Lab Exercise #1 - INSTRUCTIONS

Microscopy Basics

How to Use a Compound Light Microscope

I. OBJECTIVES:

- ✓ Learn proper use and care of a compound light microscope
- ✓ Learn how to make a wet mount slide of a specimen
- ✓ Learn how to view specimens at different levels of magnification
- ✓ Learn how to obtain and label photomicrographs of specimens
- ✓ Practice universal precautions
- ✓ Use the terminology (listed) correctly

II. TERMINOLOGY: Students should define and use the following terms:

bacterial smear	objective	total magnification
compound microscope	oil immersion	universal precautions
depth of field	ocular	wet mount
high-dry power	parfocal	
iris diaphragm	resolution	
low power	scanning power	
nosepiece	simple stain	

III. INTRODUCTION:

Principles of Microscopy

You will be using the microscope in various exercises throughout the course. It is your responsibility to take proper care of the microscope and to learn to use it correctly. When you retrieve your microscope, if you find it not in proper storage order, note that in the microscope log.

You will be working with a **compound light microscope**. Magnification is the result of two lenses: the objective and the ocular. The objectives, located on the rotary nosepiece, achieve 4 different degrees of magnification:

Name	Characteristics	Magnifying power
Scanning power	shortest objectives, red stripe	4 X
Low power	next shortest, yellow stripe	10 X
High-dry power	intermediate length, blue stripe	40 X
Oil immersion	longest, black stripe	100 X

The ocular, located at the end of the body tube, has a magnification power of 10X. The total magnification is determined by multiplying the power of the objective by the power of the ocular. (For example, 4 X times 10 X = 40 X TM). Using two sources of magnification makes this microscope a **compound** microscope.

Your microscope is referred to as a **light** microscope because the specimen is observed using visible light. The light source is an incandescent bulb which is turned on by the toggle switch at the side of the base. For proper illumination, locate the iris diaphragm, just under the stage. This lever controls the amount of light that enters the condenser which will allow for appropriate observation of contrast and depth of field. In general, close the diaphragm on low power, (allowing for reduced amounts of light to reach the stage, preventing 'burn out' of the image) and open the diaphragm for the higher power objectives.

As two small objects are moved closer to each other, a point is reached where the eye is unable to

distinguish the objects as separate entities, and only a single object is observed. The smallest distance at which two points can be seen separately is called the resolving power of the lens. The resolving power of the human eye at ten inches is 0.1 mm. This **resolution** (Figure 1) increases if you use the microscope to aid your eye, and increases as magnification is increased. However, there is a limit to the resolution of even the highest magnification lens on a light microscope, and other types of microscopes, like electron microscopes, must be used to resolve smaller structures of bacteria (like flagella) and viruses.

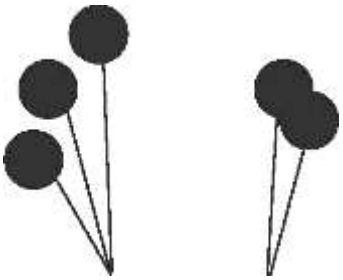

Figure 1: Levels of Resolution

As the light from your light source passes through the slide from below, and then enters air again, the light is bent, and goes off to one side or the other, rather than continuing on through the lens and to your eye. This bending (Figure 2a and b) is called **refraction**, which occurs any time light passes from material of one density to material of another density. When refraction occurs, the refracted light is lost, and it is harder for you to see your object. Refraction can be partially overcome if material of the same density is placed between the slide and the glass lens of the objective. Modern light microscopes use a particular oil for this purpose, and a particular kind of objective. These objectives are referred to as **oil-immersion** objectives, and are meant to be used with immersion oil; without oil, you can see very little through them.

