

# Laboratory 1

## Scientific Calculations and Basic Lab Techniques

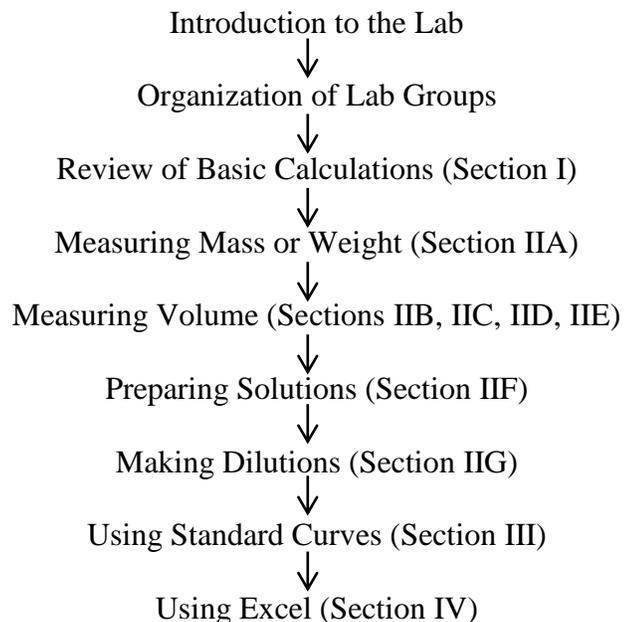
The purpose of this laboratory session is to introduce the Cell Biology Laboratory course and to organize students into groups for the rest of the semester. The basic calculations and techniques presented in this lab will be used in many of the later experiments.

In this lab, we will:

- discuss the syllabus for the course
- go through the general procedures for lab safety
- review the guidelines for laboratory reports
- organize the students into lab groups
- practice some of the scientific calculations that will be used throughout the semester
- practice some basic laboratory methods such as the use of balances, pipets, and micropipettors.
- introduce you to some of the basic features of the Microsoft Office Excel (2010 version) spreadsheet and data processing software package
- demonstrate the use of Excel to organize and process data
- look at the ways in which Excel can be used to create different types of graphs

Please read the sections on **Scientific Calculations** and **Basic Laboratory Techniques** before the lab session. We will work through the calculations and the practice exercises during the lab. While the answers to the practice problems are given at the end of this portion of the lab manual, you should complete all of the steps in each calculation. Similar calculations may be included on the Data Sheet or the next lab quiz.

The following is a flow chart for this laboratory session:



## I. Review of Scientific Calculations

The prerequisites for this course include one year of General Biology with laboratory and one year of General Chemistry with laboratory. This section reviews the metric system of units and some of the basic scientific calculations that were, in most cases, covered in those courses.

### A. The Metric System

Scientists throughout the world use the metric system of units. This section summarizes these units and gives some of the common conversion factors between metric units and their American/English equivalents.

- Metric prefixes.** The following prefixes often occur before units of length, weight, or concentration.

prefix	decimal form	exponential form	abbreviation
mega-	1,000,000	$10^6$	M
kilo-	1,000	$10^3$	k
deci-	0.1	$10^1$	d
centi-	0.01	$10^{-2}$	c
milli-	0.001	$10^{-3}$	m
micro-	0.000001	$10^{-6}$	$\mu$
nano-	0.000000001	$10^{-9}$	n
pico-	0.000000000001	$10^{-12}$	p
femto-	0.000000000000001	$10^{-15}$	f

- Units of length.** The basic unit of length is the meter, which is abbreviated m.

1.0 meter (m)	= 3.28 feet	= 1.09 yards
1.0 centimeter (cm)	= 0.01 m	= 0.394 inches (1.0 inch = 2.54 cm)
1.0 millimeter (mm)	= 0.1 cm	= 0.001 m
1.0 micrometer ( $\mu\text{m}$ )	= 0.001 mm	
1.0 nanometer (nm)	= 0.001 $\mu\text{m}$	= 10 Angstroms (A)

- Units of mass.** The basic unit of mass is the **gram**, which is abbreviated **g**. Mass and weight are often used interchangeably, but they have somewhat different meanings. Mass is an inherent property of an object, while weight reflects the force of gravity on the Earth at a certain atmospheric pressure. An astronaut who has a mass of 150 pounds weighs about 150 pounds on Earth. However, even though that person has the same mass in space, his/her weight is 0 because there is no gravitation field.

1.0 kilogram (kg)	= 1000 g	= 2.2 pounds
1.0 g	= 0.0353 ounces	
1.0 milligram (mg)	= 0.001 g	
1.0 microgram ( $\mu\text{g}$ )	= 0.001 mg	

**4. Units of volume.** The basic unit of volume is the **liter**, which is abbreviated **l** or **L**.

1.0 liter	= 1.06 quarts
1.0 ml	= 0.001 liter
1.0 $\mu$ l	= 0.001 ml

**5. Units of amount.** The basic unit of the amount of a chemical substance is the **mole**, which is sometimes abbreviated **mol**. A mole is  $6.023 \times 10^{23}$  (Avogadro's number) of molecules of a substance. The mass of this number of molecules in grams is referred to as the **gram molecular weight**. The gram molecular weight of NaCl is 58.44, so 58.44 grams represents 1.0 mole or  $6.023 \times 10^{23}$  molecules of this chemical. Some chemicals are produced in a form in which a certain number of water molecules are bound to each molecule of the compound of interest (for example,  $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ ). In the case, the mass is most often expressed as the **formula weight** (FW).

1.0 millimole (mmol)	= 0.001 mole
1.0 micromole ( $\mu$ mol)	= 0.001 mmole
1.0 nanomole (nmol)	= 0.001 $\mu$ mole

## B. Conversion of Units

It is often necessary to move back and forth between different metric units or between metric and American/English units. While you can often do this in your head, the best way to ensure that you have done the calculation correctly is through the use of conversion factors. This is sometimes called dimensional analysis. A conversion factor is simply a ratio that expresses the relationship between two units. Either unit may be used in the numerator or the denominator, depending on the calculation required. For example, there are 2.54 cm in an inch, so this can be written as either

$$\frac{2.54 \text{ cm}}{\text{inch}} \quad \text{or} \quad \frac{1 \text{ inch}}{2.54 \text{ cm}}$$

One reason for doing a conversion of units is to express numbers as simply as possible. It is easier to understand a number expressed as 8.37 than it is a number written as 8,370,000,000 or as 0.00000837. In the case of 8,370,000,000, it would be better to use a larger unit. Instead of saying you have 8,370,000,000 nanometers, it is better to say you have 8.37 meters. In the case of 0.00000837, it would be better to use a smaller unit. Instead of saying you have 0.00000837 meters, it is better to say you have 8.37 micrometers.

As an alternative to changing units, you can also write very large or very small numbers in **scientific notation** as a power of 10. For example, the number 8,370,000,000 nanometers can be written as  $8.37 \times 10^9$  nm and the number 0.00000837 meters can be written as  $8.37 \times 10^{-6}$  m.

1. **Interconversion of metric units.** Suppose you have measured an object and found that it is 3.7 cm long.
  - a. What is the length of the object in meters (m)?
  - b. What is the length of the object in millimeters (mm)?
  - c. What is the length of the object in nanometers (nm)?

To make these conversions, multiply the given value by the appropriate conversion factor. For example,

$$\text{a. } 3.7 \text{ cm} \quad \times \quad \frac{1 \text{ meter}}{100 \text{ cm}} \quad = \quad \frac{3.7 \text{ meter}}{100} \quad = \quad 0.037 \text{ m}$$

$$\text{b. } 3.7 \text{ cm} \quad \times \quad \frac{10 \text{ mm}}{1 \text{ cm}} \quad = \quad 3.7 \times 10 \text{ mm} \quad = \quad 37 \text{ mm}$$

$$\text{c. } 3.7 \text{ cm} \quad \times \quad \frac{1 \text{ meter}}{100 \text{ cm}} \quad \times \quad \frac{10^9 \text{ nanometer}}{1 \text{ meter}} \quad = \quad 3.7 \times 10^7 \text{ nm}$$

Notice that you always cancel out the unneeded units so that the final value is expressed in the desired units. If you cannot remember a specific conversion factor (for example, between cm and nm), you can always use conversion factors that relate each unit to the basic unit of measurement, a meter, and then multiply through by all of these conversion factors.

