**Light Microscopy**

# Background on Microscopy:

“In the year 1675, about half-way through September, I discovered living creatures in rain which had stood but a few days in a new tub that was painted blue....

They were incredibly small, nay so small, in my sight that even if 100 of these very wee animals lay stretched out one against another, they could not reach the length of a single grain of sand.”

***(First Reported Description of Bacteria)***

On his own and in his spare time, Antony van Leeuwenhoek founded the science of microbiology. Using a simple (one lens) microscope, which he constructed from scraps of metal and fragments of glass, Leeuwenhoek took the first bold steps in the exploration of the microbial world. Like a curious puppy that sniffs (with a total lack of discrimination) at everything around him, Leeuwenhoek put his tiny lens (which was not much larger than the head of a pin), to work. He borrowed

ox-eyes from the local butcher and marveled at the crystalline structure of the eye-lens. He carefully examined the scales of his own skin and grunted "impossible" when he first spied the stinger of a flea. Imagine his surprise as he discovered a tiny world of "little animals" inside of a single drop of rainwater!

Today you will follow in the footsteps of dear old Leeuwenhoek. You will learn how to use the light microscope to observe living things and, in the process, hone your powers of observation. After

today you will be counted among the very few individuals that have actually "seen" microorganisms. Enjoy!

**Knowing Your Microscope**

A few years ago, when you were 15 or 16 years old, you probably spent a great deal of time sitting behind the wheel of your dad’s car while it was still parked in the driveway. While the neighbors thought you a little odd, you probably were fantasizing about the "big day" when you would earn your license and hit the road for real. Indeed these "stationary excursions" serve an important function for they provided you with the opportunity to become familiar with the important parts of the car. You learned how to hold the steering wheel, where the brake and accelerator pedals are located and other important points such as how to adjust the seat, the rearview mirror and your radio stations. As the time of the driver's test approached, this informal training was supplemented by formal instruction at school or at home during which you learned the correct way to drive and how to be comfortable and confident with this complex piece of equipment.

Today you will be instructed in the correct use of the light microscope. You will learn where each part is located, what its function is and, hopefully, will become comfortable and confident with this wonderful piece of equipment.

From this day forward you should be able to use your microscope with minimal assistance from your instructor.

**Identifying the Parts of a Light**

**Microscope**

Remove the microscope from the cabinet and carry it to the bench. ALWAYS use BOTH hands when carrying the microscope. Use one hand to grip the **arm**

and another to support the **base**. Once you have placed the microscope safely on the lab bench, remove the cover and refer to Figure 1.

We will begin with a "stationary excursion" where you will learn the parts of the microscope with the help of a diagram (Figure 1). You should be able to locate the microscope parts which are in **bold** print. The general idea of a microscope is to provide an enlarged image of a small specimen, a process which usually requires light. The **light source** is located in the base of the microscope and is simply an attached bulb which emits light . The light is directed onto the specimen by a **condenser** lens and the amount of light which enters is regulated by the **iris diaphragm**. The specimen sits like a sacrificial lamb on a platform called the **stage.** If the stage has knobs, which allow you to move the slide horizontally and vertically, it is known as a **mechanical stage.** The **coarse** and **fine adjustment knobs** help you to focus the image by raising and lowering the stage (in some microscopes the objectives are raised or lowered). After the light has passed through the specimen, it contains valuable information which reaches one of the **objective lenses** that are mounted on the **revolving nosepiece**. This lens gathers the valuable light rays and performs an initial magnification.

Finally, the information reaches your eyes through the **ocular lens**. The

ocular lens performs the final magnification of the specimen and is the lens that you look through. If there is only one ocular then you are using a **monocular** microscope; if there are two, it is a **binocular** microscope.

Now that you have mastered some important microscope parts, it is time for your written test -- Complete Table 1.

# NOTES:

**Figure 1 – The Light Microscope**

Ocular lens

Revolving nosepiece

Objective lens

Slide holder

arm

stage

Fine and course

adjustment knobs

Mechanical

stage control

light

Condenser

and iris diaphragm adjustment

base

*Please note that this microscope is not exactly the same as you will be using in lab, but contains the same basic components.*

# Table 1: Parts of the Light Microscope

|  |  |
| --- | --- |
| **Microscope Part** | **Function** |
| **1. Ocular** |  |
| **2. Objective** |  |
| **3. Condenser** |  |
| **4. Iris Diaphragm** |  |
| **5. Stage** |  |
| **6. Coarse-adjustment knob** |  |
| **7. Revolving nosepiece** |  |
| **8. Light Source** |  |

necessary.

**Setting up the Microscope**

During the next part of your training, the instructor will demonstrate the correct way to use a microscope. It is a good idea to keep this handout handy for future microscopy labs.

