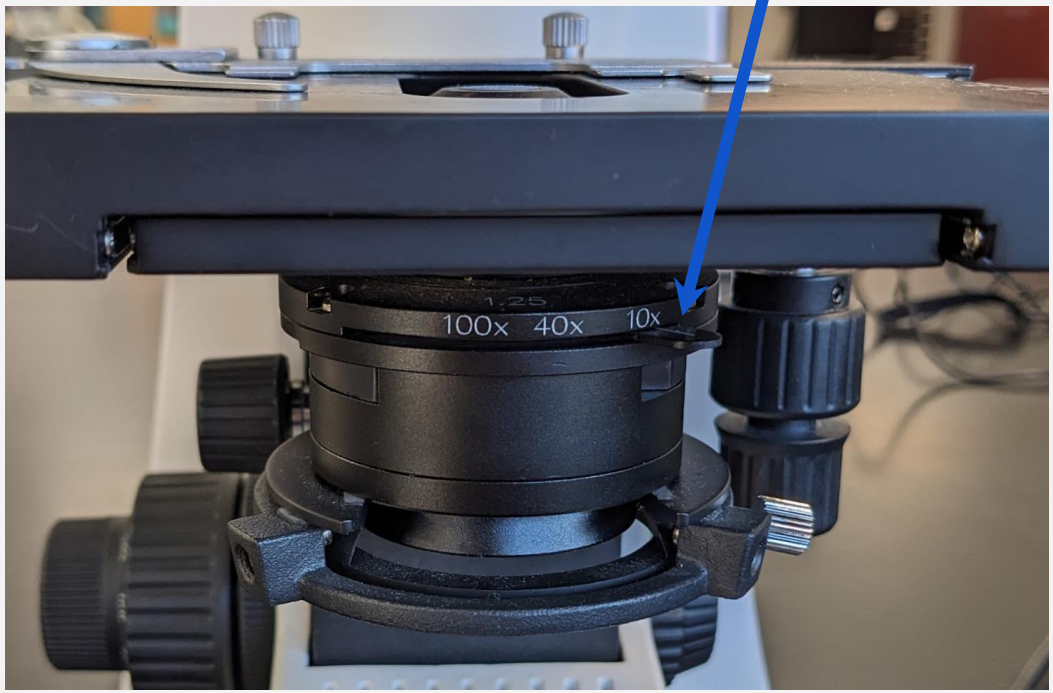
Illumination System: Diaphragm

The diaphragm controls how much light reaches the sample. It is adjusted with the diaphragm lever

2. Adjust the diaphragm and light level

When using the 4X objective, slide the diaphragm lever all the way to the right.

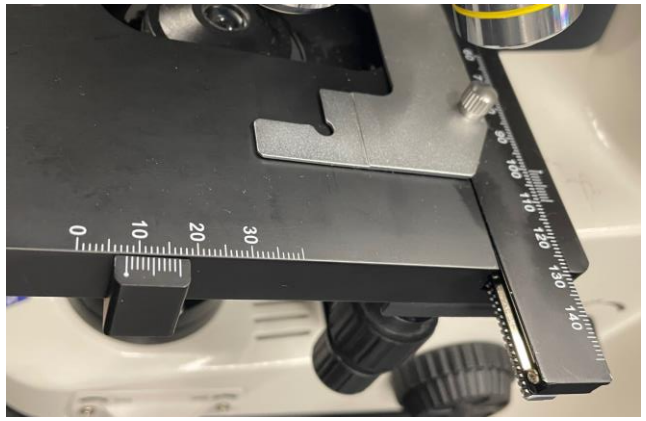
Later, we will use this to adjust the brightness.



