Name ____________________________

Pre-lab 1–Metrics and Microscopes

*Note regarding Pre-lab assignments: Pre-lab assignments should be completed after reading the lab for the upcoming week. They should be turned in at the beginning of class. All students are responsible for reading the lab manual prior to attending class.

1. Using Tables 1.1 and 1.2, complete the following metric conversions.

   3 km = ___________ cm
   2.64 mg = ___________ ng
   ___________ µl = .031 mL
   42.6 m = ___________ mm
   12 kg = ___________ g
   5.1 nl = ___________ µl

2. True or False: (circle the correct answer) The ability to clearly see an object is affected by the wavelength striking the object and by the amount of light allowed through the diaphragm onto the specimen (numerical aperture).
3. Considering that the magnification (M) of an object (based on lens magnification numbers) is inversely proportional to the amount of the specimen (D) that can be viewed through the eyepiece, complete the following calculations. An example is provided.

EXAMPLE:

The field of view at 4X magnification is 10 cm in diameter, therefore the diameter of the field of view at 10X is ______ cm, and at 40X the field of view would be ______ cm.

Consider the concept of Inverse Proportions, which means that the product of the Magnification and Diameter of the field of view at one microscope setting is equal to the product of the Magnification and Diameter of the field of view at another setting. We can write this in an equation:

$$M_1 \times D_1 = M_2 \times D_2$$

To solve the problems above, the equation becomes:

4X x 10 cm = 10X x _____ cm and for part II, 4X x 10 cm = 40X x_____cm

So, the answer is that if the field of view at 4X magnification at 10 cm in diameter, the diameter of the field of view at 10X is 4 cm (because as magnification goes up by a factor of 2.5, the amount of the specimen seen is decreased by a factor of 2.5). At 40X the field of view would be 1 cm.

SOLVE THIS PROBLEM:

If the field of view at 4X is 14 cm in diameter, then the field of view at 10X is______cm and at 40X the diameter of the field of view would be______cm. (SHOW YOUR WORK)

LABORATORY 1

Metrics and Microscopes

INTRODUCTION

The metric system and the microscope are very important tools in biology. This lab is designed to introduce you to the concept of the metric system and to the basics of microscopy and to give you practice in using both of these important tools.

PART A: THE METRIC SYSTEM

Background

The metric system is the standard in science for units of measurement. The three base units are the meter for length, the gram for mass, and the liter for volume. There are several prefixes that specify whether fractions or multiples of a unit will be used for a measurement. Table 1.1 contains the prefixes and their designations.

Table 1.1 Common metric units and their designations.

Prefix	Fractional/Multiple	Decimal	Exponential
kilo- (k)	1,000/1	1000.0	10^3
none = base unit (m, g, L)	1/1	1.0	10^0
centi- (c)	1/100	0.01	10^{-2}
milli- (m)	1/1,000	0.001	10^{-3}
micro- (μ)	1/1,000,000	0.000,001	10^{-6}
nano-(n)	1/1,000,000,000	0.000,000,001	10^{-9}

If we were measuring lengths of earthworms, our base unit would be the meter. The most practical fraction of that unit for measuring macro-invertebrates would be the centimeter. If we were aliquoting doses of medicine for injections, our base unit would be the liter, and the most practical fraction of that unit for measuring small volumes would be the milliliter. Metric conversions can be performed by a number of methods, but these all involve the memorization of equivalencies and an example of algebraic conversion follows. NOTE: the metric system is based on units of 10!

Table 1.2 Common conversion factors in the metric system.

1 km = 1,000 m	1 m = 1/1,000 km
1 m = 1,000 mm	1 mm = 1/1,000 m
1 mm = 1,000 µm	1 µm = 1/1,000 mm
1 µm = 1,000 nm	1 nm = 1/1,000 µm

Example algebraic conversion:

1,200 mm = ___________ m

$$(1{,}200mm)\left(\frac{1m}{1{,}000\ mm}\right) = \left(\frac{(1{,}200mm)(1m)}{(1{,}000\ mm)}\right) = 1.2m$$

If we know how many mm there are in a meter, then we have a conversion factor to multiply by the original measurement. Always set up the equation so that the original units cancel out. This will leave the desired units in the answer.

Here is a general rule that may help you with your calculations: If you are converting a larger unit to a smaller one (meter to cm) then move the decimal to the right; if you are converting a smaller unit to a larger one (meter to km) then move the decimal to the left.

