Lab 3: The Microscope and Cells

All living things are composed of cells. This is one of the tenets of the Cell Theory, a basic theory of biology. This remarkable fact was first discovered some 300 years ago and continues to be a source of wonder and research today. Cell biology is an extremely active area of study and helps us answer such fundamental questions as how organisms function. Through an understanding of how cells function we can discover how human ailments, such as cancer and AIDS, can be possibly treated.

The Cell Theory states the following:

1. All life is composed of cells

2. Cells are the fundamental units which possess all the characteristics of living things

3. New cells can only come into existence by the division of previously existing cells

Notice that this scientific concept about life is called a theory. In science, unlike the layman’s definition, the word theory is used for a hypothesis about which there is a large body of convincing evidence. Under experimental conditions all observations have thus far confirmed the theory. The evidence that helped formulate the theory was obtained using the microscope. The microscope is of enormous importance to biology and has extended our ability to see beyond the scope of the naked eye.

When we look at cells under the microscope, our usual measurements fail to work. In science, the metric system is used to measure objects and, as you will see, is vastly superior to our antiquated English system of measurement. Here are the basic units:

Length: 1 meter (m)

1 millimeter (mm) = 10-3 m or 1/1,000 m

1 micrometer (μm)= 10-6 m or 1/1,000,000 m

1 nanometer (nm)= 10-9 m or 1/1,000,000,000 m

Volume: 1 liter (L)

1 milliliter (ml) = 10-3 L

1 microliter (μl) = 10-6 L

Weight: 1 gram (g)

1 milligram (mg) = 10-3 g

1 microgram (μg) = 10-6 g

Temperature: 100˚ Celcius (C) = water boiling (= 212˚ F)

0˚ C = water freezing (= 32˚ F)

Converting between units can be confusing. The most effective way to do this is by using conversion factors and canceling units. For example, if you want to know how many liters are in 425 milliliters, you can set up a simple equation that looks like this.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| 425 ml | x | 1 liter | = | 425 ml = | 0.425 L |
|  |  | 1000 ml |  | 1000 ml |  |

Practice

1.2 mm = 1200 μm 0.224 m = 224 mm 225 nm = 0.000225 mm

0.023 L = 23 ml 750 ml = 0.75 L 50 μl = 0.00005 L

Part 1: Microscope Parts

The compound microscope is a precision instrument. Treat it with respect. When carrying it, always use two hands, one on the **base** and one on the **neck**.

The microscope consists of a **stand** (base +

neck), on which is mounted the **stage** (for holding

microscope slides) and lenses. The lens that you

look through is the **ocular** (paired in binocular

scopes); the lens that focuses on the specimen is the

**objective**.

Your microscope has four objectives of varying magnifications (4x, 10x, 40x, and 100x) mounted on a revolving **nosepiece**. The 100x objective is

a special oil immersion objective that needs to be used with oil - we won’t use the oil immersion objective for this course.

Positioning the specimen requires that you turn the **mechanical stage controls**, which operate the slide bracket on the surface of the stage. One control moves the specimen in the x-direction, and the

other moves the specimen in the y-direction.

Focusing on the specimen is achieved by knobs that move the stage up and down, so that it is closer or farther from the objective. There are two knobs, an outer **coarse focus** and an inner **fine focus**.

The **substage condenser** directs light through the slide into the objective. An **iris diaphragm** on the substage condenser controls the amount of light reaching the objective, and also affects the contrast of the specimen.

Part 2: Magnification

The compound microscope has two sets of lenses;

the **ocular lens** (or eye piece) which magnifies an

*Image accessed from* [*http://en.wikipedia.org/wiki/File:Optical\_*](http://en.wikipedia.org/wiki/File%3AOptical_)

*microscope\_nikon\_alphaphot.jpg on 12/20/13- in public domain*

object 10 times its normal size, and the **objective lenses** located on a revolving nosepiece. Rotate the nosepiece and notice how each objective lens clicks into place. Each objective lens has a different magnification of power written on it (such as 4, 10, 40 or 100). This number is the power of magnification for each of the objective lenses. For total magnification multiply the ocular power (10x) times the objective lens that is in place. For example, if you have a 10x ocular and a 10x objective, the total magnification is: 10x \* 10x = 100x.