Stage with Sample Slide

The stage holds the sample slide, specimen side up

Notice that micrometer readings indicate the position of the stage in two horizontal dimensions



Stage: Moving horizontally

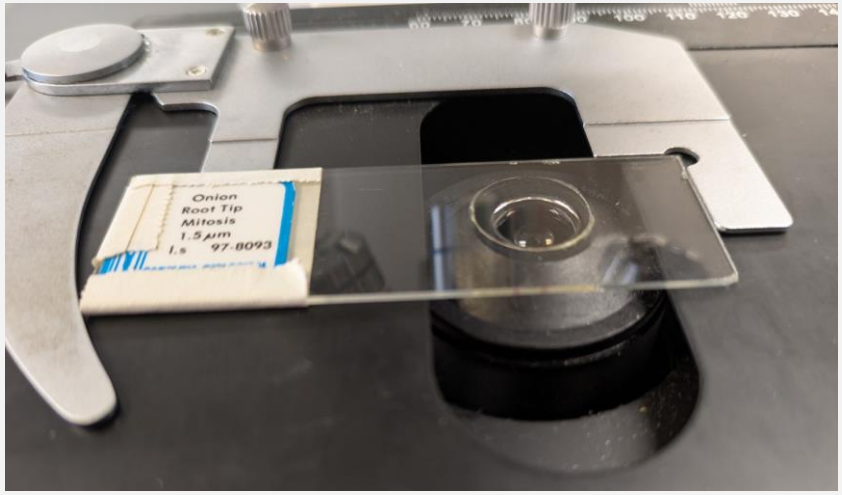
The top stage adjustment knob moves the sample slide forward and backward

The bottom stage adjustment knob moves the sample slide side to side

3. Place the sample slide

Place the slide on the stage with the **specimen side up**:

- Hold the slide by its edges
- Pull back the adjustable clip and slip the glass slide into place
- Make sure that the clip is not on top of the slide
- Use the stage adjustment knobs to center the specimen over the lens below the stage

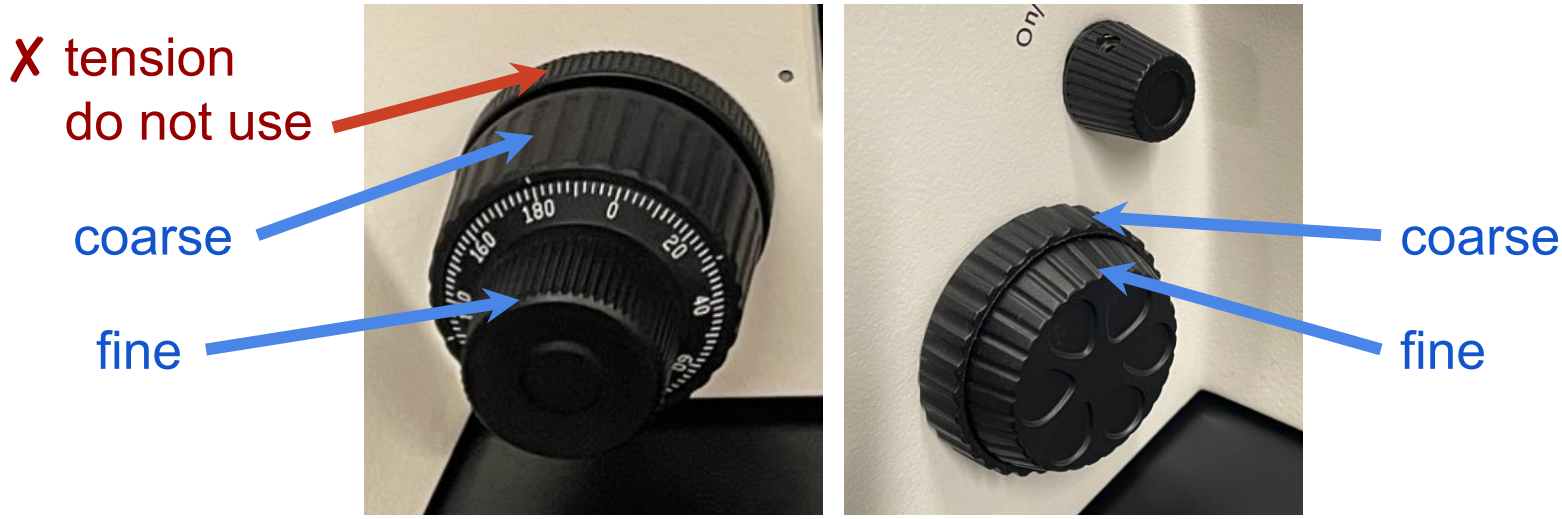


Stage: Moving up and down

Two focusing knobs move the stage up and down:

