The Light Microscope

Learning Objectives:

The student should be able to:

1. Identify parts of the light microscope and describe their functions
2. Demonstrate the correct use, care, cleaning and storage of the light microscope
3. Prepare wet mounts of live specimens for observation
4. Correctly focus on live samples and prepared slides for observation

Key Terms:

light microscope, magnification, resolution, refractive index, field of view, depth of field, ocular lens, objective lens, condenser, stage, coarse and fine focus, iris diaphragm, immersion oil, parfocal

Introduction:

Imagine being the first human to see microbial life! In the late 1600's, a Dutch businessman with no formal scientific training named Antonie van Leeuwenhoek (1632-1723) did just that. While the microscope had been invented earlier, van Leeuwenhoek perfected a technique for making single lenses that produced superior image quality as compared to other microscopes of the time. These tiny lenses, some of which were only 1 mm across, were capable of magnifying objects 200-300 times, which allowed van Leeuwenhoek to resolve details as small as 1.35 µm. With these lenses, he observed thousands of specimens including red blood cells, sperm, muscle fibers and various microbes, which he originally called "animalcules" or tiny animals. We now know that most of these microbes are single celled organisms including protozoa and even bacteria. Prior to van Leeuwenhoek, the scientific community refused to believe in the existence of living organisms too small to be seen with the unaided eye. Because of his contributions to microscopy, van Leeuwenhoek is often called the Father of Microbiology.

Light Microscopy:

The light microscope uses visible light and a series of magnifying lenses to view specimens. In today's lab, you will be using a compound light microscope to observe specimens of various sizes and shapes. The **condenser**, which sits below the stage and above the light source, gathers and focuses light as it passes through the specimen. The light then passes through various **objective lenses** where the image of the specimen is magnified. The magnified image is then further magnified by the ocular lens. The total magnification of a specimen is obtained by multiplying the magnification of the objective lens by the ocular lens. For example, if you are viewing a specimen with the 10x objective lens and a 10x ocular lens, the total magnification would be 100x.

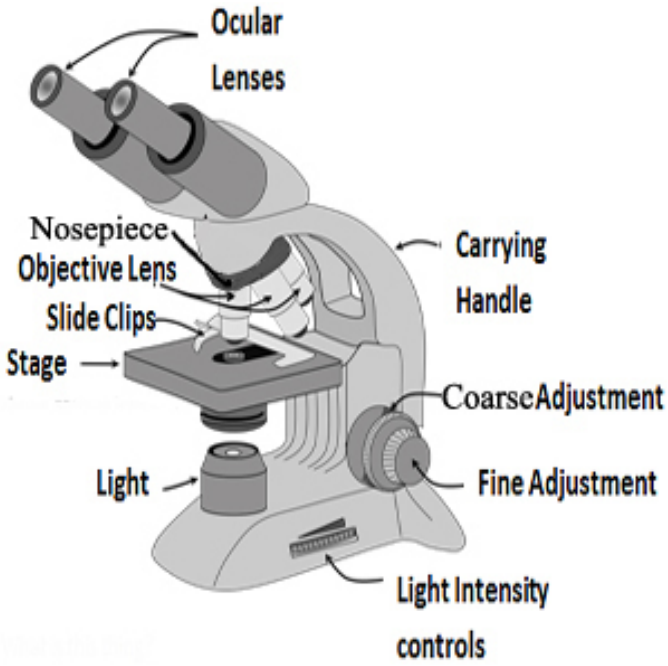
The **field of view** is the full image that you observe when looking through the microscope. You can think of this as the diameter of the circle of light when looking into the ocular lens.

Magnification and field of view are inversely proportional – that is, as the magnification increases, the field of view decreases. Using the lowest objective (4x) you may see the entire organism; however, as you increase magnification, you may only be viewing a portion of the organism. The **depth of field** is the distance from the nearest object plane in focus to the farthest object plane simultaneously in focus. Like field of view, depth of field also decreases as magnification increases.