**2. Practice problems. Record your complete calculations in your lab notebook.**

- a. How many mm are there in 5.5 cm?
- b. How many cm are there in 5.5 m?
- c. How many m are there in 555 mm?
- d. How many cm are there in 555 m?
- e. How many nm are there in 555 m?
- f. How many ml are there in 0.042 L?
- g. How many l are there in 42 ml?
- h. How many l are there in 420 ml?
- i. How many g are there in 754 ng?
- j. How many ng are there in 7.54 mg?
- k. How many kg are there in  $7.54 \times 10^5$  mg?

**3. Interconversion of metric and English units.** Suppose you have measured the weight of object and find that it is 1.85 pounds.

- a. What is the weight of the object in kilograms?
- b. What is the weight of the object in grams?
- c. What is the weight of the object in nanograms?

Again, you can solve this type of problem by using the appropriate conversion factors.

$$\text{a. } 1.85 \text{ pounds} \times \frac{1 \text{ kilogram}}{2.2 \text{ pounds}} = \frac{1.85 \text{ kilogram}}{2.2} = 0.841 \text{ kg}$$

$$\text{b. } 1.85 \text{ pounds} \times \frac{1 \text{ kilogram}}{2.2 \text{ pounds}} \times \frac{1000 \text{ gram}}{1 \text{ kilogram}} = \frac{1.85 \times 1000 \text{ gram}}{2.2} = 841 \text{ g}$$

$$\text{c. } 1.85 \text{ pounds} \times \frac{1 \text{ kilogram}}{2.2 \text{ pounds}} \times \frac{1000 \text{ gram}}{1 \text{ kilogram}} \times \frac{10^9 \text{ nanogram}}{\text{gram}} = \frac{1.85 \times 1000 \times 10^9 \text{ nanogram}}{2.2} = 8.41 \times 10^{11} \text{ ng}$$

#### 4. Practice problems. Record your complete calculations in your lab notebook.

- How many inches are there in 16.3 cm?
- How many m are there in 16.3 inches?
- How many mm are there in 1.63 inches?
- How many feet are there in 163 cm?
- How many mg are there in 2.27 ounces?
- How many ounces are there in 2.27 gram?
- How many g are there in 2.27 pounds?
- How many kg are there in 227 pounds?
- How many ml are there in 1.98 quarts?
- How many l are there in 198 gallons?
- How many ml are there in 1.98 gallon?

### C. Significant Figures

An important issue in any calculation is the number of significant figures. Most electronic calculators will give numbers with 8 digits, such as 23463864 or 22.056948. Are all of these figures significant and should they be included in your calculations? The short answer is **no**. For most scientific calculations, the key issue is the accuracy of the instruments used in a particular measurement. For example, the balances you will use in today's lab are only designed to weigh material to 0.01 g. While you can report a measurement of 12.67 g, you cannot assume a higher level of accuracy and say there were 12.67003 g.

Suppose you repeated a particular weight measurement five times and found values of 12.67, 13.01, 12.40, 12.93, and 13.08. You should report that the average is 12.82, even if your calculator displays a value of 12.818. You should round up to the next higher number if the third digit to the right of the decimal point is  $\geq 5$  and round down if the third digit is  $< 5$ . Likewise, a P-100 micropipetter (described below) that is used to transfer small volumes of liquid has only 3 digits on the dial, so if you set it to 0.74, you can report that you used 74  $\mu\text{l}$  of solution, but not 74.034  $\mu\text{l}$  of solution.

For most of the calculations in this course, it will be sufficient to include 2 digits to the right of a decimal point (for example, 12.67 g), or just two digits if it is a number less than 1.0 (for example, 0.0013). If you make measurements with several different instruments, the number of significant figures is determined by the accuracy of the least accurate instrument.

## II. Basic Laboratory Techniques

### A. Measuring Mass or Weight

The mass of an object is normally determined by weighing a sample on a laboratory balance. Technically, mass is an inherent property of an object while weight is the apparent mass of an object under certain gravitational forces or atmospheric forces. Since we are relatively close to sea level in Arizona, we will take the weight of an object to be the same as its mass in this course

#### 1. Top Loading Balances

Most laboratory measurements of mass or weight are made on relatively simple top-loading balances. Each balance has a metal pan on which samples to be weighed can be placed and a digital display that shows the weight of the object in grams. The display allows you to read the weight of an object to 0.01 g. While the display can be set to display weights in ounces or grams, we will only use the metric units in this course.

In using these balances, always place a plastic weighing dish, a beaker, or glassine paper on the metal pan and then place your sample on this container. Never place samples, particularly chemicals, directly on the metal pan because they can cause corrosion. These balances have a "tare" or "rezero" function, which means that after placing the weighing dish, beaker, or paper on the metal pan, you can reset the display to 0.00. In this way, you can directly measure the weight of the sample of interest. Look closely at the balance at your station and familiarize yourself with its controls.

#### 2. Measuring the Weight of Solids

- a. Set the balance to 0.00. Then place a plastic weighing dish on the balance. Record the weight of the dish below.

weight of dish = \_\_\_\_\_

Now press the reset or tare key and set the balance to 0.00.

- b. To determine the consistency of weight measurements, measure the weights of 10 individual pinto bean seeds. Using a forceps, place each seed individually onto the weighing dish and

record its weight in the table shown below. Use the tare function to reset the balance to 0.00 after adding each seed. Record the results in the following chart.

Seed	Weight (g)
1	
2	
3	
4	
5	
6	
7	
8	
9	
10	

- c. Now calculate the arithmetic mean of the seed weights. The mean or average can be calculated by adding the individual values together and then dividing by the total number of values.

The formula for the mean ( $\bar{X}$ ) is

$$\text{mean } (\bar{X}) = \frac{\sum x_i}{N}$$

where  $\Sigma$  indicates sum,  $x_i$  indicates the individual value, and  $N$  indicates the total number of values.

- d. In this case, since there are weight values for 10 seeds, you would total up the ten individual values and divide by 10. Record the result below.

mean = \_\_\_\_\_

- e. Now calculate the variance ( $s^2$ ) and the standard deviation (SD) of the seed weights. These values give you a sense of how variable the data in a particular set of measurements actually are.

The variance is determined using the following formula:

$$s^2 = \frac{\sum (\bar{X} - x_i)^2}{N-1}$$

It is calculated by subtracting each individual value ( $x_i$ ) from the mean ( $\bar{X}$ ), squaring it (so that all values are positive), and adding all of these squared deviations from the mean together. The total is then divided by  $N-1$ .

- f. The standard deviation (SD) is the square root of the variance.

$$SD = s = \sqrt{\text{variance}}$$

The actual calculations of means, variances, and standard deviations can be done either with a standard laboratory calculator or using a spreadsheet program such as Excel.

- g. Give the variance and standard deviation of the pinto bean seed weights.

variance = \_\_\_\_\_

standard deviation = \_\_\_\_\_

- h. Now repeat the weight analysis using one of the other types of beans or other dried seeds available in the lab.

