Laboratory 1: Microscope

A. The Compound Microscope

The microscope is a delicate, precision instrument. It must be treated gently; the slightest bump or jar may damage the alignment of its lenses. Carry the microscope with care by grasping its arm with one hand, keeping the instrument close to your body and supporting its base with the other hand. **Always carry the microscope with two hands.** Microscope lenses are to be cleaned before and after each use with lens paper. **Never wipe a lens with paper toweling, handkerchief, or Kleenex.** If you wear corrective glasses for astigmatism you must use them with the microscope; if your glasses are for near-sightedness or far-sightedness, you may use the focus of the microscope rather than your glasses.

Parts of the Compound Microscope

1. The **ocular lenses** are located at the upper end of the inclined body tube. The magnification of the ocular lens is 10X.

2. The inclined **body** and the **nosepiece** hold the ocular lenses and objective lenses at a set distance from each other. The body stays in place and the **stage** raised or lowered for proper focusing).

3. The **rotating nosepiece** is attached to the lower end of the focusable nosepiece and the four **objective lenses** are mounted on the rotating nosepiece. The rotating nosepiece may be turned to either direction, so the objective may be changed while viewing a specimen. When the objective lens is in the correct position it will click into place.

4. The four **objective lenses**, so called because they are closest to the object or specimen, have individual magnifying powers of **4X, 10X, 40X and 60X**. We will not use the 60X objective in this class. The 4X objective lens is called the **low power objective**, the 10X objective lens is the **medium power objective**, and the 40X objective lens is the **high power objective**. When an objective is placed over the specimen, the objective lens will produce a magnified image which will be viewed through, and further magnified by, the ocular at the top of the body tube. A **combined magnifying action makes this instrument a compound microscope.** The magnifying power of the ocular multiplies or compounds the magnifying power of the objective. The **total magnification** is magnification of the ocular multiplied by the magnification of the objective, for example on low power the total magnification is 4 x 10 = 40.

5. The **stage** is mounted perpendicularly from the arm and below the nosepiece. This part supports the specimen slide over a hole that admits light for specimen illumination.

6. The **mechanical stage** has a metal spring-clip to hold the specimen slide in place. The spring clip should gently caress the slide. **Do not** put the spring clip on top of the slide. Two control knobs (micromanipulators) move the mechanical stage (and therefore the specimen slide) for viewing.

7. The **arm** supports the body tube. The arm is connected to the base and is used to carry the microscope.

8. The **base** bears the weight of the microscope. There is also a handle on the front of the base.
9. The **light source** or **in-base illuminator** built into the base, illuminates the specimen. The illuminator has a curved outer lens that directs light through the stage opening. The **light switch** is located on the arm. The **light adjusting knob** below the light switch adjusts the brightness of the light.

10. The **iris diaphragm** is located on the underside of the stage. It may be opened or closed to regulate the amount of light reaching the specimen. **Best detail is seen with the lowest light intensity.** This lever also affects the **depth of field.**

11. The **under-stage condenser** further directs the light from the illuminator onto the specimen. You’ll see a knob for the condenser under the stage. Normally you won’t ever have to adjust the condenser.

12. The **coarse focus adjustment** knob moves the stage up or down in initial focusing. Since a small turn of the coarse focus moves the stage a large distance, it is used **only** to bring the specimen into view, not into fine focus. **The coarse focus knob is never used when the 40X objective is in position above the specimen.** If the coarse focus is used with the 40X objective, the objective could ram the slide causing damage to it or even worse, damage to a the objective lens itself.

13. The **fine focus adjustment knob** permits exact focusing by moving the stage up or down very slightly. The fine focus also allows focusing at different levels within the specimen. All slide material has depth, no matter how thin the specimen; this third dimension can only be seen by continually focusing up or down through the material. This is critical focusing, a very important technique in good microscopy.

**Focusing**

1. Put the low power objective lens in place (the one with the red band) by turning the nosepiece on the microscope.
2. Clean the slide.
3. Place the slide on the stage
4. Use the micromanipulator to center the object over the light source (through the condenser). Don’t look through the oculars yet, tilt your head to the side so that you can see the stage while moving the slide.
5. Bring the stage to the top using the course focus knob.
6. Adjust the ocular lenses so that you can see a perfect circle while looking through them with both eyes.
7. Look through the ocular lenses and slowly scroll down with the course focus knob until the object comes into focus.
8. Use the nosepiece to change to the medium power objective lens.
9. Use the fine focus to adjust the view.
10. Use the nosepiece to change to the high power objective lens.
11. Use the fine focus to adjust the view. **NOTE: NEVER USE THE COURSE FOCUS KNOB WITH THE HIGH POWER OBJECTIVE LENS!** There is no "stop" when focusing with this objective and too great a downward movement may result in the cracking of the slide or the scratching of the lens
Procedure 1: Examining the letter “e”
Step 1: Obtain a letter "e" slide
Step 2: Before you place a slide of the letter "e" on the stage, take note of the position of the letter. Is it upside down, rightside up, backward or forward?
Step 3: Place the slide on the stage and follow the focusing procedure above. Note: Do Not Use the course focus knob with the high power objective lens.

Question 1: What is the position of the letter e BEFORE you look at it with the microscope?

Question 2: What is the position of the letter e AFTER you look at it with the microscope?

Question 3: Can you see more area of the slide on the low or high power objective lens?
Procedure 2: Examining colored threads

Step 1: Obtain a prepared slide of colored threads.
Step 2: Focus these slides as described above.

Question 1: What color is on the top? ___________; on the bottom? ________

Procedure 3: Examining prepared slides

Step 1: Obtain one of the 4 prepared slides.
Step 2: Focus this slide as described above.
Step 3: Draw what you see on the slide.

Repeat steps 1 through 3 with the 3 remaining prepared slides!

Procedure 4: Focus on Blood at 400x

Demonstrate in front of your instructor that you can focus the microscope on a prepared blood slide. Each student must do this on their own, without the help of their lab partners!