Resolution (or resolving power) is the ability to distinguish between two points a specific distance apart. That is, the greater the resolution, the greater ability to see fine details in the specimens you observe.

Light "bends" or refracts whenever it travels into a substance with a different **refractive index**, which is the measure of the light bending capacity of a medium (such as air, water, oil). So, the light will bend as it moves from air to glass to water when you use the light microscope. The angle of refraction depends on the type of media the light passes through (air, glass, water, oil). To achieve high magnification with good resolution, the opening in the objective lens must be small, such as with the 100x objective. As the light moves from glass to air to glass, much of it is refracted and lost – especially with the small opening. To prevent this, your microscope has a special 100x **oil immersion** lens. This lens is immersed in a drop of oil that one places directly on the slide containing a specimen. The oil has the same refractive index as glass allowing for the maximum amount of light to be captured by the objective lens.

NOTE: The **working distance** is the distance between the specimen/slide and the objective lens. As you increase magnification, the working distance greatly decreases. Since the 100x oil immersion objective is immersed in oil and very close to the slide, it is important to never use the course focus with this objective. Also, oil should only be used with the 100x objective.



Materials:

Prepared slides: miscellaneous

Procedure:

1. ALWAYS carry the microscope upright by holding on the arm of the microscope with one hand and supporting the base with the other hand.
2. Clean the ocular lenses and objective lenses before you begin using LENS PAPER. You should avoid using Kimwipes or paper towels on lenses. However, you may use Kimwipes for cleaning dirty slides, stage, etc.
3. Plug in your microscope, switch it on and adjust the light intensity.
4. Place a slide over the opening on the stage under low power (4x) and center the specimen over the light.
5. Before looking into the microscope, turn the course adjustment so that the stage is at the lowest position, and ensure that the substage condenser is raised close to the slide.
6. Looking into the microscope, adjust the position of the oculars to match the distance between your eyes.
7. Gradually turn the coarse adjustment knob until the object comes into view. You may need to adjust the position of the slide on the stage using the stage adjustment knobs. Then, use the fine adjustment knob to get the sharpest focus of your specimen. NOTE: you should reserve use of the coarse adjustment knob for the 4x objective! From this point forward, you should only need to use the fine adjustment knob.
8. Use the iris diaphragm to change the amount of light coming through the specimen on the slide as needed. You may also need to adjust the substage condenser to provide a clear white background. Practice moving the condenser to see the effect it has on image quality.
9. Your microscope is parfocal. That is, if the image is in focus at low power it will be nearly in focus as you increase magnification. To view a specimen at higher magnification, first focus on 4x, center the specimen in the center of the field of view, and then slowly rotate to the next higher objective lens (10x). You should only need to focus slightly with the **fine adjustment knob**. Adjust the light as needed. Avoid using the course adjustment knob from this point forward.
10. Repeat the previous step to move to the next higher objective (40x). You should note the working distance is now greatly decreased. Never use the course adjustment knob with this objective!
11. The 100x objective is for OIL IMMERSION only. Your instructor will demonstrate the proper use of the oil immersion objective in more detail. Briefly, rotate the 100x objective slowly toward the slide, but not quite in place. Place a drop of immersion oil directly onto the slide in the light path, and then slowly click the 100x objective into

place. Use the fine adjustment knob to bring the image into focus. NEVER USE OIL ON ANY OTHER LENS, AND DO NOT ATTEMPT TO USE THE 100X OBJECTIVE WITHOUT OIL. Be very cautious not to drag the 40x objective through oil. Always clean the objective lenses after using oil immersion!

