USING THE MICROSCOPE TO OBSERVE CELLS

*****IMPORTANT!!!!!
BEFORE VISITING YOUR LEARNING CENTER TO CARRY OUT THIS LAB ACTIVITY PLEASE READ BELOW

Before you visit your Learning Center to use the microscope, it is important that know what you need to carry out the lab activity. Please read the lab description carefully before attempting the lab activity.

Also bring the following items with you to your Learning Center when you go to do the lab activity: (1) your dissecting tools from your fetal pig dissection kit (you will need your forceps, scissors, and teasing needle); (2) a piece of paper with small text printed on it (e.g., a page from a newspaper, book, or brochure - paper must be thin, not thick); and (3) something tiny (e.g., pollen grains from a flower, flea, mosquito, tiny spider, hair shaft, etc. - it must be small and thin enough for viewing under the microscope) to look at under the microscope.

Introduction

Of all the tools that scientists use, perhaps the microscope is the distinctive tool of the biologist. Since Anton van Leeuwenhoek (1632-1723) first peered at tiny "animalcules" in a drop of pond water and Robert Hooke (1635-1703) first observed the tiny compartments of cork he called "cells", the microscope has revealed a universe too small to be seen with the unaided eye.

This microscopic universe is incredibly diverse, consisting of bizarre forms of life that challenge the imagination of science fiction novelists. This is the universe of bacteria, protozoa, and fungi. It also includes very small plants and animals that, while obscure to our eyes, have a tremendous impact upon human health and the ecosystem. Finally, even large plants and animals have life history components that can only be seen through the aid of a microscope.

Through the eyepiece of the microscope, we can examine and begin to understand the fundamental units of living things: cells. An individual cell is perhaps the smallest component of a living thing that we can easily identify as being alive. While cells vary greatly in size, structure and function, all cells share some major characteristics: (1) cells are separated from the external environment by a cell membrane that maintains the internal integrity of the cell by regulating what enters and what leaves the cell; (2) cells possess a genetic instruction set in the form of DNA; (3) to stay alive, cells process energy and materials in such a way as to yield energy less capable of doing work; and (4) cells grow and reproduce.

In this laboratory activity you will become familiar with the use and care of both the standard compound microscope. In addition, you will learn how to prepare materials for observations under the microscope. Finally, you will also be introduced to the basic types of cells and their components.

The Parts of the Compound Microscope

BASE AND ARM

These components, the horizontal base and the vertical arm, form the supporting mechanism for the optical portion of the instrument. Some microscopes are hinged at the point of attachment of the base and arm to permit tilting of the instrument. Other models are constructed in a fixed position and cannot be inclined.
BODY TUBE

This portion is attached to the arm and supports the lenses. In many modern microscopes, the body tube may be inclined for easier viewing.

REVOLVING NOSEPIECE

At the bottom of the body tube is the revolving nosepiece. When rotated, objective lenses of various magnifying capacities will be brought into position. Rotate the nosepiece and note the decided "click" as each objective lens comes into place.

OBJECTIVE LENSES

The lenses attached to the nosepiece are known as the objective lenses for they are nearest to the object being viewed. They may vary in number from two to four, depending upon the make and model of microscope. Your scopes have four objective lenses: (1) the 4X scanning objective used to examine relatively large objects or to scan the slide for smaller objects to view; (2) the 10X low power objective; (3) the 40X high power objective; and (4) the 100X oil immersion objective to be used only to view very small objects (e.g., bacteria) under oil immersion procedures (oil immersion lenses have a black ring around the barrel).

OCULAR LENSES OR EYEPIECES

These are the lenses you look into. Your scopes have two ocular lenses and are called binocular compound microscopes. Other microscopes may have only one ocular lens, hence they are called monococular scopes. When you use a binocular microscope, you look through both lenses at the same time. You will note that you can adjust the distance between the lenses to fit the distance between your eyes.

MECHANICAL STAGE

The stage is the flat surface upon which you place your slide under the objective lens. The hole in the center of the stage allows light rays to pass through the object to be viewed on your slide. Your microscope has a mechanical stage that holds the slide and moves it by means of two knobs at the edge of the stage.

CONDENSER

This mechanism, located immediately below the stage of many microscopes, focuses the light in a concentrated beam onto the object being viewed. The condenser may be of the variable focus type, having a milled condenser adjustment knob for raising and lowering the mechanism.
**IRIS DIAPHRAM**

Below the condenser, or fused to it, is another mechanism for light adjustment, the iris diaphragm, which opens and closes by means of a small lever at the side of the instrument. This adjustment varies the amount of light that enters the microscope. It is the most important adjustment on your microscope for controlling the amount of light entering the instrument. The rule of thumb is that you should use the minimum amount of light necessary to view the object. Too much light can impair resolution.

**LIGHT SOURCE**

The light source for your microscope will be one of two general types; a mirror, or a built-in lamp. The mirror reflects light from some outside source up through the material set on the stage, and into the body tube. Some microscopes have a built-in lamp whose intensity is controlled by a rheostat, while others may merely be switched either on or off.