Use this information to fill in the following table:

OCULAR LENS OBJECTIVE LENS TOTAL MAGNIFICATION

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| 10 | x | 4 | (scanning) | = | 40 |
| 10 | x | 10 | (low power) | = | 100 |
| 10 | x | 40 | (high power) | = | 400 |
| 10 | x | 100 | (oil immersion) | = | 1000Ə |

Part 2: Using the Compound Light Microscope

After the instructor explains the proper carrying procedures, each student should get out a compound microscope and place it before them on the bench. The instructor will then go over the procedures for using your scope. You will not need to memorize its parts.

**Complete the following procedure EVERY TIME you get your microscope out and**

**EVERY TIME you put it away.**

Getting Started

**1. Get your microscope out of the cabinet in the lab. Carry it with TWO HANDS to your table.**

**2. Before plugging in your scope, always make sure that the voltage control is at its lowest level and the light switch is off.**

**3. Plug in the microscope and turn on the light source.**

**4. Raise the substage condenser to its top position and open the iris diaphragm all the way.**

**5. Turn the nosepiece so that the 10x objective is lined up with the light source.**

**6. Place a slide on the stage and use the mechanical stage controls to move it into place.**

**7. Turn up the light to a comfortable level.**

Getting a Focused Image

**8. Adjust the interocular distance (distance between the oculars) by gently pressing the oculars together or pulling them apart until you see a single circular field of view.**

**9. Look through both oculars (i.e., keep both eyes open), but think right eye and adjust focus until the**

**specimen is clear in your right eye.**

**10. Now think left eye and turn the diopter adjustment (the moveable ring) on the left eyepiece to adjust the focus for your left eye. You should have a sense of the image suddenly “popping out” at you, sharp and clear.**

Optimizing Resolution and Contrast

**11. Resolution is the ability to distinguish two closely spaced points on your specimen, and it is always best with the iris diaphragm wide open. Contrast is the magnitude of difference between light and dark objects, and it increases as you close the aperture of the iris diaphragm. Getting the best image, then, requires that you find the right balance. Slowly open and close the iris diaphragm to get a feeling for the effect this has on your image.**

Changing Magnification

**12. Always start with the lowest power objective (4x) to get oriented and locate an area of interest, and then switch to higher power to examine interesting regions more closely. To change magnification, simply rotate the nosepiece to bring one of the other objectives into the light path.**

Finishing Up

**13. In this order: Turn down the illumination; turn off the power; switch back to the 4X objective; remove your slide; unplug the power cord and wrap it around the base of the scope; lower the stage to hold the cord in place; return your scope to the cabinet.**

Part 3: The Letter ‘e’

Materials

Light microscope Letter “e” slides

**1. Center the slide of the letter “e” on the stage with the “e” in its normal upright position. Bring the letter into focus under low power using the procedures described above.**

A. Draw what you see through the eyepiece.

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**2. Note the position of the letter “e” on the slide (using your eyes only). Compare this to what you see through the eyepiece.**

A. What do you notice about the position of the “e”?

e

The “e” is flipped and backwards.

**3. While looking through the microscope, move the slide to the left, notice which way the letter “e” moved. Now move the slide to the right. Notice which way the letter “e” moved. Do the same with moving the slide away and towards you.**

A. When you move the slide to the left on the stage, what direction does the image appear to move?

The slide moves to the right.

B. When you move the slide away from you on the stage, what direction does the image appear to move?

The slide moves toward me.

C. Why is it important to explore this?

Knowing how the controls will move the slide will help you to locate objects on your slide much easier and faster.

Part 4: Colored Threads

Materials

Light microscope Colored thread slides

**1. Obtain a slide of colored threads and view them under the scanning power.**

A. Which thread is on top? Which is on bottom?

Each slide had a different thread order.