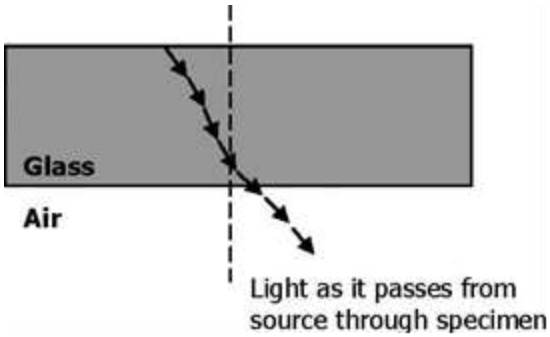

Figure 2a: Refraction of Light Rays

Pathway of Light Rays with No Refraction

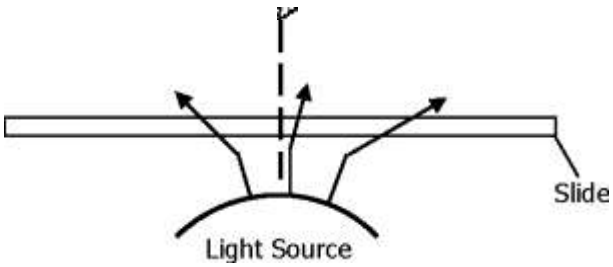

Figure 2b: Refraction of Light Rays

The term **depth-of-field** refers to the vertical distance that is in focus at any one time. Higher magnification lenses have smaller depths of field – that is, only a thin horizontal slice of your sample may be in focus at any one time. Notice this effect when you examine the elodea leaf in this lab.

The term **field-of-view** refers to how much of your sample, or the area of your sample, you can see at any one time. The field of view decreases with increased magnification. Notice this effect when you examine the items to be examined in this lab. You may need to center your specimen, using the stage adjustment lever, as you increase magnification because the item you want to observe is no longer in the center of the field of view.

A set of objectives on a microscope are said to be **parfocal** if you can change from one to another and still have your specimen in focus without having to focus more than a little. This is a very convenient characteristic of a set of lenses, because as the magnification increases, the depth-of-field decreases. If you had to find your specimen using a high-magnification objective, it would probably take quite awhile, because you would tend to miss the particular position where the specimen was in focus. With parfocal lenses, you can find your specimen using low-power, long depth-of-field lenses, and then switch to a high-magnification objective, knowing your specimen will be in focus (or very close to it) under the high-magnification objective.

In many of the exercises in the following weeks it will be important for you to see individual cells under the microscope. As you look at things under the scope today, distinguish those things that you are supposed to observe from **artifact** – things inadvertently introduced. This is a fancy way to say that you should be able to distinguish what it is you should see from junk. Be able to explain how you distinguish your sample from artifact.

Retrieving the Microscope

To get your microscope out of the storage cabinet, place one hand on the arm and another supporting the base of the scope and carry the scope to your bench top. Remove the dust cover, fold it and place it out of the way. Plug your microscope into the nearest outlet (CAUTION: Avoid placing the cord where it can become easily entangled in student paraphernalia. Unwrap only a length of cord necessary to reach the electrical outlet). You will probably find a finger cot covering the 40X objective. This finger cot is a reminder to you that you should not use the 40X objective if there is oil on your slide. Remove the finger cot when you set up your scope, and put it back on the 40x objective when you use oil and when you store the scope. Note any difficulties or improper storage on microscope in the log as needed.

Focusing the Microscope

1. Place the specimen on the slide and secure the slide to the microscope using the stage clip. Check that the slide is held by the stage clip by moving it with the mechanical stage control.
2. Click the scanning power (4X) objective into place on the nosepiece (it should already be there).
3. Use the mechanical stage control to position the specimen over the light source.
4. Use the coarse adjustment to raise the stage to its highest position. Looking through the microscope ocular, turn the coarse adjustment slowly away from you, lowering the stage until your specimen comes into focus. Remember to adjust the iris diaphragm for the best image.
5. "Fine tune" your image with the fine adjustment knob.
6. Click the low power (10X) objective into place. You will probably have to use only the fine adjustment knob to focus your object well because the objectives are parfocal.
7. Click the high-dry power (40X) objective into place on the nosepiece. Using **only** the fine adjustment knob "fine tune" the image.
8. (**Note**: When focusing, always start at low power and work your way up. This not only helps find and focus on specimens quickly but also alleviates the potential of ramming a long, high-powered objective through a slide when trying to focus with the course adjustment knob under high power. Pay attention to the **working distance** of the lens. This is the distance between the lens and the slide when the specimen is seen in sharp focus. The higher the magnification, the smaller the working distance. To avoid ramming a long objective into a slide, observe the **Working Distance Rule**: Use the coarse adjustment knob on low power only.)
9. You only use the oil immersion lens (black and white banded lens) when viewing bacteria. We will cover this in Lab 2.

Storing the Microscope

When putting your microscope away, you need to be sure it is in proper storage order. Proper storage order consists of the following:

1. Microscope stage is clean.
2. Oil cleaned from the oil immersion lens and all other parts of the microscope.
3. 4 X objective clicked into place (shortest lens).
4. Stage is in the lowest possible position.
5. Dust cover is on.