Materials

meter stick
10 mL graduated cylinder
water
plastic ruler
triple beam balance

PROCEDURE

1. Obtain a meter stick and a smaller plastic ruler and answer the following questions.
   How many cm are in a m? ___________
   How many mm are in a cm? ___________
   What is the smallest unit on the meter stick? ___________ on the ruler? ___________
   What is the largest unit on the meter stick? ___________ on the ruler? ___________
   Would you use the meter stick or the plastic ruler to measure an object in mm? ______
   What is your height in m? ___________
   What is your height in cm? ___________
2. Using a triple beam balance, a ruler, a 10 mL graduated cylinder and water, measure the mass, length, width, and then the volume of 5 objects specified by your instructor. Record your observations in Table 1.3, then calculate the mean and standard deviation for each category of measurements. Do you think everyone in the class would obtain the exact same results? Why or why not? When you finish, your instructor may want you to pool all of the data for the class for further analysis.

   Variance formula: $$s^2 = \frac{\Sigma\,(x - \bar{x})^2}{n - 1}$$

   Standard deviation formula: $$s = \sqrt{\frac{\Sigma\,(x - \bar{x})^2}{n - 1}}$$

   Σ = sum, x = an individual number, $\bar{x}$ = mean, n = number of groups

Table 1.3 Measurements of Object 1 (class results).

Object ______	**Mass**	**Length**	**Width**	**Volume**
Your Group				
Group ____				
Group ____				
Group ____				
SUM				
MEAN				
SD				

Table 1.4 Measurements of Object 2 (class results).

Object ______	**Mass**	**Length**	**Width**	**Volume**
Your Group				
Group ____				
Group ____				
Group ____				
SUM				
MEAN				
SD				

Table 1.5 Measurements of Object 3 (class results).

Object ______	**Mass**	**Length**	**Width**	**Volume**
Your Group				
Group ____				
Group ____				
Group ____				
SUM				
MEAN				
SD				

Table 1.6 Measurements of Object 4 (class results).

Object _______	**Mass**	**Length**	**Width**	**Volume**
Your Group				
Group ____				
Group ____				
Group ____				
SUM				
MEAN				
SD				

PART B: MICROSCOPY BASICS

Background

The successful practice of microscopy depends on understanding how your microscope works, and a lot of patience and perseverance. This section will cover basic microscope types and their functions, microscope use, slide preparation and size estimation.

There are three major classes of microscopes: the stereo- or dissecting microscope, the compound microscope, and the electron microscope. In this lab we have dissecting microscopes for examining whole objects and compound microscopes for examining very small or very thin specimens on slides. Pictures taken using electron microscopes are also available. These are used for studying extremely small or extremely thin objects or features of specimens.

All microscopes require some source of energy to make specimens visible. Dissecting microscopes require an external light source to illuminate surface features of objects. Compound microscopes have either a mirrored reflector or a light below the stage to shine light through a slide. Electron microscopes use beams of charged electrons that either bounce off of the object (scanning electron microscope) or pass through the object (transmission electron microscope). Specimens may be fixed in plastic, stained with metals and sliced very thin, or a very thin coating of gold is applied. In TEM, the electrons that go through the specimens form an image on a film or screen like the shadow of the object. In SEM, the electron beam excites electrons in the gold plating, and these electrons are collected on a film or screen producing a 3-dimensional image.

The **resolving power** of the microscope is affected by the wavelength of energy used and the light gathering ability of the lens (**numerical aperture**). The equation stating the relationship between numerical aperture and wavelength is

$$R = \frac{\lambda}{N.A.}$$

For a given lens, the numerical aperture is fixed but resolution can be improved by decreasing the wavelength of the energy source. **Resolution** is the ability to clearly see details of an object at a specific distance. Visible light used in dissecting or compound microscopes has wavelengths in the range of 380 to 750 nm, and can resolve objects up to about 2 μm. The wavelength of the electron beams in electron microscopes is around 0.005 nm and can resolve up to 20 nm. **Magnification** is power of a microscope to increase the size that an object appears to be when it is viewed through a microscope.