Type of seed \_\_\_\_\_

Seed	Weight (g)
1	
2	
3	
4	
5	
6	
7	
8	
9	
10	

mean = \_\_\_\_\_

variance = \_\_\_\_\_

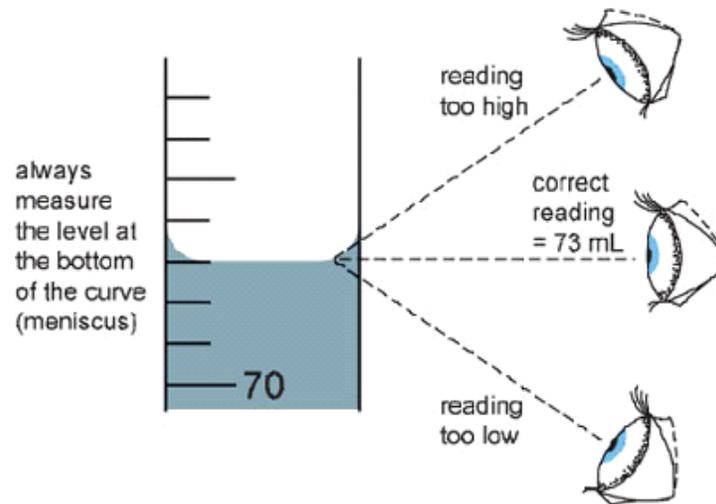
standard deviation = \_\_\_\_\_

## B. Measuring Volume

### 1. Graduated Cylinders

There are several different ways to measure the volume of a liquid or to transfer a specific volume of liquid from one container to another. For relatively large volumes (greater than 10 ml), graduated cylinders are most often used. A graduated cylinder has markings on the side of the glass or plastic which indicate the volume. Depending on the total capacity of the cylinder, the meaning of the markings will vary. For a 10 ml, 25 ml, or 50 ml cylinder, they are most often 1 ml apart. For a 250 ml or 500 ml cylinder, they are most often 10 ml apart. One of the problems with most cylinders is that an aqueous solution (that is, one made up in water) often shows a pronounced meniscus or curvature when it is placed in the cylinder. This is due to the binding of water molecules to one another and to the sides of the glass. The volume in a cylinder is read most accurately by looking at it straight on and reading the volume at the bottom of the meniscus (Figure 1). A graduated cylinder can be used either to measure the volume of unknown solution or to measure out a certain volume of

solution and then to transfer it to another container. Note that measurements with a cylinder are most accurate when the scale is viewed straight (as in **b**) on rather than on an angle (as in **a** or **c**).

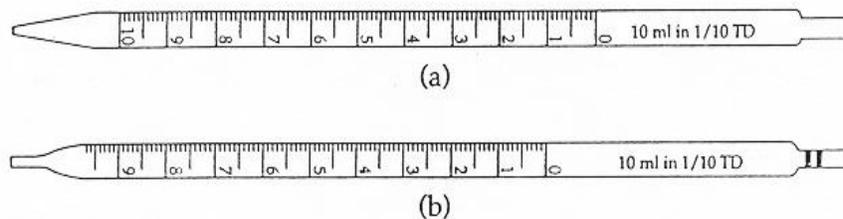


**Figure 1.1.** Picture of a graduated cylinder showing the meniscus and the best way to read the volume (**b**) rather than (**a**) or (**c**).

## 2. Pipets

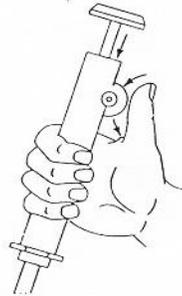
For intermediate volumes (between 1 ml and 25 ml), pipets are most often used to transfer liquids from one container to another. A pipet is a calibrated piece of glass or plastic tubing with markings along the side. Again, depending on the total capacity of the pipet, the meaning of the markings will vary. For a 1 ml pipet, the markings may be either 0.1 ml or 0.01 ml apart. For a 5 ml or 10 ml pipet, the markings are usually 0.1 ml apart. Each pipet usually is marked at the top so that the gradations are clear: it will say 5 in 1/10 ml or 1 in 1/100 ml. As with graduated cylinders, there is often a meniscus with aqueous solutions. A certain volume of liquid may be transferred from one container to another by drawing the liquid up into the pipet and then allowing a volume of liquid between two specific markings to flow out.

Mohr or measuring pipets (**a**) are calibrated only to a point near the bottom of the glass tube. A specific volume is delivered by measuring the amount of liquid between two markings. Serological pipets (**b**) are calibrated all the way to the tip and so must be "blown out" in order to deliver all of their volume (Figure 1.2).



**Figure 1.2.** Picture of a Mohr pipette (**a**) and a Serological pipette (**b**)

To use a pipet, it is necessary to draw liquid up into it. In the past, this was often done by sucking on the end of the tube with your mouth (as with a straw) and covering the end with your index finger. However, this type of mouth pipetting is no longer considered safe and various types of bulbs, pipet aids, or propipets are used instead. **MOUTH PIPETTING IS NOT ALLOWED IN THE COURSE.** In this class, plastic pipet pumps will be used (Figure 1.3).



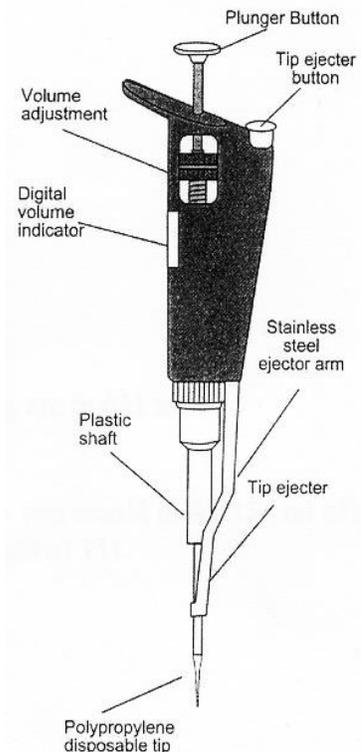
**Figure 1.3. Picture of a pipette aide and illustration of proper use.**

A green pump should be used with 5 ml or 10 ml pipets; a blue pump should be used with 1 ml pipets. To use this type of pump, insert the top end of the pipet into the pump and **twist** it so that it seals against the plastic. Then rotate the plastic wheel to draw the liquid up into the pipet. To let liquid out of the pipet, rotate the wheel in the opposite direction. Pipets are most often used to transfer a certain volume of liquid from one container to another, although with care they also can be used to measure the volume of a solution.

### 3. Micropipettors

For small volumes ( $1.0 \text{ ml} = 1000 \text{ }\mu\text{l}$  or less), calibrated micropipettors are most often used. In this course, a type of micropipettor called a Finn Pipet and sold under the name of a FisherBrand micropipettor will be used. A diagram of a similar type of instrument is shown in figure 1.4.

Micropipettors come in various sizes. For the pipettors to be used in this course, a P-1000 is used to transfer from  $100 \text{ }\mu\text{l}$  to  $1000 \text{ }\mu\text{l}$  ( $1.0 \text{ ml}$ ) of liquid, a P-100 is used to transfer from  $10 \text{ }\mu\text{l}$  to  $100 \text{ }\mu\text{l}$  ( $0.1 \text{ ml}$ ) of liquid, and a P-10 is used to transfer up to  $10 \text{ }\mu\text{l}$  of liquid. It is important to use the appropriate pipettor for each needed volume. Never try to set a pipette above or below the maximum or minimum limits. **A plastic pipet tip should always be used with these pipettors.** Sterile pipet tips will be provided in plastic boxes. **Use the larger blue or white tips for the P-1000 pipettors and the smaller yellow or white tips for the P-100 and P-10 pipettors.**



**Figure 1.4. Parts of a Pipette**

To set a P-1000 to a certain volume, look at the digital display on the side and rotate the inner wheel. There are four numbers on the display. For example, to set the pipetter to 1000 l, set the display to **1 0 0 0** by turning the wheel. To set the pipet to 650 l, rotate the wheel to set the display to **6 5 0**. Never rotate the wheel so that the reading is over 1 0 0 0 or under 1 0 0. To set a P-100 to a certain volume, again look at the digital display on the side and rotate the inner wheel. To set the pipetter to 100 l, set the display to **1 0 0** by rotating the wheel. To get a volume of 72 l, set the display to **7 2** by rotating the wheel. Again, never rotate the wheel so that the display is over 1 0 0 or under 1 0. To set a P-10 to a certain volume, look at the display and rotate the inner wheel in the same way. However, in this case, the last digit on the display indicates 0.1 l. To set the pipetter to 10 l, set the display to **10.0**. To get a volume of 2 l, set the display to **0 2.0**. Note that 0 0.2 indicates 0.2 l, a volume you will not use in this course. You can set the P-10 micropipetter to intermediate volumes: **6.7** is used to deliver 6.7 l.