**FOCUS ADJUSTMENT KNOBS**

On the arm of the microscope you will find two adjustment knobs. These will be variously located, depending on the make and model of your microscope. The larger of these knobs is the coarse adjustment and is used for bring the image into coarse focus. The smaller of the two knobs is the fine adjustment and is used for bringing the image into fine focus.

The Proper Handling of Microscopes

When transporting the microscope, always carry the instrument upright with both hands, one hand under the base and the other on the arm. The ocular (eyepiece) lenses may be loose in the body tube and may easily fall to the floor if the scope is tilted too much.

After gently placing the scope down at your station, examine it to make sure it is in good working order. Carefully remove any slides that may have been left on the stage from a previous lab activity. If necessary, use a moist tissue to clean any dirt on the stage, base, or body tube -- but do not wipe any of the optics with this tissue. Should the lenses need cleaning, remove this dirt with lens paper (moistened lens paper may be used for stubborn smudges). Wipe gently. Be sure the switch for the light source is off.

Before using the scope, adjust the coarse focus adjustment knob to maximize the distance between the revolving nosepiece and the stage. Rotate this nosepiece such that the lowest power objective (4X scanning objective lens) is in place as the selected power.

Plug in the scope and turn on the light source. Adjust the ocular lenses to fit the width between your eyes. Note the number selected by rotating each ocular lens. This number should be close to the interpupilar distance selected on the head of your scope, although some adjustment may be necessary for differences in the focusing abilities of your eyes. The iris diaphragm should be closed down such that a minimal amount of light reaches the objective lens through the condenser.

Place the microscope slide onto the mechanical stage and position it so that the object to be viewed is centered beneath the objective lens. Looking at your scope such that you can clearly see the top of the stage, rotate the coarse focus adjustment knob to bring the slide nearer to the objective lens (as near as possible without making contact). From this point on, focusing should involve turning the knob to move the stage away from the objective lens.

After bringing the object to approximate focus in the field of view,
adjust the light to the minimum amount necessary to clearly resolve the object. From this point on you should only need to use the fine focus adjustment knob for further focusing.

Do not change to a higher power objective lens until you have the desired object centered and focused in the field of view at lowest power (4X scanning objective lens). When changing objective lenses, always switch to the next highest magnification, center and focus the object before moving to even higher magnification. Note that if the object was in focus under a lower magnification, it will be in approximate focus under the next highest magnification. When rotating the objective lens, always watch that the end of the lens does not hit the slide. Do not use the 100X oil immersion objective unless you are viewing very small objects (e.g., bacteria) prepared for oil immersion viewing.

Before putting the microscope away, remove the slide from the stage and wipe up any dirt or fluids left on any part of the scope as described above. Replace the cover.

**Magnification on the Compound Microscope**

The magnification of the image under observation is the result of both the ocular lens magnification and that of the objective being used. To calculate the magnification, just multiply the ocular magnification by the objective magnification. Thus if your ocular lens magnification is 10X and you are using the 4X scanning objective, your image magnification will be 40X.

**Procedures and Assignments**

When you arrive at the Learning Center you should be that the following materials are present: microscope, box of clean, blank microscope slides for making your own slides, box of cover slips for making your own slides, box of labeled commercially-prepared microscope slides, dropper bottle filled with water, lens paper (for cleaning microscope lenses if needed), box of KimWipe tissues for cleaning up small messes, and a slide disposal box for disposing of the slides that you prepare (DO NOT DISPOSE OF COMMERCIALLY-PREPARED SLIDES - return these to their box).

Be sure to carry out all tasks and answer all questions asked during the assignment below. When answering questions, answer with complete sentences.

**I. USING THE COMPOUND MICROSCOPE**

Obtain a commercially-prepared letter "e" slide from the slide box provided and set it up for viewing under the microscope at the lowest power (4X scanning objective).

A. Effect of Microscope Optics on Image Orientation

After focusing and adjusting the light intensity for optimal viewing, note the position and orientation of the image of the "e" relative to its actual position and orientation on the stage. How has the optics of the microscope changed the appearance of the image orientation from its actual orientation on the stage?

B. Effects of Moving the Slide

Using the knobs that adjust the position of the slide on the mechanical stage, move the slide to the right. What happens to the image when you make this movement? What happens to the image when you move the slide to the left? What happens to the image when you move the slide away from you? Towards you?

C. Magnification and Objective Changes
Change the objective lens to the 10X low power objective. What is the magnification of this image using this objective? About how much bigger does the image appear to you than it did using the scanning objective? How much bigger should it appear?

Change the objective lens to the 40X high power objective. What is the magnification of the image using this objective?

D. Adjusting the Iris Diaphragm

Set up the slide for viewing the letter “e” using the 40X objective. Adjust the iris diaphragm so that the aperture is at it’s smallest diameter then increase the aperture until you have just enough light to see the “e”. Adjust the focus so the edges of the “e” are sharp. Now increase the aperture diameter until it is wide open. How does increasing the aperture diameter affect the resolution of the image (as determined by the sharpness of the image)?

