Microbiology Laboratory 2

Microscopy Background

Microorganisms are too small to be seen with the naked eye. Thus a microscope is used to magnify objects so they can be observed. A lens consists of one or more pieces of ground glass. A **simple microscope** contains only one lens and is essentially a magnifying glass. The lens functions by *refracting* (bending) light rays coming from an object and focusing them to form an image of the object. When using a **compound microscope** the subject is observed through more than one lens. Our compound microscopes use two lenses: an objective lens and an ocular lens.

Light microscopes use visible light as their illumination source. All light microscopes are limited in their magnification by the wave lengths of visible light. Therefore the total magnification has a limitation. To go beyond this limitation a different illumination source is required such as electrons in an electron microscope.

Our compound microscopes have three lens systems: ocular, objective, and condenser. The **ocular** lenses (eyepiece) is found at the top of the microscope and are what you look through. Our microscopes have two ocular lenses and are therefore *binocular* microscopes. The ocular lenses magnify the image 10×.

The **objective** lenses are mounted on the rotating nosepiece. The four objective lenses in our microscopes magnify the object 4×, 10×, 40×, and 100×. They are referred to as scanning, low power, high power (high dry), and oil immersion, respectively.

To calculate the **total magnification** (the amount the image has been enlarged) of a compound microscope the magnification of the ocular lens and the magnification of the objective lens are multiplied together. Thus when viewing a specimen under low power the total magnification is:

$$10 \times 10 = 100\times$$

Since light microscopy is based on the visible light wavelengths, the limit for light microscopy is 1000×.

The third lens system is the **condenser**, which is found below the stage. This lens system collects and directs the light from the illumination source to the object being viewed. The condenser does not affect the magnification of the object viewed.

An important component of a microscope is the **resolving power**. The resolving power is the ability of a lens to completely separate two objects being magnified. In other words, the resolving power is how close two objects can get and still be able to distinguish each as a separate object. For a light microscope the limit is about 0.2 μm. That means that objects closer than 0.2 μm would not be seen as two distinct objects. Since bacterial cells are about 1.0 μm, you can see individual cells with the light microscope. But internal structures of a bacterial cell are less than 0.2 μm so you will not be able to observe cellular structures.

One way to increase the resolving power of a microscope is to lessen the amount of light rays that are lost. When light travels from one medium to another the rays are bent. So when light passes from the microscope slide to the air and then to the glass lens, light rays are bent and lost. Using **immersion oil** on the 100× objective lens improves the resolving power. This oil has the same refractive index as glass. So when light passes from the glass microscope slide to the immersion oil and then to the objective lens no light rays are refracted away. Thus the increase of light rays helps improve the resolving power of the microscope.

A third concept that aids microscopy is **contrast** - the ability to observe detail against the background. Many biological specimens have no color, so they are difficult to see, since the light simply passes through them. One way to increase contrast is to stain the specimen with a dye so that can be distinguished from the background. Another way to increase contrast is to use special optics such as phase contrast lens to distinguish objects from the background.

Using the Microscope

Transport

The microscopes are kept in the cabinets in front of the lab. The instructor will not assign students to a specific microscope unless there are problems (like not being returned properly).

- When carrying your microscope around the lab use both hands to hold the instrument. Place one hand on the bottom and the other hand on the arm of the microscope.
- Once at your workstation: remove the dust cover and place it in a drawer.
- Unwrap the cord and plug it in.
- If you wish you may rotate the head around to use the microscope.

Procedures

- Make sure either the scanning or low power objective lens is in-line with the head (clicked into position).
- Position the slide on the stage with the subject on the upper surface of the slide. You will need to move the retainer lever to position the slide.
- Turn on the light source. If necessary position the slide so that the stained material is located in the center of the light source.
- Check to make sure the iris diaphragm is set to the correct position.
- Turn the coarse adjustment knob to bring the stage all the way up. A built-in stop will prevent the slide from running into the objective lens.
- While looking down through the oculars bring the object into focus by turning the course adjustment know. You may also need to adjust the interocular distance to match your eyes. (Slide the oculars apart or together.)
- Once you have achieved the best focus, use the fine focus adjustment knob to achieve the best focus.
- You may move the slide around with the mechanical stage control to search for what you are looking for.