Universal Precautions

At various times in your Health Careers labs you will use blood or body fluids as a sample. In the clinical environment exposure to blood and body fluid is common. As you begin your preparation for the clinical environment you must understand and practice universal precautions. Universal precaution is an approach to infection control that treats all blood and body fluids as if it is contaminated with a lethal infectious microbe (i.e. HIV, Hepatitis B, etc). Due to the highly infectious and lethal nature of such agents we must be extremely cautious. It is your responsibility to ensure that

- ➔ **No one** comes in contact with your body fluids. Properly dispose of any material that is contaminated with your body fluid **immediately.**
- ➔ Do not come in direct contact with another person's body fluids.
- ➔ Always wear gowns, gloves and safety glasses when engaging in risky laboratory procedures such as obtaining or handling blood products. (Our lab does not contain any procedures where this is an issue, but it is best to be aware of the rule.)

In this lab you will sample your cheek cells. This sample is considered to be a biologically hazardous material and should be treated accordingly.

IV. MATERIALS (In addition to supplies found in your supply drawer):

Newspaper print
Microscope
Biohazard bags
Dropper bottle of DI water
Dropper bottle of physiological saline
Dropper bottles of Methylene Blue & Iodine (stains)
Elodea leaf
Onion bulb
Toothpicks
Disinfectant tray
Crystal violet stain

V. PROCEDURE: Lab Exercise #1

Observe the Letter "e"

A. To familiarize yourself with the workings of the microscope, perform the following exercises:
   1. Cut a small case letter "e" from the newspaper. Prepare a "wet mount" using the following technique:
      a. Get a clean microscope slide
      b. Place a drop of water on the slide
      c. Place the "e" in the drop of water
      d. Apply a cover slip
   2. Observe the "e" under scanning, low and high power. Draw or take a photomicrograph (depending on which version of the lab report you are completing) of what you see at 100xTM and 400xTM and label with the magnification power. (Note: Always label micrographs with the total magnifying power).
   3. What happened to your field of view as you increased your magnification? (Think about how much of the '"e" you see as you go from scanning to high power.)
   4. Compare the '"e" that you observe with your unaided eye to the view through the microscope. What is different about it? (i.e. Look at the "e" the way it is mounted on your slide and then view it through the microscope. Does its orientation change?)
   5. Create a micrograph of your "e" at 100xTM and 400xTM. A micrograph is a photo of what you see through the microscope.

Observe Plant Cells (eukaryotic cells that have a cell wall)

B. Observe the following and describe what you see:
   1. Elodea -Mount using water and a cover slip.
   2. Onion skin – Mount using a drop of iodine stain and a cover slip.

One lab partner should prepare one type of plant cell and the other lab partner, the other type. Then look at each others samples so that you are able to view both types. Then make a micrograph of both cell types at 100xTM and 400xTM. Label as many cell parts as you can recognize under magnification.

Observe Animal Cells (eukaryotic cells with NO cell wall)

C. To review the structure of eukaryotic cells, obtain cell scrapings from the inside of your cheek. You will use a toothpick to scrape your cheek and then rub the toothpick in a drop of saline on a microscope slide. Take special note of all the items that pose a 'body fluid' hazard and dispose of them properly as directed.
   1 Obtain a clean microscope slide.
   2 Place a drop of physiological saline (0.9% NaCl) on the slide.
   3 Using the flat end of a toothpick, **gently** scrape the inside of your cheek and then swirl the toothpick through the saline.
   4 **Dispose of the toothpick in hazardous waste.**
   5 Obtain the methylene blue. Apply a drop and then add the coverslip.
   6 Make a micrograph of the stained epithelial cells at low and high power. Label as many parts to the cell as possible.
   7 Discard this microscope slide and coverslip into the disinfectant tray on your bench.

Proper Storage of Your Microscope

D. To practice proper care of your microscope, make certain to clean it and put it away properly at the end of this exercise.
   1. Make certain the slide is removed from the stage.
   2. Clean all lens with **Lens Paper** if there is excess oil.
      a. Obtain a clean sheet of lens paper.
      b. Rub oculars to clean as demonstrated.
      c. Rub objectives to clean, starting with the scanning power objective through the high power objective. Clean the oil immersion objective last.
   3. Put the scanning power objective in place.
   4. Pull the body tube away from the stage (i.e. lower the stage as far as possible)
   5. Wrap the cord.
   6. Return the proper storage location in the cabinet.