To use a pipetter, place a plastic tip on the end and **depress the plunger button just to the first stop (Figure 1.5)**. Draw the desired liquid up into the pipet tip by slowly allowing the plunger button to return to its original position. **DO NOT "POP" THE Plunger button BACK.** Then place the tip against the inside of the container in which you wish to place the liquid. Depress the plunger button past first stop and **down to the second stop**. The extra pressure will allow of the liquid in the pipet tip to be ejected. **NOTE: IF YOU DEPRESS THE PLUNGER BUTTON TO THE SECOND STOP WHEN YOU DRAW THE LIQUID UP INTO THE TIP, YOU WILL HAVE MORE THAN THE CALIBRATED VOLUME.**

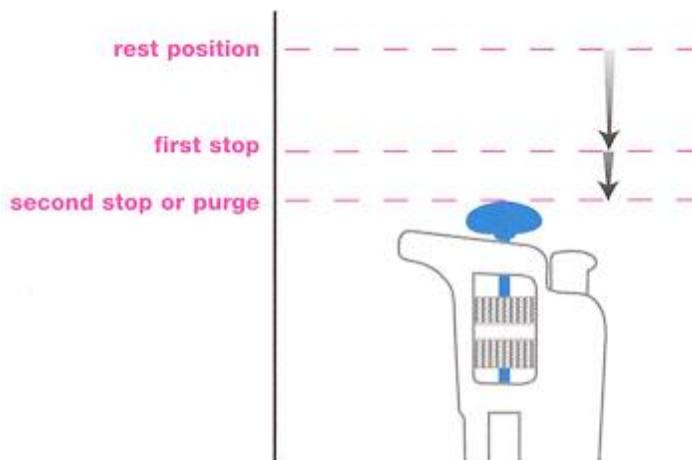


Figure 1.5. Micropipetter Stop Positions

### C. Measuring Volume with a Graduated Cylinder

1. Take a 100 or 150 ml beaker and fill it about half full with the red colored liquid in a flask at your station. The liquid is just water with some food coloring added to it so you can see the liquid easily. Note that while the stock flask or the beaker may have markings on their sides, these values are only approximate and are never to be used for an actual measurement. Pour the liquid into a 100 ml graduated cylinder. Look at the meniscus, measure the volume. Record the results below.

volume of red liquid: \_\_\_\_\_

- To test the accuracy of this measurement, place a clean dry beaker on the top-loading balance and reset it to 0.00 g. Pour the liquid into the beaker and measure the mass in grams. Record the weight of liquid below. The density of pure water is 1.00 g/cm<sup>3</sup> or 1.00 g/ml. How far off was the graduated cylinder?

weight of red liquid: \_\_\_\_\_

- difference between weight as determined by volume and weight as determined on the balance:

\_\_\_\_\_

### D. Measuring Volume with a Pipet

- Now fit a 10 ml serological pipet with a green pipet pump. Draw some of the liquid up into the pipet and look at it carefully. Rotate the wheel and then transfer 10.0 ml of this liquid back into the original container.
- Repeat this process with volumes of 6.7, 5.8, 4.2, and 3.1 ml. Note that you can use the markings anywhere along the pipet to transfer a certain volume. For example, 6.7 ml could be measured from 1.0 to 7.7 ml or from 2.5 to 9.2 ml or from 3.3 to 10.0 ml.
- Once you have figured out how to read the markings correctly, place a clean dry beaker on the top-loading balance and reset it to 0.00. Using the pipet, transfer 6.7 ml of liquid to the beaker and measure the mass in g. The transfer will be most accurate if you touch the tip of the pipet to the glass and allow the liquid to run into the container.
- Repeat the process nine more times and record your values below. Use the tare function to reset the balance to 0.00 after adding each sample. Then calculate the arithmetic mean or average value of the mass of liquid. Also calculate the variance and the standard deviation. Record the results in a chart like that shown below.

**Transfer of 6.7 ml**

Replicate	Weight (g)
1	
2	
3	
4	
5	
6	
7	
8	
9	
10	

mean = \_\_\_\_\_

variance = \_\_\_\_\_

standard deviation = \_\_\_\_\_

## E. Measuring Volume with Micropipetters

1. practice transferring liquids with a micropipetter, set a P-1000 to 1 0 0 0 (1000 l = 1.0 ml) and place a pipet tip firmly on the end. Depress the piston to the first stop and slowly draw some of the red liquid up into the pipet tip. **Be careful not to get any air bubbles in the tip.** Now place the tip against the side of a beaker and depress the piston down to the first stop and then down to the second stop. Watch the liquid flow out of the pipet tip.
2. To test the accuracy of the pipetter, set the P-1000 to 460 l (**4 6 0**). Place a plastic weighing dish on the top loading balance, and transfer 10 replicate samples of the red liquid to the dish. Measure the weight after adding each sample to the dish and record the results in the following table. Use the tare function to reset the balance to 0.00 after adding each sample. Calculate the mean, the variance, and the standard deviation. Record the results the chart shown on the next page.

Transfer of 460  $\mu$ l

Replicate	Weight (g)
1	
2	
3	
4	
5	
6	
7	
8	
9	
10	

mean = \_\_\_\_\_

variance = \_\_\_\_\_

standard deviation = \_\_\_\_\_

3. Now repeat this process using the P-100 micropipetters. For these pipetters, be sure to use the smaller plastic tips. **Pick a specific volume** to use (from 10  $\mu$ l to 100  $\mu$ l for the P-100) and measure the weight of 10 replicate samples. Use the tare function to reset the balance to 0.00 after adding each sample. Record the results in the chart on the following page and calculate the mean, the variance, and the standard deviation.

P-100 volume = \_\_\_\_\_

Replicate	Weight (g)
1	
2	
3	
4	
5	
6	
7	
8	
9	
10	

mean = \_\_\_\_\_

variance = \_\_\_\_\_

standard deviation = \_\_\_\_\_

## F. Preparing Solutions

In this course, most of the solutions you will need for the experiments will be prepared for you in advance. However, you still will need to understand the meaning of the concentration units given on each solution and to have a sense of how that solution might be prepared.

### 1. Concentrations

- The term concentration refers to the amount of a solute in a solution. Concentrations can be expressed in several ways. Because solid solutes may contribute to the final volume of solution, solutions containing large amounts of solute are usually made up by the dissolving the solid in a smaller volume and then making the solution up to the designated total volume.
- Weight/Volume solutions. A weight/volume solution is one in which a concentration is expressed as the amount of a solute in a certain volume. Weight/volume solutions usually have units of mg/ml or g/ml for small volumes or g/l for large volumes. For example, a solution in which 5 mg of a solute is dissolved in 10 ml of water has a concentration 5 mg/10 ml or 0.5 mg/ml. A solution in which 25 g of a solute is dissolved in 1000 ml of water has a concentration of 25 g/l, which can also be expressed as 25 mg/ml.
- Molar solutions. A molar solution is one in which the concentration of a solute is expressed in terms of the number of moles of that solute in a total volume of 1 liter (1000 ml). A solution in which 58.44 g of NaCl is dissolved in a total volume of 1000 ml has a concentration of 1.0 M. For solutions containing smaller amounts of solutes, concentrations may be expressed as mM (millimolar) or  $\mu$ M (micromolar). For example, a solution in which 0.058 g (58 mg = 1 millimole) of NaCl is dissolved in a total volume of 1000 ml has a concentration of 0.001 M or 1.0 mM.
- Percent solutions. A percent solution is one in which the concentration of a solute is expressed as a percentage of a total of 100 parts. For liquids, percent concentrations are

usually given in terms of a volume/volume ratio. 50 ml of solute in a total volume of 100 is a 50% solution; 1 ml of solute in a total volume of 100 ml is a 1% solution. For solids, percent concentrations are usually given as a weight/volume ratio. A solution in which 25 g of solute is dissolved in a total volume of 100 ml is a 25% solution; a solution in which 2 g is dissolved in a total volume of 1000 ml is a 0.2% solution.