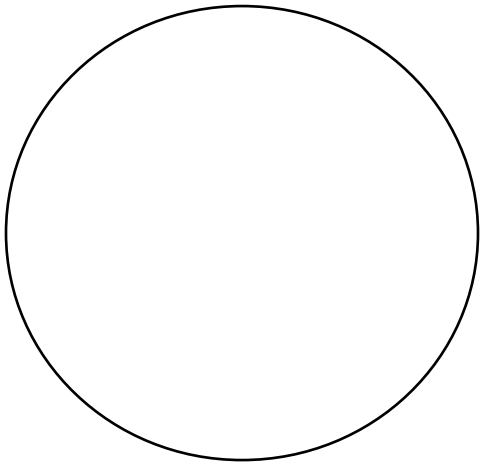
12. Before putting your microscope away: turn off the light, remove all slides, clean the stage, rotate the 4x objective into place, move the stage to the maximum distance away from the objectives, unplug the microscope and wrap the cord around the coil holder. If oil was used, be sure to clean the objective lenses.
13. Return the microscope to the appropriate cabinet and numbered slot with the oculars facing in. (see below)

Safety and disposal:

1. ALWAYS carry the microscope upright by holding on the arm of the microscope with one hand and supporting the base with the other hand.
2. Make sure power cord is securely wrapped.
3. Depending on which lab room you are in, you may have to rotate the oculars/head in order to properly place microscope into cabinet.

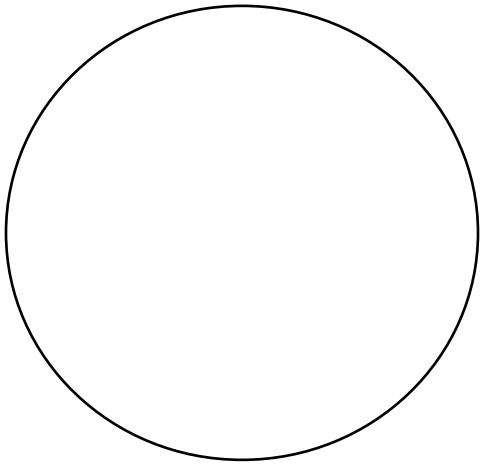
Observations:

1. Draw your observations below.



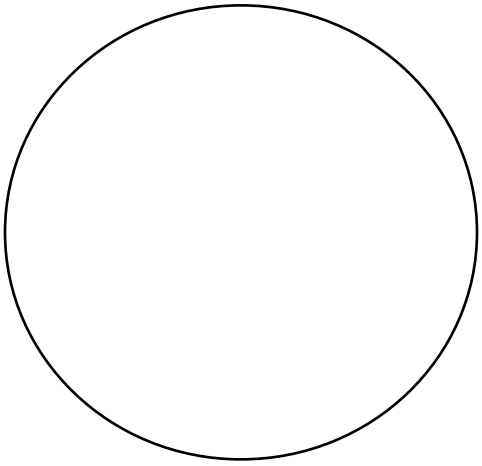
Organism:_____

Magnification:_____



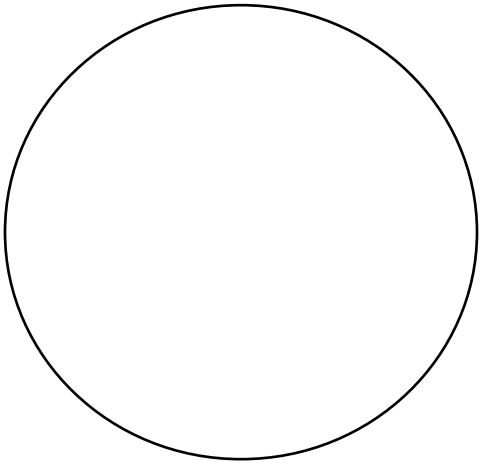
Organism:_____

Magnification:_____



Organism:_____

Magnification:_____



Organism:_____

Magnification:_____

Questions:

1. What is the function of the condenser?

2. What is the function of the iris diaphragm?

3. When should you use the course adjustment knob? How about the fine adjustment knob?

4. What is the highest possible total magnification you are able to achieve with your light microscope? How does this compare to van Leeuwenhoek's microscopes?

5. Suppose you failed to add immersion oil when using the 100x objective. Would you be able to focus on a specimen? Why or why not?

6. What was the best specimen you observed today? Why?