**2. View the threads under high power (not oil immersion). Use the fine focus to figure out the order of the threads from top to bottom. As you rotate the fine focus, different strands will go out of focus while others will become more sharply focused.**

A. Are all of the threads in focus at the same time?

No

B. What is the order (from top to bottom)?

The order is different for different slides.

**3. “Depth of field” refers to the thickness of the plane of focus. With a large depth of field, all of the threads can be in focused at the same time. With a narrower depth of field, only one thread or a part of one thread can be focused at a time. In order to view the other threads, you must focus downward to view the ones underneath and upward to view the ones that are above.**

A. What happens to the depth of field when you increase to a higher magnification (increases, decreases, or remains the same)?

Decreases

B. Explain how the slide with threads could be used to answer the question above.

Knowing that each thread was layered one on top of the other and knowing their order on the slide helped me to see the thickness of the plane of focus. The plane of focus isn’t just flat. I can look at many different levels by focusing on each different level. With low magnification all the threads and levels were in focus, but with high magnification only part of the plane of focus (one thread) was in focus.

Part 5: Plant Cells

Preparing a Wet Mount

If you want to look at something small under the microscope, you must know how to prepare a wet mount of the specimen.

**1. Place a drop of water on the center of a microscope slide.**

**2. Pull off a single Elodea leaf (also called Anacharis in the aquarium trade) and place it within the drop of water.**

**3. Carefully place a coverslip at an angle against the water droplet. Then drop the coverslip onto the water and the leaf. This will reduce the number of air bubbles caught under the coverslip.**

**4. Make sure the scanning power objective is selected. [Always begin on scanning power!]**

**5. Place your slide onto the stage and secure with the clip.**

**6. Do not look through the ocular lens. Use the mechanical stage knobs to center the specimen under the scanning objective. Crank the coarse adjustment so that the scanning lens is close to the slide (look directly at the slide).**

**7. Now look through the ocular lens and slowly crank the coarse adjustment back until something comes into focus. Use the mechanical stage knobs to search for your specimen. Once the specimen is positioned in the center of the field of view, use the fine adjustment knob to resolve in more detail.**

**8. Search for any cellular organelles, such as chloroplasts, that you can find.**

**9. Remember, the leaf is alive! Can you spot cytoplasmic streaming?**

Estimating the size of objects in the field of view

To determine the size of the object you are viewing, you must know the distance across the field of view (the diameter of the total circular area you see when looking through the microscope). Millimeters (mm) are used to measure distances across the field of view on scanning power, whereas micrometers (μm) are used for greater magnification. The fields of view and approximate distances across for scanning, low, and high power are as follows:

0.5 mm

5 mm

2.5 mm

1. Drawing: Using the space below, carefully draw your Elodea at all three magnifications. Determine the length of your specimen at each magnification and place this number under the measurement bar that you draw under the specimen. Include any organelles you see.

100 µm



chloroplast

cell wall

2. There are three structures that distinguish plant cells from animal cells. Label these structures in your high power drawing.

Cell wall, chloroplast, and central vacuole (can’t see)

Part 6: Animal Cells

Materials

1 toothpick/ person Tap water Methylene blue Slide Coverslip

Procedure

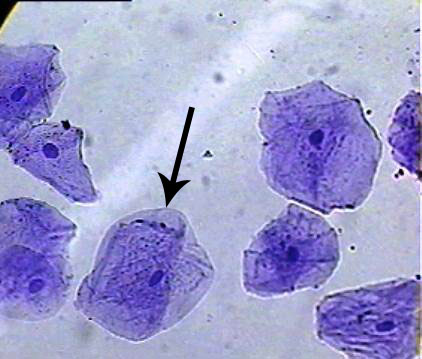
**1. Take the flat end of a toothpick and gently scrape the lining of your cheek inside your mouth.**

**2. Spread the sample on a drop of water you have already placed on a microscope slide.**

**3. Place a coverslip on top and carefully add one or two drops of methylene blue dye to the edge of your coverslip.**

**4. Allow the dye to diffuse across the slide as you examine your cells under the microscope.**

**5. Draw a typical cheek cell that has been stained with dye and label all visible parts. Include a scale bar in your drawing.**



Cell membrane

nucleus

100 µm