## 2. Solution Calculations

Solutions are usually prepared by dissolving a certain amount of a solute in a particular solvent. While percentage solutions can be prepared using English units (for example, 1 pound of solute in 1 gallon of solution), most solutions in biology are prepared using metric units. Solutions of a particular molarity are prepared by 1) calculating the amount of solute needed to prepare a particular volume of solution at a specified molarity; 2) weighing out the desired amount of solute and dissolving in the desired solvent; and 3) making the final solution up to the desired total volume.

For example, suppose you are told to make 0.5 liter of a 0.25 M solution of Calcium Chloride, which has a gram molecular weight of 111. You can calculate the needed amount of calcium chloride as follows:

$$0.5 \text{ liter} \times \frac{0.25 \text{ mole}}{\text{liter}} \times \frac{111 \text{ g}}{\text{mole}} = 13.88 \text{ g}$$

Notice that the desired volume is multiplied by the desired concentration, which is then multiplied by a conversion factor relating weight to moles. The unneeded units cancel out, so that the final value is expressed in grams.

- a. What is the concentration of this solution in mmoles/l?

$$\frac{0.25 \text{ mole}}{\text{liter}} \times \frac{1000 \text{ millimole}}{1 \text{ mole}} = \frac{250 \text{ millimole}}{\text{liter}}$$

$$= 250 \text{ mM}$$

- b. How many mg of calcium chloride would you use to make only 5 ml of solution?

$$5 \text{ ml} \times \frac{0.25 \text{ mole}}{\text{liter}} \times \frac{1 \text{ liter}}{1000 \text{ ml}} \times \frac{111 \text{ g}}{\text{mole}} \times \frac{1000 \text{ mg}}{1 \text{ g}}$$

$$= 138 \text{ mg}$$

## 3. Problems. Record your complete calculations in your lab notebook.

- How many g of NaCl (gram molecular weight = 58.44) should you use to make 100 ml of a 0.37 M solution?
- How many g of NaCl should you use to make 37 ml of a 1.0 M solution?
- How many g of NaCl should you use to make 5.8 l of a 3.7 mM solution?

- d. If you have weighed out 5.84 g of NaCl, what would be the total volume of a 1.0 M solution?
- e. How much NaCl should you use to make 250 ml of a 5% solution?
- f. What percent solution would you have if you dissolved 3.7 g of NaCl in a total of 250 ml?
- g. If you dissolve 37 mg of NaCl in a total volume of 25 ml, what is the molar concentration?
- h. If you dissolve 5.84 g of NaCl in a total volume of 37 ml, what is the molar concentration?
- i. If you have a 10% solution of NaCl, what is its molar concentration?
- j. If you have a 2.5 M solution of NaCl, what is its percent?
- k. What is the concentration of a 0.025 M solution of NaCl in mg/ml?

## G. Making Dilutions

### 1. Dilution Factors

It is often convenient to prepare stock reagent solutions in a concentrated form and then dilute them for use in a particular experiment. Likewise, it is often necessary in determining the amount of material in an unknown solution to test several different dilutions of it. The liquid (for example, water) that is used to dilute a stock solution or an unknown solution is called the diluent. The amount of dilution or the dilution factor can be defined in several ways, but the most useful method is in terms of the ratio:

$$\frac{\text{initial volume of solution}}{\text{final volume of solution}}$$

For example, if 2.5 ml of a stock solution is combined with 7.5 ml of water, the dilution factor is 1/4:

$$\frac{2.5 \text{ ml}}{2.5 \text{ ml} + 7.5 \text{ ml}} = \frac{2.5 \text{ ml}}{10.0 \text{ ml}} = \frac{1}{4}$$

Dilutions are often made serially, that is, by making several dilutions sequentially. Suppose that 1.0 ml of an unknown solution is added to 2.0 ml of diluent; this makes a 1/3 dilution. If 1.0 ml of this 1/3 dilution is then added to another tube containing 2.0 ml of diluent, this makes a 1/3 dilution of a 1/3 dilution or a 1/9 dilution. If 1.0 ml of the 1/9 dilution is added to another tube containing 2.0 ml of diluent, this makes a 1/3 dilution of a 1/9 dilution or a 1/27 dilution. Note that sequential dilution factors are **multiplied** to give the final dilution factor.

Suppose you add 1.0 ml of a sample to 9.0 ml of water. You then make 4 serial 1/2 dilutions of this solution. What is the final dilution factor?

$$1/10 \times 1/2 \times 1/2 \times 1/2 \times 1/2 = 1/160$$

## 2. Making Serial Dilutions

- a. Obtain 6 16 x 150 mm tubes and label them 1, 2, 3, 4, 5, and 6. Using a green pipet pump and a 5 ml pipet, add 5.0 ml of **water** to each of the tubes. In this exercise, water is the diluent.
- b. Using green pipet pump and another 5 ml pipet, add 5.0 ml of the **red liquid** to the first tube to make a 1/2 dilution. Mix the solution carefully by drawing the liquid up and down in the pipet.
- c. Now add 5.0 ml of the liquid in tube 1 to tube 2 and mix the solution by drawing the liquid up and down in the pipet. What is the dilution factor in this tube?

\_\_\_\_\_

- d. Continue the process until you have made all six dilutions. Record the total dilution factor for each tube and note the change in the intensity of the color.

Tube	Total Dilution Factor
1	
2	
3	
4	
5	
6	

- e. Now obtain 4 clean 16 x 150 mm and label them 1, 2, 3, and 4. Using a green pipet pump and a 10 ml pipet, add 9.0 ml of **water** to each of the tubes.
- f. Using a P-1000 micropipetter and a tip, add 1000  $\mu$ l or 1.0 ml of the **red liquid** from the flask on your bench to the first tube. Mix the solution carefully by drawing the liquid up and down in the pipet. What is the dilution factor in this tube?

\_\_\_\_\_

- g. Now add 1000  $\mu$ l or 1.0 ml of the solution in the first tube to the second tube. What is the dilution factor in this tube? Continue the process of making serial dilutions until you have made all four dilutions. **Record the total dilution factor for each tube and note the change in the intensity of the color.**

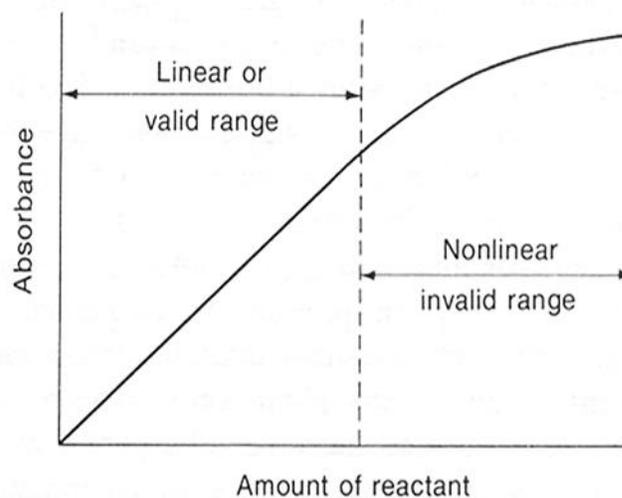
Tube	Total Dilution Factor
1	
2	
3	
4	

- h. How quickly did the color dilute out in the 1/10 dilutions compared to the 1/2 dilutions? Which tube in the 1/2 dilution series has a color intensity most similar to the second tube in the 1/10 dilution series? Which tube in the 1/10 dilution series has a color intensity to the last tube in the 1/2 dilution series? Why does this make sense?