1. Remove the microscope from the cabinet and carry to the bench.
	1. ALWAYS use BOTH hands when carrying the microscope.
	2. Use one hand to grip the

**arm** and another to support the **base**.

1. Wind the electric cord around a pipe and plug in. This will prevent the cord from ensnaring some poor unsuspecting passerby.
2. Turn on light.
3. Clean lenses. It is a good idea to clean the ocular and objective lenses before AND after use.
	1. ONLY LENS PAPER AND LENS CLEANER SHOULD BE USED!
	2. DO NOT USE Bibulous paper - it is not designed for this purpose.
	3. Gently wipe the dirty lens with a clean piece of lens paper. DO APPLY EXCESSIVE PRESSURE.
	4. If the lens does come clean, simply moisten a small piece of lens paper with the lens cleaner and gently wipe the lens. Then wipe with a dry piece. The instructor will supply you with the lens cleaner as necessary. Overuse of the lens cleaner can damage the lens so it should be used only when

**Step-by-step Instructions on Using the Microscope**

1. Place lowest objective (10x) in position (you should hear it click)
2. Place slide on **stage** and center specimen.
3. Turn the **coarse adjustment knob** to lower the objective until the objective stops. (a built-in stop should prevent the objective from hitting the slide).
4. Look into the eyepiece - focus with the **coarse adjustment knob**.
5. Focus with the **fine adjustment knob.**
6. Adjust light with the **iris diaphragm**

as needed.

1. While watching from the side, rotate the **nosepiece** to bring the next objective (10x) into position.
2. Focus with the **coarse** (if needed) and **fine adjustment knobs.**

After using the 10X objective lens, do not use the course adjustment knobs to focus. Using the course adjustment can damage the slide or the objective lens.

# Using the Oil-immersion lens

In order to get a good look at most bacterial cells, you will need to use the **oil- immersion** lens. First center the specimen and focus with the high-dry objective (43x) then follow the procedure below.

* 1. Rotate the **nosepiece** so that the specimen is between the **high-dry** and **oil-immersion** lenses.
	2. Place a SINGLE drop of immersion oil on the slide directly over the specimen.
	3. Watching from the side, rotate the **nosepiece** to bring the **oil- immersion** lens into position.
	4. Focus with the **fine focus adjustment knob** ONLY.

Note: If the image is too dark you might want to open the **iris diaphragm** a little.

(only about four times more powerful than Leeuwenhoek's microscope which was constructed over 300 years ago!)

The **ocular lens** magnification of your microscope is **10x.**

1. Examine the smallest objective and locate the magnifying power.

(you should see a number on the side of the objective)

1. Enter this number in Table 2 below.
2. Now find and record the magnifications of each of the other objectives.

# Table 2: Magnifying Power of Objectives.

**Magnification**

Magnification refers to the apparent increase in size of an object when viewed through a microscope. Leeuwenhoek's simple microscopes had a magnifying power of about 270 times the diameter of the specimen. The modern compound microscope combines the magnifying power of the **ocular lens** and the **objective lens** and provides a magnifying power of about 1,000 x

**Magnifying Power of Objectives**

**Low-Power**

**High-Dry**

**Oil-Immersion**

The **Total Magnification** is found by multiplying the magnifying power of the ocular lens by the magnifying power of the objective lens that you are using.

# Q: Calculate the total magnification when the high-dry objective is used.

When working with a microscope there are three important concepts that you need to be familiar with. The first of these concepts is field of view. The field of view is the area that you can actually see when looking through the lens of the microscope. There is a relationship between the field of view and the magnification. As the magnification increases, the field of view decreases.

**ANS:**

**Some Important Information**

Basically what this means is that as you increase the magnification you are looking at less of the specimen.

The second concept you need to be familiar with is working distance. As you increase magnification by using the different objective lens, you will notice that as the magnification power of the lens increases, so does the length of the lens, which means that the lens is closer to the specimen. The rule here is that as the magnification increases, the working distance decreases. This why you need to be careful when using the oil immersion lens as it will be only a fraction of a millimeter from the microscope slide when it is at its proper working distance.

The third concept you need to be familiar with is depth of field. With any lens as you reach the proper working distance, you will notice that the specimen will come into focus. If you were to continue getting closer to the specimen there would come a point when the specimen would go back out of focus. The range of distance that the specimen is actually in focus is referred to as the depth of field. The relationship between depth of field and magnification is as the magnification increases, the

depth of field decreases. Once again if you think about this it makes sense.

With the increase in magnification you are actually looking at a smaller portion of the specimen and as such the working distance decreases, the field of view deceases, and the depth of field decreases. You literally are getting closer and closer to the portion of the specimen you are looking at as you increase the magnification.

Keeping these principles in mind you will come to the realization that there is a limit to how small of an object that can be viewed with a typical light microscope. This limit is more correctly referred to as the limit in the resolving power of the microscope. The resolving power of the microscope is the ability of the microscope to allow for closely spaced objects to be clearly distinguished. The limit to the resolving power is related to the wavelength of light used to illuminate the specimen and the medium through which the light passes. Generally, the light is not as critical as the medium. For higher magnifications oil is used and as such increases the resolving power and allows you see smaller objects.

In this and most microbiology labs the oil immersion lens is used to examine specimens, particularly specimens containing bacteria. The lower magnification lens may be helpful in finding a specimen on a microscope slide, but will yield very little useful information about the specimen.

**Observing Prepared Slides of Different Microorganisms**

1. Obtain prepared slides of different microorganisms.
2. In order to closely examine individual bacterial cells, you will need to use the **oil- immersion** lens. (Follow the instructions provided previously)
3. Examine the slide using the **oil-immersion** lens and sketch your observations in the space below.