- The large knob is for **coarse** focus adjustment (moves stage)
- The smaller knob is for **fine** focus adjustment (sharpen focus)

Make sure you can identify both knobs on both sides of the microscope:

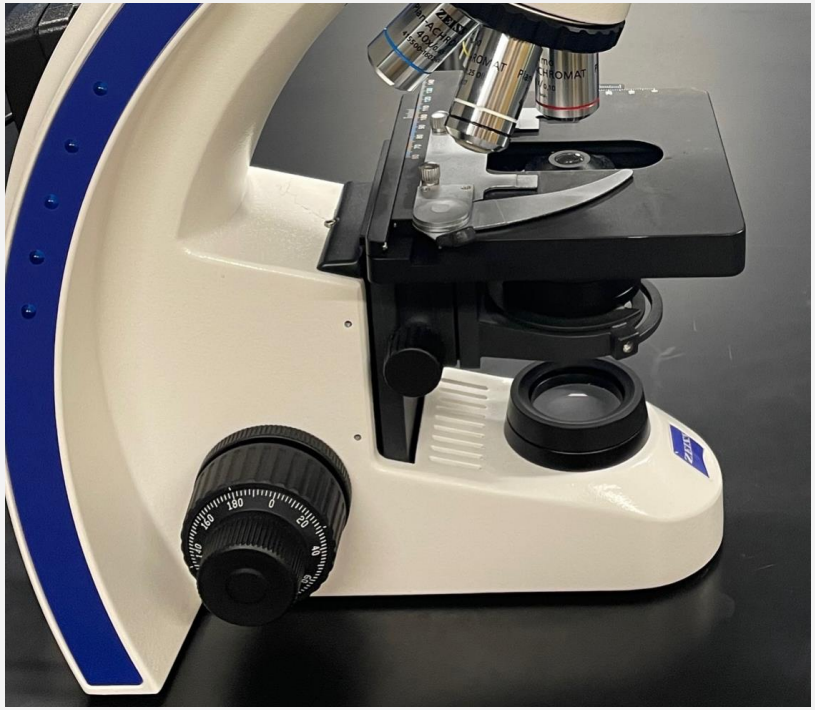


4. Raise the stage

Gradually raise the stage using the coarse focus knob until you reach a stop

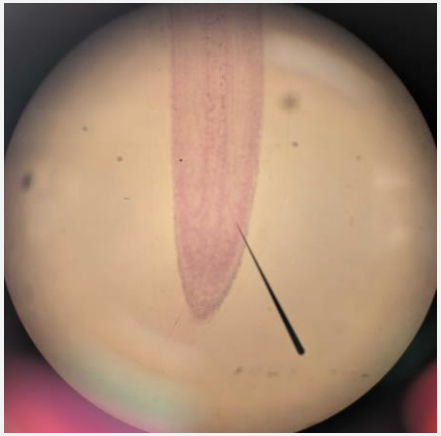
Watch from the side to make sure the objective lens does not hit the slide

Notice which direction raises the stage, and which direction lowers it



5. Focus by lowering the stage

Look through the eyepiece with the pointer. You should see a pink or brown shape:



Very slowly lower the stage using the coarse focus knob until you see the specimen and the pointer in focus