### III. Using a Standard Curve

#### A. What is a standard curve?

A standard curve is a graph that shows the relationship between the amount of a particular compound in a solution and the absorbance of that solution. This absorbance may be an intrinsic property of the molecules of interest or it may result from treating the molecules of interest with a particular set of reagents. A standard curve is constructed by setting up a series of solutions that contain varying amounts of the compound of interest in exactly the same volume. The amount of a compound may be expressed in molar units (for example, nmoles or  $\mu$ moles) or in weight units ( $\mu$ g or mg). The absorbance of each solution is then measured against a reference solution or blank that lacks the compound of interest. The absorbance values of the solutions are then plotted as a function of the amount of compound (Figure 1.6)



**Figure 1.6. A typical standard curve. Shown is a standard curve for a color-forming quantitative reaction.**

In most standard curves, there are both linear and nonlinear regions. In the linear region, the absorbance increases in direct proportion to the amount of the compound of interest. Within this region, it is quite easy to interpolate between the data points and to infer the amount of the compound of interest in any similar solution of known absorbance. In the nonlinear region, however, the relationship between absorbance and amount is not simple and interpolation between the data points is more difficult. Nonlinearity in a standard curve can result from the loss of sensitivity of the instrument at high concentrations of the compound of interest or from a failure to completely convert the compound of interest to a detectable material.

## B. Using a standard curve

**Once a standard curve has been created, it can be used to determine the amount of the compound of interest in an unknown solution.** The absorbance of this unknown solution is first measured using the same instrument at the same wavelength as the standards. There are then two ways to estimate the actual amount. One is graphically. That is, you can simply find the absorbance on the Y axis, move over to the linear region of the standard curve, and then read down to the X axis to get the corresponding amount. The other is mathematically. You can use the equation for the linear region of the standard curve to create a conversion factor for relationship between absorbance and amount. That is, within the linear region, the straight line has the formula

$$y = mx + b$$

where **m** is the slope of the line and **b** is the Y intercept. If **b** = 0, that is, the line goes through the origin at 0,0, you can use the slope of the line (**m**) as the conversion factor since it directly gives the relationship between **x** and **y**. You can then divide the absorbance of the unknown sample by the conversion factor to determine the corresponding amount.

For example, suppose you construct a standard curve for compound Z by setting up a series of tubes containing varying amounts of Z in  $\mu\text{g}$  and by measuring the absorbance of each tube. Suppose you then determine the slope of the line and get the equation  $y = 0.062x$ . This means that y (the absorbance) increases by 0.062 for increment in x (the number of  $\mu\text{g}$ ). In other words, there is 0.062 A/ $\mu\text{g}$ . If you then measure the absorbance of an unknown solution containing Z and find that the absorbance is 0.198, you can calculate that the number of  $\mu\text{g}$  of Z in this tube is:

$$0.198 \text{ A} \quad \times \quad \frac{1 \mu\text{g}}{0.063 \text{ A}} \quad = \quad 3.19 \mu\text{g}$$

The graphical method is easiest if only a few values need to be determined. The mathematical method is easiest if there are many values that need to be determined.

### 1. Standard Curve Practice Problems (Record your complete calculations in your lab notebook)

Use Figure 1.7 to answer the questions on the following page

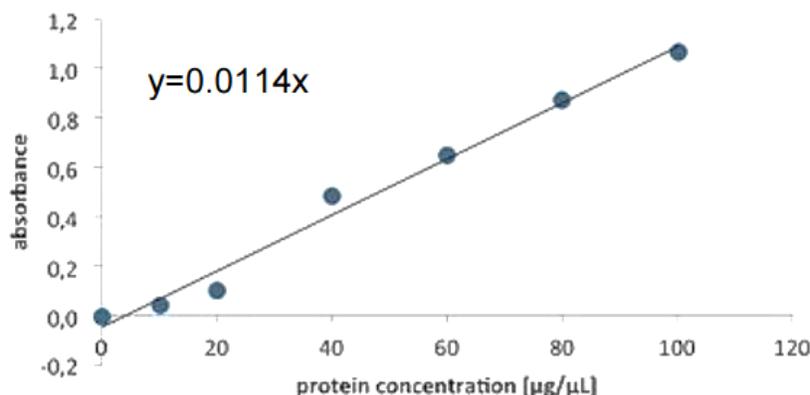


Figure 1.7. Sample Standard Curve

- a. A sample of BSA of unknown concentration had an absorbance reading of 0.502 A. Using the graphical method of analysis, determine the approximate concentration of BSA in the unknown sample.
- b. A sample containing a mixture of proteins had an absorbance of 0.611 A. Using the graphical method of analysis, determine the approximate concentration of protein in the unknown sample.
- c. A sample contains a mixture of proteins at a concentration of 65  $\mu\text{g}/\mu\text{l}$ . Using the BSA standard curve, what is the approximate absorbance reading you expect for this sample.
- d. A sample containing a mixture of proteins had an absorbance of 0.384. Using the mathematical method of analysis and the equation  $y = 0.0114x$ , determine the concentration of protein (in  $\mu\text{g}/\mu\text{l}$ ) in the unknown sample.
- e. A sample containing a mixture of proteins had an absorbance of 0.477. Using the mathematical method of analysis and the equation  $y = 0.0114x$ , determine the concentration of protein (in  $\mu\text{g}/\mu\text{l}$ ) in the unknown sample.

## VI. Using Excel

As part of this laboratory course, you will need to process numerical data and prepare various types of graphs. These graphs will be included in your Data Sheets and Lab Reports. The third part of this lab session will focus on Excel, one commonly-available software package that can be used for these purposes. The most common versions of Excel used today are Excel 2007 and Excel 2010. Although they allow you do many of the same things, the positions of the task buttons and the procedures for doing certain steps in preparing graphs may be slightly different.

### A. Introduction

Excel is a spreadsheet, calculation, and graphics program that is part of the Office suite of software programs developed by Microsoft. All of the computers on the ASU campus now have the Office 2010 version of this program. Most students will have this version on their home computer as well, although some people may still use the Office 2003 or 2007 version. While Excel is not actually the best program for many scientific applications, it is commonly used at ASU and is sufficient for the laboratory work to be done in this course.

The following are some links to free tutorials for using Excel 2010 and 2007

#### Excel 2010 Tutorials

<http://office.microsoft.com/en-us/excel-help/getting-started-with-excel-2010-HA010370218.aspx>  
<http://www.gcflearnfree.org/excel2010/1>

## Excel 2007 Tutorials

<http://office.microsoft.com/en-us/training/HA102189871033.aspx>

<http://www.fgcu.edu/support/office2007/Excel/Gettingstarted.asp>

The book by Karin Knisely mentioned on page 10 of the **Student Lab Manual** has a very good appendix on the use of Excel 2007 to make Graphs.

Knisely, K. (2009) **A Student Handbook for Writing in Biology**, 3/e. Sinauer Associates/ W. H. Freeman and Company, Sunderland, MA.

If you have a computer with Excel 2010 on it and are familiar with the software, you can jump ahead to the two exercises in Section C. If you have not used Excel 2010 before or are unfamiliar with some of its features, you may want to purchase the Knisely book or spend some time with the tutorial links above.

## B. Excel Exercises

The following two exercises are designed to help you learn to use the basic features of Excel that will be most important in this class. As part of the first exercise, you will process some data related to seed weights and make a bar graph to summarize the results. As part of the second exercise, you will analyze some data related to the length of the flagella on a protist called *Chlamydomonas* and make a scatter-plot of the results. As with most software programs, there are several ways to do many of the steps, so do what is easiest for you.

### 1. Exercise 1 (Include a copy of your graph in your lab manual)

#### *The data set*

- a. Suppose that you do a series of weight measurements of various plant seeds like you did as part of Laboratory 1 using a balance that reads to 0.001 g (1 mg). You obtain the following results.