II. A TEMPORARY MICROSCOPE SLIDE (WET MOUNT)

A. Make Your Own Letter “e” Slide

Obtain a blank glass slide and coverslip and, if they are dirty, clean them with soap and water, rinse thoroughly with water, and wipe dry. Handle the slide by its edges to prevent smudges due to fingerprints which could obscure the final image you see through the microscope. Be careful -- coverslips are very fragile!

Cut or tear a piece of paper about 1/4 inch square with a typewritten "e" on it.

With a dropper or pipette, put one or two drops of water on the center of the slide.

Place the paper into the drop of water with the "e" right side up.

While holding the coverslip by the edges, carefully lower it to the slide at a 45° angle so that the edge of the coverslip just touches the drop of water. The, slowly lower the coverslip so that it lies flat on the slide over the letter "e". This method should prevent air bubbles from being trapped beneath the coverslip.

Examine your slide at different magnifications on your compound microscope. Does your preparation look different through the microscope than did the commercially prepared slide?

B. Your Own Wet Mount Specimen

Using the specimen you brought in to examine, prepare another wet mount and examine this specimen under the appropriate magnification and lighting for the specimen provided.

Draw a labeled line diagram of the specimen as it appeared to you under the microscope. This diagram should take up at least half a sheet of white paper. Give this diagram a figure number and descriptive title (following the rules for presenting figures).

III. OBSERVATIONS OF COMMERCIAL-LY-PREPARED SLIDES

For each of the commercially-prepared microscope slides listed below draw a labeled line diagram that exhibits the significant features observed using the 40X objective. Each diagram should take up at least half a sheet of white paper. Label significant features observed (e.g., nucleus, chloroplast, cell wall, plasma membrane, etc. - see below). Give each diagram a figure number and descriptive title.

Answer any questions presented.

A. Marine Diatoms

Diatoms are single-cell plants that secrete a glass (silicate) outer shell. In the slide the cytoplasm may be stained green.
You may also see a darker central nucleus. There are several different types of diatoms present on the slide. Be sure to label the test (shell), cytoplasm, and nucleus.

B. Typical Plant Cells

This slide portrays a plant specimen that has been sliced so thin that only thin sections of the cells appear. The cells of interest appear round or oval in the slide. In addition you should be able to see purple-stained chloroplasts around the periphery of each cell just inside of the cell wall and plasma membrane (may be difficult to see the cell membrane). You may also be able to see lightly stained nuclei in some of the cells (depends upon whether or not the slice cut through a nucleus in a cell). Be sure to label the cell wall, chloroplasts, cytoplasm, and nuclei in your diagram.

C. Human Blood Smear

The red blood cells are most easily observed at high power (40X objective). They will be relative small and faintly pink. They will also be the most numerous cells on the slide. If a red blood cell is oriented at the right angle, then it may have a clear area in the middle. This phenomenon is a result of the biconcave shape of the cell - they are thinner in the middle. What three features observed in your plant cells are missing in the red blood cells?

You may also see some larger cells with dark purple, irregular nuclei inside of them. These are white blood cells. Notice that the white blood cells are relatively rare on the slide.

Be sure to label the red blood cells and white blood cells in your diagram.

D. Frog Blood Smear

Like human blood, the most common blood cell in frog blood is the red blood cell.

In what two ways do the frog red blood cells differ from the human red blood cells?

Be sure your diagram labels the nucleus, cytoplasm, and plasma membrane of a typical cell.

E. Human Strat Squamous Epithelium

The slide presents squamous cells that have been gently scraped from the cheek inside the mouth. The smear was then fixed and stained (light blue stain). The cells will be very flat and irregular. In some cases, the cells may be folded over. Then central nucleus will be oval-shaped and stained a darker blue. Your diagram should label the nucleus, cytoplasm, and plasma membrane of a typical cell.

F. Human Simple Cuboidal Epithelium

This slide portray many large round follicles containing fluid. Each follicle is surrounded by a single layer of simple cuboidal epithelium. The round, darkly-stained nuclei should be present in each cell. Your diagram should portray a typical follicle with its surrounding layer of simple cuboidal epithelium. Be sure to label this epithelium as well as the nucleus, plasma membrane, and cytoplasm of a typical cuboidal cell within the epithelium.

G. Human Ciliated Columnar Epithelium

This slide portrays a cross section of a tubule whose innermost lining is a ciliated columnar epithelium. You may have to move the slide around a bit to find a suitable location for optimal viewing of this epithelium. Using the 40X objective you should be able to see the cilia on the surface of this epithelium. Your diagram should illustrate this epithelium labeling the cilia, nucleus, cytoplasm, and plasma membrane.
VOCABULARY

cell
compound microscope
base
arm
body tube
revolving nosepiece
objective lens
scanning objective
low power objective
high power objective
oil immersion objective
ocular lens
binocular microscope
monocular microscope
mechanical stage
condenser
iris diaphragm
focus adjustment knob
course focus
fine focus
resolution
interpupilar distance
wet mount
slide
coverslip
stain
plasma membrane
cytoplasm
nucleus
nucleolus
vacuole
cell wall
chloroplast