High-Dry examination

- To observe a specimen at a higher magnification you need to rotate the nose piece so that the next objective lens clicks into place.
- In addition, if necessary you may need to adjust the aperture (iris diaphragm).
- You may need to adjust the fine focus to obtain the best view.
- It is important not to use the coarse adjustment knob under the high-dry objective lens. The microscope slide is so close to the objective lens it is now possible to physically run the slide into the lens and damage either one.
- Our microscopes are **parfocal**. This means that the image will remain in focus when changing from one objective lens to another. Only minimal focusing with the fine focus adjustment knob would be necessary.

Putting the microscope away

- Remove the slide
- Clean the oil immersion lens if used. Clean the 40× lens if it is dirty.
- Turn the head so it faces back over the arm if necessary.
- Turn the nosepiece to the lowest power (scanning lens).
- Unplug the cord and wrap it on the back.
- Cover the microscope with the dust cover.
- Using both hands return the microscope to the correctly numbered slot in the cabinet.

Cleaning the objective lenses

- If you cannot get your subject into clear focus or if you had the subject in focus and it becomes very blurry when switching to a new objective lens, your lens maybe dirty.
- First try wiping the lens with lens paper.
- If the image is still blurry use lens cleaner.
- Obtain a cotton swab and place a few drops of lens cleaning fluid on the swab.
- Rub the bottom of the objective lens with the cotton swab.
- Dry the lens with a fresh piece of lens paper.

Observations from Last Week's Lab

Observations of microbial growth

- Describe the colonies isolated from the environment.

- Describe the colonies grown from your finger tips.

Using scanning and low power

- Obtain a slide with a louse on it
- Under scanning power (4×), how many legs does the louse have?

- Using the low power (10×), what is at the end of the legs?

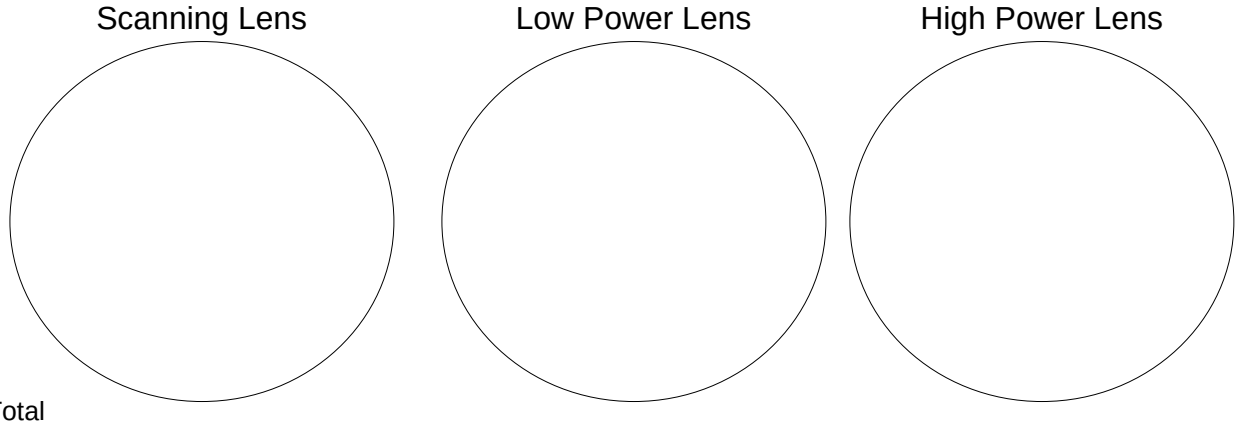
Specimen Orientation

- Obtain a slide with the letter "e" on it.
- When placing the slide on the stage notice which way the "e" is facing.
- Position the slide with the mechanical stage so the "e" is directly over the condenser.
- View the slide under the microscope under the scanning lens. Using the mechanical stage position the "e" so that it is in the center of your field of view.
  - Draw (and label magnification) the relative size of the "e" in the circle below.
  - Compared to your earlier observation which way is the "e" now?