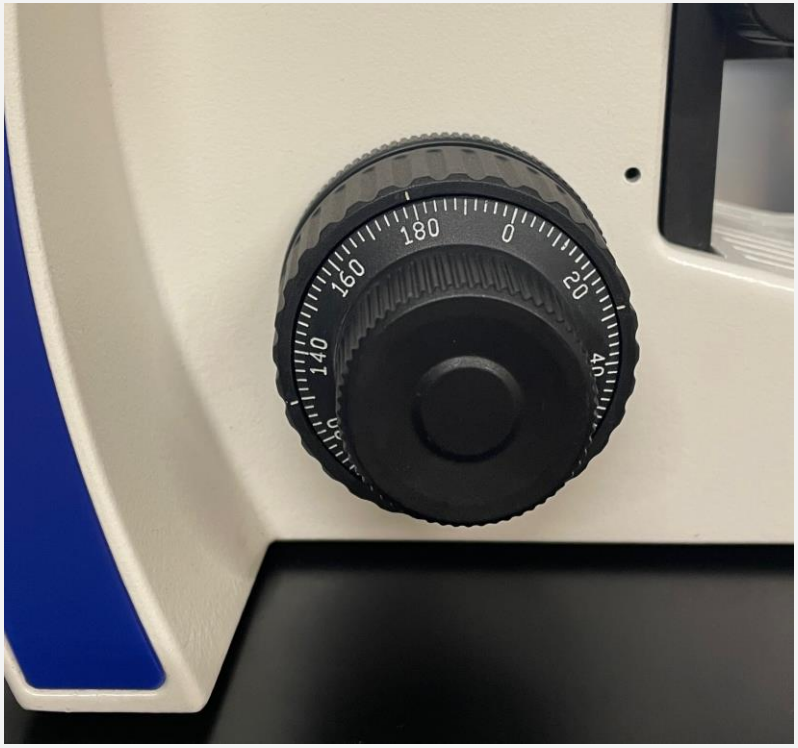
When looking through the eyepieces:

- Always lower the stage away from the objective lens
- Never raise the stage. You could smash the slide into the objective lens and ruin them both.

6. Adjust the fine focus

Looking through the same eyepiece, adjust the fine focus knob until both the specimen and pointer are sharp and clear

You can also adjust the light intensity, if necessary



7. Adjust the focus for your other eye

Make sure the specimen is in focus for the first eye

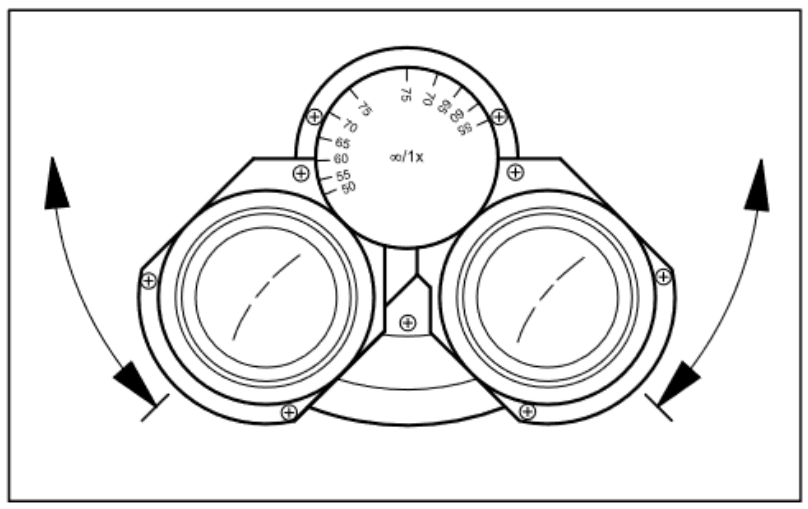
Now, looking through your other eye, adjust the telescoping knob on the eyepiece to focus on the specimen