<u>Pinto beans</u>	<u>Lima Beans</u>	<u>Great Northern Beans</u>	<u>Peas</u>
0.263	0.895	0.562	0.188
0.278	0.951	0.549	0.134
0.246	1.004	0.565	0.142
0.255	0.875	0.534	0.122
0.279	0.953	0.597	0.152
0.261	0.804	0.539	0.102
0.249	0.837	0.548	0.143
0.252	0.984	0.535	0.139
0.285	1.017	0.561	0.153
	0.917	0.573	0.136

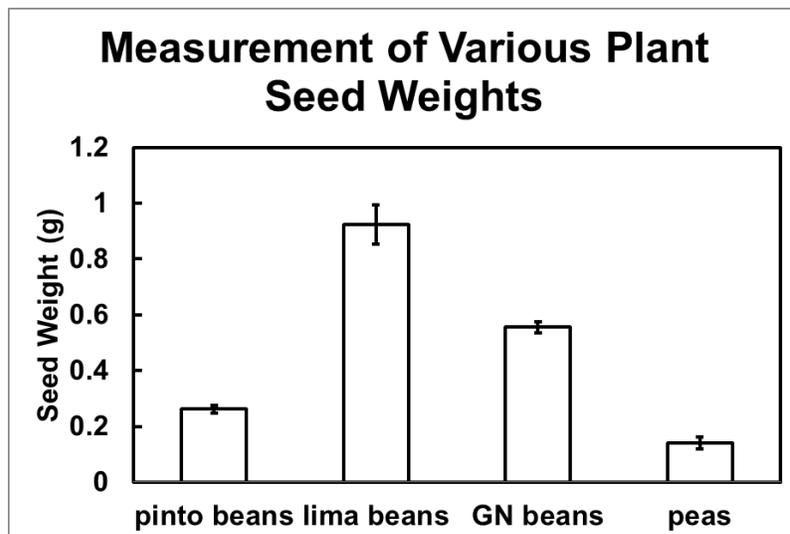
- b. To analyze these results, you will first calculate the mean, the standard deviation, and the variance for each type of seed. You will then display results graphically as a bar graph.

### *Numerical calculations*

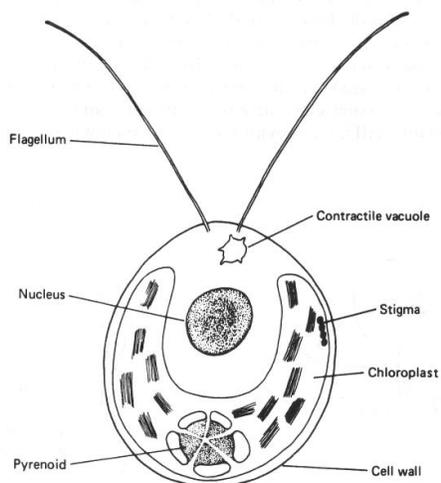
- a. Open Excel 2010 for Windows on your computer; be aware that if you are using a different version of Windows or Excel for Mac the detailed instructions here may not work the same way as are suggested. You will see a worksheet consisting of a series of rows and columns. You will also see a Ribbon across the top which has a series of Tabs labeled Home, Insert, Page Layouts, Formulas, Data, Review, and View. When you click on each of the Tabs, a series of additional menu options will appear.
- b. Enter the data for the pinto beans in cells A1 to A10, the data for lima beans in cells B1 to B10, the data for Great Northern Beans in cells C1 to C10, and the data for peas in cells D1 to D10. In order to save time, you can also use the prepared worksheet containing these data, which is available on the Blackboard course web site.
- c. To calculate the Mean or Average for the Pinto Beans, move the cursor down to cell A12 and click once. The outline of the cell will appear in bold. From the list of tabs at the top, click on Formulas. This will bring up a new Tool Bar. Click on Insert Function ( $f_x$ ) on the left end of the tool bar. From the list of options in the dialog box, select AVERAGE and click OK. This will bring up a new box. Enter A1:A10 in the box labeled Number 1 to designate the cells with the numbers to be included in the average. Click OK. You also can start with the cursor in the A1 cell and scan down to the A10 cell. The set of cells will be outlined with a dotted line. You also can insert =AVERAGE(A1:A10) in cell A12 or into the  $f_x$  function bar at the top. In each case, you should get the same result. Repeat the process to calculate the Average of each of the other sets of seeds.
- d. To calculate the Standard Deviation for the Pinto Beans, move the cursor down to cell A13 and click on it. The outline of the cell will appear in bold. From the list of Tabs at the top, click on Formulas. This will bring up a new Tool Bar. Click on Insert Function ( $f_x$ ) on the left end of the tool bar. This will bring up a new box. Enter STANDARD DEVIATION in the box under Search for a Function and click GO. From the list of options in the box, select STDEV and click OK. This will bring up a new box. You can enter A1:A10 in box Number1 to designate the cells with the numbers to be included in the standard deviation calculation. Click OK. You also can start with the cursor in the A1 cell and scan down to the A10 cell. The set of cells will be outlined with a dotted line. You also can insert =STDEV(A1:A10) in cell A13 or into the  $f_x$  function bar at the top. Repeat the process to calculate the Standard Deviations of each of the other sets of seeds.
- e. To calculate the Variance for the Pinto Beans, move the cursor down to cell A14. The outline of the cell will appear in bold. From the list of Tabs at the top, click on Formulas. This will bring up a new Tool Bar. Click on the More Functions button and then go over to Statistical. Scroll down the list of functions to VAR.S. Click on it to insert this function. Again enter A1:A10 in box Number1 and click OK. You can also scroll over the cells that you want to include and click OK. Alternatively, you can type =VAR.S(A1:A10) in cell A14 or into the  $f_x$  function bar at the top or you can type =A13\*A13. This means to multiple the value of the standard deviation by itself, which will give you the variance. Repeat the process to calculate the Variance for each of the other sets of seeds.
- f. Finally, enter the names of various seeds in cells A11, B11, C11, and D11 as pinto beans, lima beans, GN beans, and peas. To save your worksheet with these calculations, click on the FILE button at the top. Click on the SAVE AS function and save the worksheet with the filename Excel Exercise 1 somewhere convenient on your computer.

### *Preparation of a bar graph*

- a. To make a bar graph of these data, click on the Insert tab in the ribbon at the top. You will see a series of options. In the section labeled Charts, click on the tab labeled Column. Then click on the 2-D column graph on the upper left-hand side. A blank chart will appear on the worksheet. Then click on the Design tab under Chart Tools. Click on the box labeled Select Data. A new box will appear. Enter =Sheet1!\$A\$11:\$D\$12 in the box to indicate that the names and means of the various seed weights are to be included in the chart. You can also use the cursor to scan across cells A11, B11, C11, D11, A12, B12, C12, and D12 to identify these cells as the basis of the chart. Click OK. When you do this, a series of blue bars will appear on the Chart. These will be identified as Series 1. The names of the seeds will appear along the X axis.
- b. To add errors bars to the bar graph, click on the chart. Then click on the Layout tab under Chart Tools. Then click on the Error Bars box in the section labeled Analysis on the right side of this menu. Go down to the bottom and click on More Error Bar options. For vertical error bars, click on the Both Direction button at the top. Then click on the custom button at the bottom. Click on the specify value box. A new dialog box will appear. For the positive error bar values, select the standard deviations in cells A13, B13, C13, and D13 which you have already calculated. You can insert =Sheet1!\$A\$13:\$D\$13 or scan across the cells as before. In the same way, insert the same function into the negative error bar values. Click OK. The error bars will appear on your graph.
- c. The rest of the manipulation of the chart layout can be done either on the worksheet itself or on a separate sheet. If you want to create a sheet with the chart alone, go back to the Design tab under Chart Tools. On the right hand side, there is box says Move Chart. Click on this box and place the chart on a new sheet labeled Chart 1. Then click OK. At this point, you will have a full-size chart to work with. You can manipulate many of the features of the Chart by clicking on the Layout tab. You can add a chart title, label the axes, change the fonts, remove gridlines, position the tick marks inside or outside the axes, and so on. Most of these formatting issues are matters of personal preference. In most scientific papers, chart titles are not used and information about the chart or graph is presented in a figure legend which is printed below the chart. Once a graph like this has been completed, it can be copied and pasted directly into a Word document. Here is one version of a chart based on the data provided in this exercise.