- Move the slide to the left and right with the mechanical stage. Which way does the "e" move when viewed through the microscope?

- Move the slide away from you with the mechanical stage. Which way does the "e" move when you view it through the microscope?

- Now switch to the low power lens. Draw the "e" in relation to the field of view below.
- Now switch to the high dry power lens. Draw the "e" in relation to the field of view below.

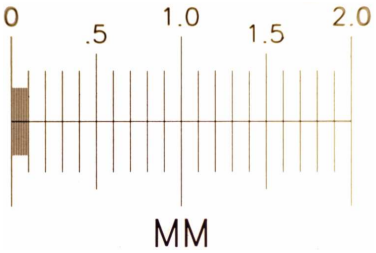


Depth of Focus

- Obtain a slide with three colored threads mounted one over another.
- Using low power (10×), find the point where all three threads cross. Then switch to high power (40×).
- Slowly focus up and down with the fine adjustment knob to observe the depth of the threads.
- Notice that when one thread is in focus the others seem blurred.
- Determine the order of the colored threads and enter the results below.

Top = Middle = Bottom =

Field of View



- Obtain a microscope slide with a ruled stage-micrometer.
- Focus on the slide under scanning power till you can read the numbers.
- Determine the diameter of the field of view for scanning, low, and high power objective lenses.

Scanning = Low = High =

Prepared slides

Obtain from the front desk the following slides and sketch the specimens under the best magnification. Don't forget to include the final magnification of your sketch.

Euglina
a protozoan

Mixed marine
diatoms
algae

Trichinella
a helminth

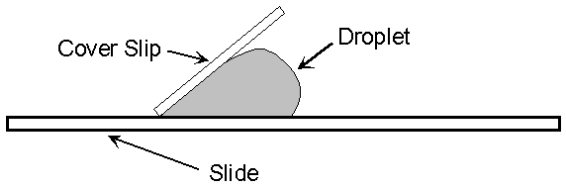
Hydra
an animal

Wet mount

Wet mounts are often used with live organisms when the observer needs to view the subject while it is in motion or is reacting to some sort of environmental stimulus. Often times wet mounts are used to observe eukaryotic cells which are much larger than prokaryotic cells. Living specimens do not survive long in the heat from an intense microscope illuminator bulb, usually because the specimen dries up. The wet mount increases the amount of time the organisms survive so they can be observed longer.

Procedure

- Remove a clean slide from your slide box and place it flat on the lab bench top.
- If the sample is not already in liquid you will need to place a drop of water or culture media on the center of the slide. This is the "wet" part of the wet mount.
- If examining an animal cell, physiological saline must be used, because if plain water is used, the cell will explode from osmotic pressure. Unlike plant cells and bacteria, animal cells have no cell wall to structurally support them.
- Place the sample to be observed in the center of the slide or in your drop of liquid on the slide.
- If the specimen is transparent, such as onion skin or check cells, stain should be added to increase contrast. Do not use stain if viewing photosynthetic cells (which already appear green due to chlorophyll), or living organisms, such as protozoans in pond water (stains will kill them).
- Place the cover glass next to the droplet along one edge. The side resting against the glass will act as the pivot point as you lower the cover glass over the sample.



- Lower the cover glass into place. As you hinge the glass downward, the drop will spread outward and suspend the sample between the slide and cover glass. Air will be pushed out. This will reduce the amount of bubbles in the wet mount.

Prepare a wet mount of pond water and *Saccharomyces* sp. (this is a unicellular fungus, but chains may be visible). Sketch what you observe and be sure to include the magnification.

Pond Water

Yeast

Label the parts of the microscope