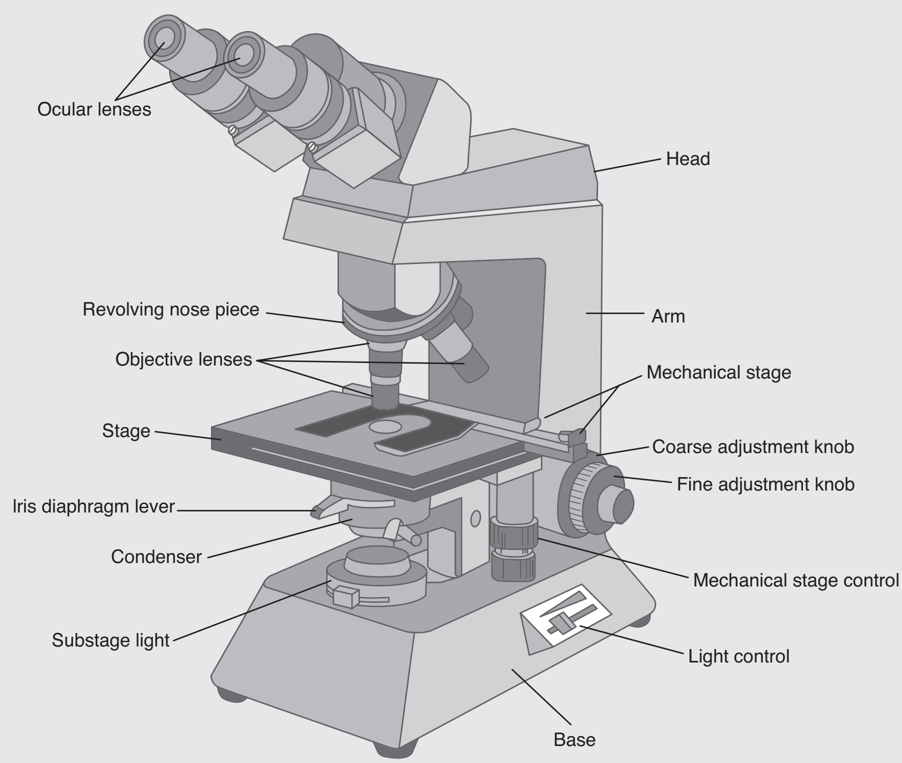

Figure 1.1 The Compound Microscope

List the function of the following parts of the light microscope:

a. Ocular–
b. Objectives–
c. Stage–
d. Condenser–
e. Iris diaphragm–
f. Coarse adjustment–
g. Fine adjustment–
h. Light–

Guidelines for microscopy:

These guidelines should help you keep your microscope in good condition as well as make your microscopy experience more rewarding.

1. Carry microscopes with two hands; one holding the neck and one supporting the base.
2. Leave the lowest powered objective facing the stage when returning scopes to the cabinet.
3. Use kimwipes and distilled water for cleaning the specimen slides.
4. Scan specimens under low power before increasing magnification.
5. When using the course focus knob, always move the objective lens away from the stage, never toward the stage.
6. Start with the least amount of light that is necessary to observe details.
7. Clean your slides with kimwipes and distilled water after use and return them to your box.

Size Estimation

PROCEDURE

Perform the following; first with dissecting scope, then with the compound scope. Record your results in the space provided.

1. Place a ruler on the stage.
2. What is the diameter of the field of view at the lowest magnification?

   dissecting ________________ compound ________________

3. What is the diameter of the field of view at the next highest magnification?

   dissecting ________________ compound ________________

4. Move the ruler to the right, which way does it appear to be moving?

   dissecting ________________ compound ________________

5. Move the ruler away from you, which way does it appear to be moving?

   dissecting ________________ compound ________________

6. On low power, focus on the numeral 4 on the ruler. Describe the orientation of the numeral on the stage, versus the apparent orientation when viewed through the microscope.

   dissecting ________________ compound ________________

Several times throughout Biology for Majors Lab I and II, you will be asked to determine the sizes of cells and structures. In biology, it is often desirable to measure cells or structures in order to distinguish different objects or to describe them—perhaps for the first time.

1. At the lowest magnification, measure the width of the field of view.
2. Set up an inverse proportion to determine the width of the field of view at other magnifications. (If the magnification increases 2.5 times then the field of view will decrease by a factor of 2.5).
3. By knowing the width of the field of view at all magnifications, you can estimate the size of any object by approximating its proportion of the diameter.

   Example: If the field of view = 10 mm at 4X; then at 40X the field of view = 1 mm.

How to Prepare a Wet-Mount Slide:

1. Begin with a clean slide.
2. Place a drop of liquid or a specimen and a drop of liquid.
3. Put the edge of a coverslip near the liquid and lower it slowly. See Figure 1.2.
4. Examine the specimen.