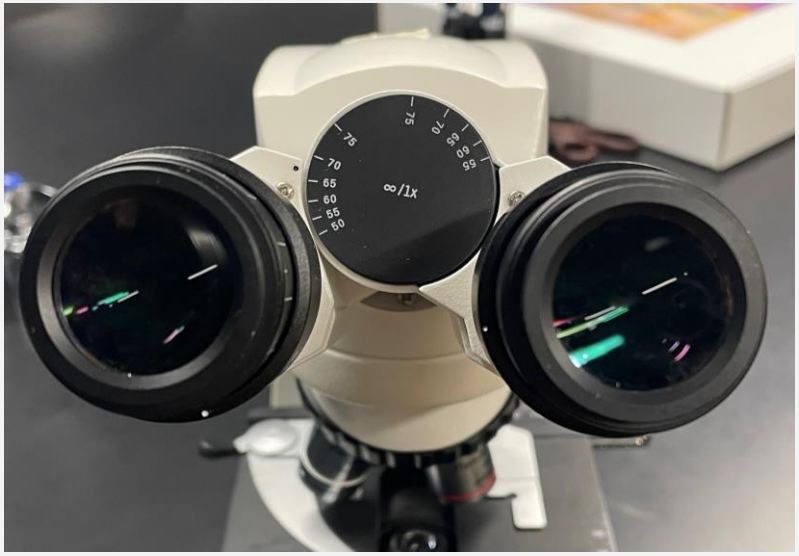
Check that the microscope is now in focus for you when looking through both eyes

- Ask an SLC consultant for help if necessary!

8. Adjust the eyepiece distance

Adjust the distance between the eyepiece tubes so that you see only one round image while looking through both eyepieces





WARNING

Do NOT use the COARSE FOCUS from this point forward FOR ANY REASON!

Touching the coarse focus knob can lead to severe damage to the microscope and will result in module failure.

How do I change the magnification?

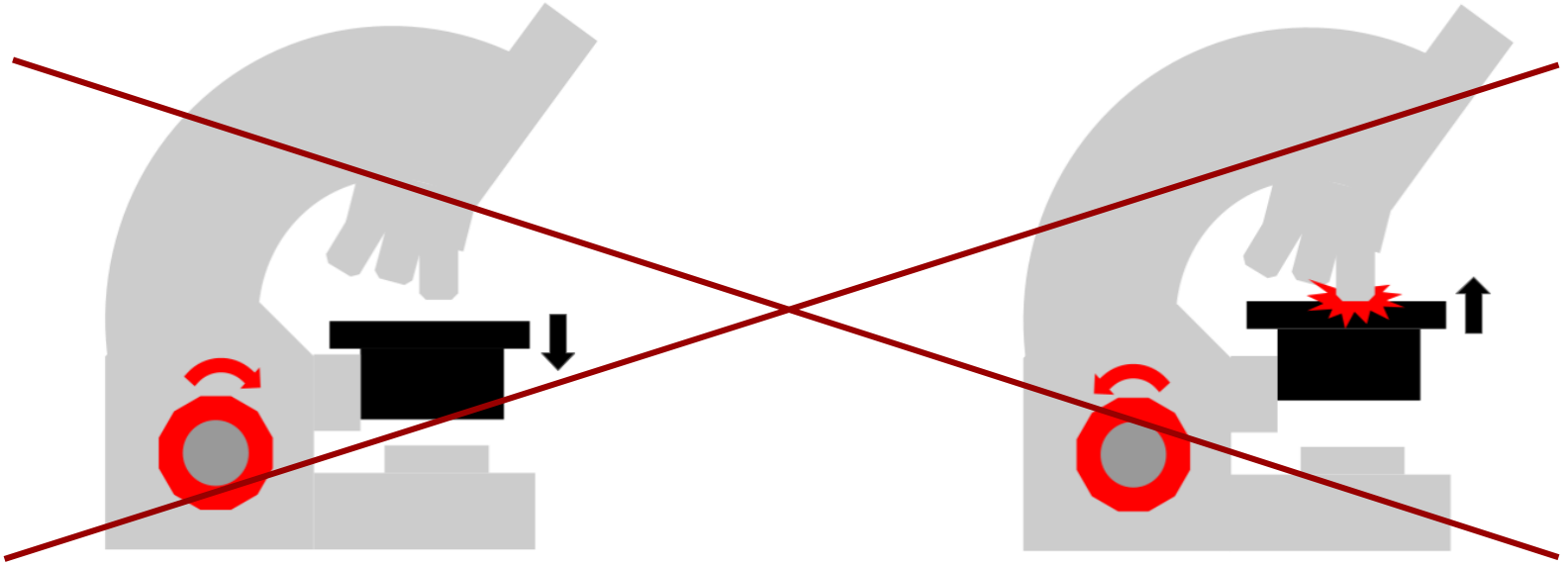
Most modern microscopes are parfocal. This means:

- Once the specimen is in focus with one objective, it should remain in focus if you change to a different objective
- When you change to a different objective, the lens should not hit the slide. But watch to be sure!

Don’t lower the stage

Don’t lower the stage.
If you move the stage down “out of the way”

You will have to move it back up to focus, risking severe damage



If it looks like the objective may get too close, ask an SLC consultant for help

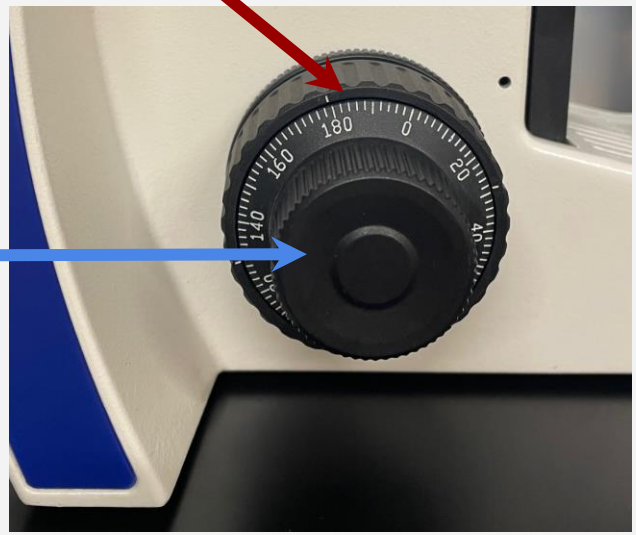
9. Change to the 10X objective

Without touching anything else, change to the 10X objective lens by rotating the turret and feeling the click as the objective comes into place.

Do not touch the coarse focus knob. Changing the stage height can smash the objective lens.

To focus, you may need to

- adjust light using the diaphragm lever
- adjust the fine focus knob
- Center the specimen using the two stage adjustment knobs



10. Change to the 40X objective

Have an SLC consultant check your slide before continuing

Repeat Step #9 with the 40X objective.

Adjust if necessary:

- the diaphragm lever
- the fine focus
- the horizontal stage position

These are the only adjustments you should make when changing objectives

Clean-up procedure

1. Rotate through the lower power objective lenses until the lowest-power objective is in place
2. Lower the stage all the way down with the coarse focus knob
3. Remove the slide
4. Turn the light source off

Summary

1. Start with the lowest-power objective
   - Raise the stage
   - Focus by lowering the stage
   - You want a clear, sharp image while looking through both eyes

2. Change to a higher-power objective, adjusting only:
   - the diaphragm lever
   - the fine focus
   - the horizontal stage position

Do not touch the coarse focus during or after changing objectives!