2. Exercise 2 (Include a copy of your graph in your lab manual)



**Figure 1.8. Chlamydomonas is a unicellular protist that moves using its two flagella.**

**The data set**

- a. When these organisms are exposed to a low pH of 4.5, the flagella fall off and movement stops. If the pH is then restored to a pH of 7.0, the flagella regenerate (Figure 1.8). The process of flagellar growth can be seen microscopically in a good light microscope. Eventually, the flagella are long enough that active motility resumes. Suppose that you treat a culture of *Chlamydomonas* acetic acid to lower the pH and then after 30 seconds, restore the pH to about 7.0 with a dilute solution of KOH. You place the deflagellated protists in a flask in a nutrient medium and then remove samples every 10 minutes for a total of 60 minutes. You place the samples in a fixative solution to stop further metabolism and preserve the cells. You then make a slide of the protists in each sample and measure the length of the flagella on 10-15 different cells. You obtain the following results, where the numbers in the table are in  $\mu\text{m}$ . Note: the cells in each column are **not** same cells each time because they are different samples from the main culture.

	Time (minutes)					
<u>0</u>	<u>10</u>	<u>20</u>	<u>30</u>	<u>40</u>	<u>50</u>	<u>60</u>
1	3	4	7	12	16	25
2	2	6	8	11	18	23
0	1	4	10	15	9	24
1	3	5	1	8	19	19
0	2	7	5	9	14	20
0	4	6	8	11	17	19
1	1	1	9	10	17	15
3	2	5	6	13	15	22
0	3	6	7	12	20	21
1	2	4	5	10	18	19
2	2	7	9	3	14	20
1	3	6	6	12	13	21
0	3	5	8		18	16
	2		9		21	
			11			

- b. To analyze these results, you will first calculate the mean, the standard deviation, and the variance for each time point during the regeneration process. You will then display results graphically as a scatter plot and put a trendline onto the graph.

### *Numerical calculations*

- a. Click on the File button in Excel and open a new workbook (Click on New followed by Blank Workbook). Again, you will see worksheet consisting of a series of rows and columns. Enter the data for the 0 time sample in the A column, the data for the 10 minute sample in the B column, and so on. In order to save time, you can also use the prepared worksheet which is available on the Blackboard course web site.
- b. Calculate the Mean for each of the samples as you did in Exercise 1 and enter the results in cells A17, B17, C17, D17, E17, F17, and G17. Calculate the Standard Deviation for each of the samples as you did in Exercise 1 and enter the results in cells A18, B18, C18, D18, E18, F18, and G18. Finally, enter the times at which the samples were taken in cells A16, B16, C16, D16, E16, F16, and G16.
- c. To save your worksheet with these calculations, click on the FILE button at the top. Click on the SAVE AS function and save the worksheet with the filename Excel Exercise 2 somewhere convenient on your computer.

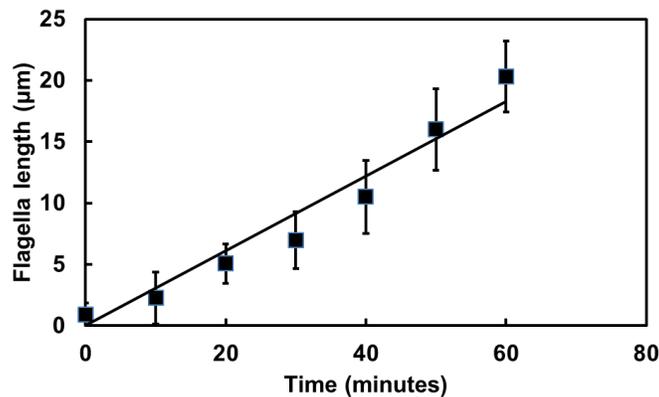
### *Preparation of a scatter plot*

- a. To make a scatter plot or a dot plot, click on the Insert tab in the ribbon at the top. You will see a series of options. In the section labeled Charts, click on the tab labeled Scatter. Then click on the graph in the upper left hand corner. A blank chart will appear on the worksheet. Then click on the Design tab under Chart Tools. Click on the box labeled Select Data. A new box will appear. =Sheet1!\$A\$16:\$G\$17 in the box to indicate that the times and means of the flagellar lengths. You can also use the cursor to scan across cells A16 to G16 and A17 to G17 to identify these cells as the basis of the chart. Click OK. When you do this, a series of blue dots will appear on the Chart. These will be identified as Series 1. The times will appear along the X axis.
- b. To add errors bars to the bar graph, click on the chart. Then click on the Layout tab under Chart Tools. Then click on the Error Bars box on the right side of this menu. Go down to the bottom and click on More Error Bar options. For vertical error bars, click on the Both Direction button at the top. Then click on the custom button at the bottom. Click on the specify value box. A new dialog box will appear. For the positive error bar values, select the standard deviations in cells A18, B18, C18, D18, E18, F18, and G18 which you have already calculated. You can insert =Sheet1!\$A\$18:\$G\$18 or scan across the cells as before. In the same way, insert the same function into the negative error bar values. Click OK. The vertical error bars will appear on your graph. Close the Format Error Bars Dialog Box. Unfortunately, Excel also will sometimes automatically add horizontal error bars (relative to the time points) to the graph as well. This will shift the scales on the X and Y axes. However, all of this can be fixed.
- c. The rest of the manipulation of the chart layout can be done outside of the worksheet. Go back to the Design tab under Chart Tools. On the right hand side, there is box says Move Chart. Click on this box and place the chart on a new sheet labeled Chart 1. Then click OK.

At this point, you will have a full-size chart to work with. You can manipulate many of the features of the Chart by clicking on the Layout tab. You can add a chart title, label the axes, change the fonts, remove gridlines, put the tick marks inside or outside, change the symbols, add a trendline, and so on. Most of these formatting issues are matters of personal preference.

- d. The easiest way to make many of the more substantive changes is to use the Change Current Selection area in the upper left corner of the Format menu. In this area, you can select (in the scroll-down menu) any of the major components of the graph. Click first to identify what you want to work on and then click on Format Selection to change the features of that component. For example, if you bring up the Horizontal Axis and click on Format selection, you can change the X axis scale so that it starts at 0. Likewise you can change the Y axis scale so that it starts at 0. To remove the horizontal error bars, bring up Series 1 X error bars and format this selection. Set it to a fixed value of 0 and the error bars will go away.
- e. Click on the Layout menu and click on the Trendline tab to add a line to the data points. In this case, it is easiest to click on more Trendline options. You can then select a linear trendline and force the line to go through the origin at 0,0 (Enter 0.0 in the Set Intercept box).
- f. Once a graph like this has been completed, it can be copied and pasted directly into a Word document. Here is one version of this graph. It seems to indicate that there is a direct relationship between flagella and time, although a more complex function might also fit the data.

**Time Course of Flagella Regeneration  
in *Chlamydomonas***



Time (min)	Average Value	Standard Deviation
0		
10		
20		
30		
40		
50		
60		

## V. Answers to Practice Problems

### Interconversion of metric units

- a. 55 mm
- b. 550 cm
- c. 0.555 m
- d.  $5.55 \times 10^4$  cm
- e.  $5.55 \times 10^5$  nm
- f. 42 ml
- g.  $4.2 \times 10^4$   $\mu$ l
- h. 0.42 l
- i. 0.754  $\mu$ g
- j.  $7.54 \times 10^6$  ng
- k. 0.754 kg

### Interconversion of metric and English units

- a. 6.42 in
- b. 0.414 m
- c. 41.4 mm
- d. 5.35 ft
- e.  $6.43 \times 10^4$  mg
- f. 0.080 oz
- g. 1032 g
- h. 103 kg
- i. 1868 ml
- j. 747 l
- k. 7472 ml

### Preparation of Solutions

- a. 2.16 g
- b. 2.16 g
- c. 1.25 g
- d. 99.9 ml
- e. 12.5 g
- f. 1.48%
- g. 0.025 M
- h. 2.70 M
- i. 1.71 M
- j. 14.6%
- k. 1.46 mg/ml

### Standard Curve

- a. Approximately 50  $\mu$ g/ $\mu$ l
- b. Approximately 60  $\mu$ g/ $\mu$ l
- c. Approximately 0.7 A
- d. 33.68  $\mu$ g/ $\mu$ l
- e. 41.84  $\mu$ g/ $\mu$ l