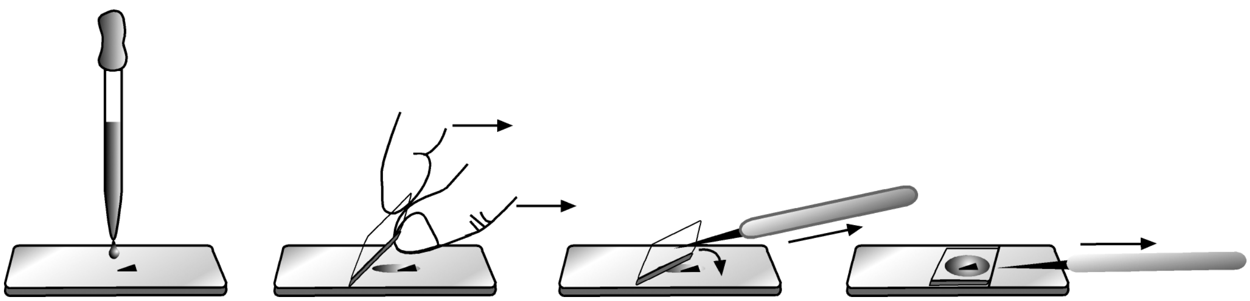

Figure 1.2 Wet Mount Slide Preparation.

PART C: OBSERVING MICROORGANISMS

Materials

hay infusion	
small petri dish	dissecting needle
dissecting microscope	tap water
compound scope	slides and coverslips

Procedure

1. With a pipette, siphon some of the liquid from the hay infusion into a small petri dish, and examine under the dissecting microscope.
2. Record the types of organisms you find in your samples (find at least 3 different types). Average several estimated lengths for each of the three of the types you find.
3. Prepare a wet mount slide of some of the debris from the hay infusion and observe with a compound microscope.
4. For one of the organism types that you determined mean length using the dissecting microscope, repeat the determination using the wet mount slide and the compound microscope.
5. How does this estimate compare to the first estimate?

PART D: LEARNING THE RESOLUTION OF ELECTRON MICROGRAPHS

Materials

electron photomicrographs ruler

PROCEDURE

1. Using the magnification bars found on some electron micrographs determine the magnification at which four prints were made using the following equation:

$$MAG(X) = \frac{Measured\ Value}{Stated\ Value}$$

2. Using the magnification bars or the stated magnification determine the actual size of some structure on four of the prints using the following equation:

$$Size = \frac{Measured\ Value}{MAG(X)}$$

PART E: DESIGNING A CELL GROWTH EXPERIMENT (FOR NEXT WEEK)

Algae are photoautotrophic eukaryotic organisms that can be either multicellular or unicellular. We will use freshwater unicellular algae (*Dunaliella* or *Chlamydomonas*).

As a class, make a list of the types of things you think might be important for cell growth:

Among many factors that affect growth, the easiest to study are light and nutrients quality and availability. Algae need light to accomplish photosynthesis; they also need carbon dioxide (CO_2) to build organic molecules using energy obtained from light. (Discuss in which steps of photosynthesis are light and CO_2 required). You can vary light intensity, light color or CO_2 supply and observe the effect of these changes on algae growth. For example, if you chose to study the effects of light color and intensity, your treatments can be: "no filter" (control), "green filter" and "red filter." IF you chose to study the effects of CO_2 supply, the treatments can be "low" and "high" CO_2.

AS A CLASS, choose which experiment you would like to do, and decide on which levels of your variable (for example, light intensity) should be used. Your lab instructor will help.

END OF CLASS DISCUSSION

Discuss the measurement results from PART A. Why is it important to know the mean and standard deviation?

Explain why you use an inverse proportion to determine the width of one field based on measurements of another field of view? In other words, if you observe and measure the size of an object using one magnification, how would you need to adjust your size measurement if you then switched to a different magnification?

What microorganisms did you and the class see in PART C? What were their sizes? Which microscope was better for this study?

Compare and contrast the use of a compound microscope with the use of a dissecting microscope.

Metric Calculations

1. 1 mm = ___________ m
2. 1 m = ___________ mm
3. 555 nm = ___________ µm
4. 1 km= ___________ m
5. 1 nm= ___________ m
6. ___________ cm = 1 km
7. 1 inch = 2.54 cm. How many inches in 1 m? ___________
8. 1 mg= ___________ g
9. 2.5 µg = ___________ mg
10. 42 g = ___________ mg
11. 1 ng = ___________ g