3. When finished, change to the lowest-power objective and lower the stage

Safely transporting a microscope

When carrying a microscope always use two hands:

one on the carrying handle

one on the base

Summary view of our Fixed Köhler microscope:

Parts to know:

1 Eyepieces

3 Illumination-intensity indicators

4 Rotary knob for switch ON/OFF and adjustment of illumination intensity

5 Fine focusing drive (right side)

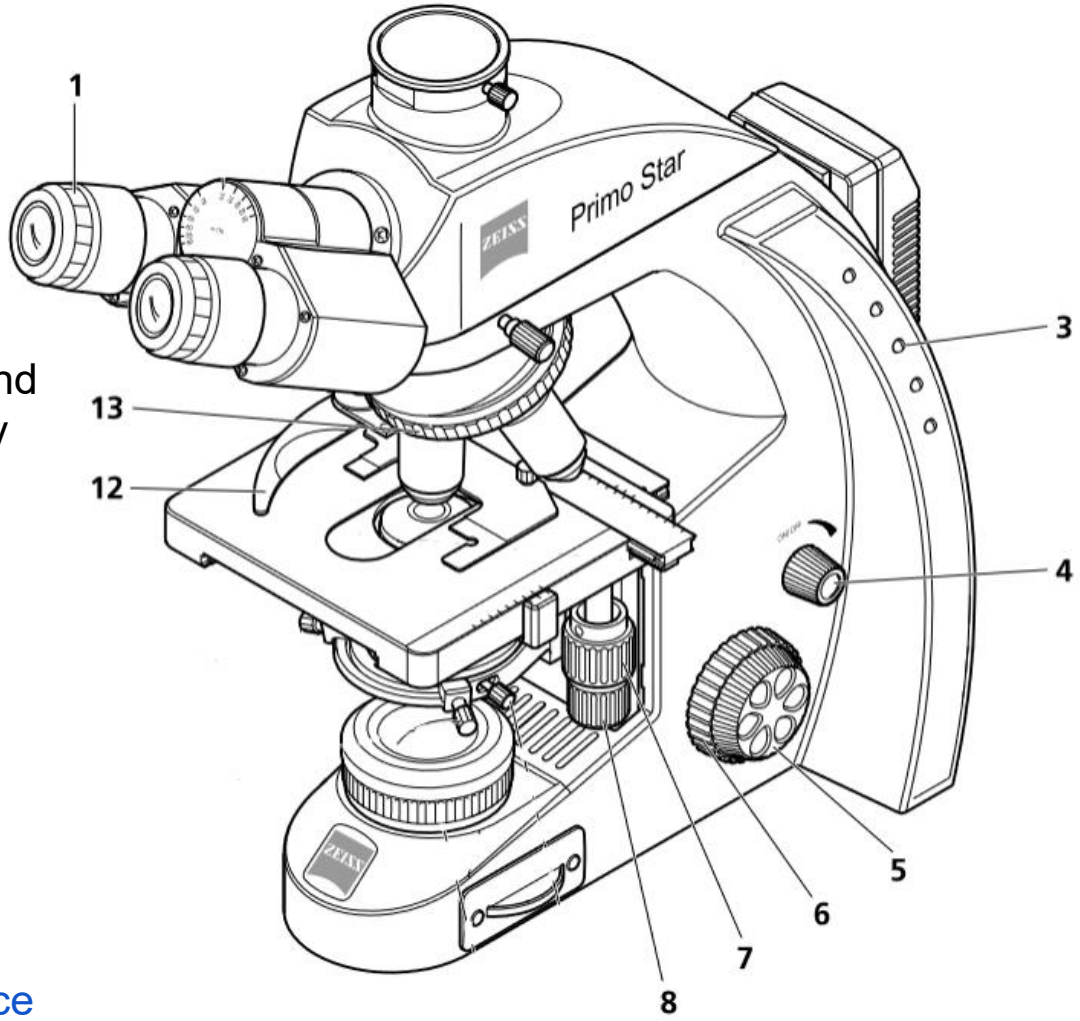
6 Coarse focusing drive (right side)

7 Control knob for X travel of mechanical stage

8 Control knob for Y travel of mechanical stage

12 Spring lever of specimen holder

13 Knurled ring of objective nosepiece



Post-Test

You have now completed the
Use of the Binocular Microscope packet

Obtain a post test from the consultant at the desk