WEEK #2: Introduction to Lab Techniques

I. INTRODUCTION

In this laboratory, you will be introduced (or reintroduced) to some basic techniques of modern biology—microscopy, spectrophotometry, and micropipetting. In today’s lab we will practice the general use of the compound light microscope. In addition, we will conduct an experiment that will introduce/reinforce the use of spectrophotometers and micropipettors, as well as exercise your computer skills.

II. THE COMPOUND MICROSCOPE

We will be using both dissecting and compound microscopes throughout the term, so it will be good to make sure that everyone is at the same level. If you feel that microscopes are an intimate part of your life, then consider this exercise a review.

The magnification system of a compound microscope consists of the **objective** and **ocular lenses**. The objectives are three or four lenses mounted on a revolving nosepiece. Each objective is actually a series of several lenses that magnify the image and improve resolution. The magnifying power of each objective is etched on the side of the lens (e.g., 4x, 10x, 40x). The **ocular** (also called an eyepiece) is the lens that you look through. The Nikon Alphaphot microscope (shown below in Figure 2.1) is a binocular microscope because it has two oculars. The oculars on this microscope magnify the image an additional ten times.

The **slide holder** secures the glass slide onto the **stage**. The slide is then moved using the **stage motion knobs**.

Figure 2.1. Components of the Nikon Alphaphot 2 compound microscope.
A. Adjusting the Microscope (also called Critical Focus adjustment)

**Do this before using your microscope**

The most common complaint from students first using a microscope is that they either can not see an image clearly or that the image is not in focus. The main causes of these problems are either that the microscope is out of adjustment or that it is being used improperly. We have very high-quality microscopes that provide excellent images - adjusted and used properly they are a joy to use.

What follows are instructions which are to be used before you use a compound microscope in the lab. Once this is done, your microscope will be set up for optimal resolution and high quality viewing of any specimen.

1. Remove the microscope from its cabinet and carry it upright with one hand grasping the arm and your other hand supporting the microscope below its base.

2. Plug in the microscope and turn on the light source. Adjust the light switch so that the lamp is set to a medium intensity setting.

3. If it isn’t already in position, rotate the nosepiece until the "low-power" (i.e., 4x or 10x, whichever is the lowest number on your scope) objective is selected. You’ll feel the objective click into place when it is positioned properly. Always begin examining slides with the low-power objective.

4. Obtain a microscope slide and, using a finepoint permanent marker, make a small “e” in the middle of the slide.

5. Pull open the arm on the slide holder and clip the slide into place. Using the stage-motion knobs, move the slide so that the ‘e’ is below the low-power objective.

**CAUTION! Use caution when viewing through the lowest power objective. It permits the most light through the system and if the light is set too high an intensity, you may hurt your eyes.**

6. Set the diaphragm iris lever about 2/3 open – i.e., so that the lever is about 1/3 from the brightest position.

7. Look through both oculars and widen or narrow the distance between the oculars to match your interpupillary distance (i.e., the distance between your eyes). If you wear glasses, remove your glasses when looking through the microscope (unless you have severe astigmatism).

8. Using the **coarse adjustment knob**, focus on the “e” until it is a sharp as you can get it. If the image through one of the oculars is out of focus, see "**Adjusting the ocular diopter**" in part B below.

You have now adjusted the lens system and ensured it is aligned. Now you will adjust the light for optimal resolution.

9. Remove the slide from the stage. At this point do not touch anything on the microscope.

10. Using a small object such as a pencil or pen point, place it directly on top of the lamp housing.

11. Locate the small knob attached directly to the sub-stage condenser (this knob adjusts the height of the condenser). Using this knob ONLY, focus the point or object as clearly as you can.

12. Now place the slide you wish to examine in place on the microscope stage.

You have just "critically focused" your microscope. Using this technique, you will have a well illuminated image with optimal resolution.
B. Adjusting the ocular diopter (i.e., matching the oculars to your eyes)

1. Look into the right eyepiece with your right eye and focus your image.

2. Now close your right eye and look into the left eyepiece with your left eye. Focus the image with the diopter ring on the ocular.

3. Repeat steps 1 and 2.

C. Cleaning the microscope

In case you think your microscope is dirty, here some directions for cleaning.

The ocular and objective lenses should be kept clean, but they should not be cleaned unnecessarily. If the image seems poor, first make sure that the fault is not with the microscope slide, cover glass, focus, condenser adjustment, etc. If the image is still poor after checking these things, turn the nosepiece to another objective. If the image quality is improved, then the objective is dirty and should be cleaned. If the image does not improve, then the oculars are probably dirty. To check, look at the oculars so that overhead light is reflected on the glass surface – dirt and fingerprints should be obvious. Clean lenses by GENTLY wiping the lens with a clean Kimwipe (a lab tissue). If the lens still appears to be dirty, re-clean the lens with another clean kimwipe wetted with a small amount of distilled water or glass cleaner. Then dry the lens with a dry kimwipe.

Use ONLY Kimwipes to clean a microscope lens. Lens paper, paper towels, fingers, etc., can permanently damage the lenses.

D. Observe some organisms

1. If it isn't already in position, rotate the nosepiece on your microscope until the "low-power" objective is selected.

2. Obtain a new slide and coverslip from the supply on your lab bench. If the slide and/or coverslip seem dirty, clean them at the sink with a kimwipe and some window cleaner (blue liquid available in a dropper bottle). Be careful with the coverslips because they are extremely fragile.

3. Using an eyedropper, transfer a drop of the pondwater sample to the center of your slide. Touch one edge of the coverslip to the slide, about 1 mm away from your sample, and release the slide – let it fall onto the sample drop like it is a hatch-door closing (you might want to ask your instructor or TA to demonstrate this – this is a case where a written description doesn’t work too well).

4. Place the slide in the slide holder and move the slide so that the cover slip is directly below the low-power objective.

5. Rotate the coarse adjustment knob to move the objective within about 1 cm of the stage.

6. Using the coarse adjustment knob, find some organisms or algae and focus to make a clear image. If you are unsure if you are focused on your sample, move the slide while looking through ocular lenses. If you are focused on your slide, your image should move. Note also that sometimes it is possible to be focused on the bottom of the slide or on the top surface of the cover slip instead of your sample (this is especially a problem if you have a dirty slide or coverslip).

7. Now turn the nosepiece and select the next higher power objective, which should be 10x. Use the coarse adjustment knob if necessary to bring your specimen into clear focus. Note that these microscopes are parfocal, which means...
that once the microscope is in focus, you shouldn’t have to move the stage (or not very much) to maintain focus when changing to another objective.

8. Now turn to the 40x objective. This time, use the fine adjustment knob to focus your specimen – don’t use the coarse adjustment knob at 40x and higher power!

9. Now choose one organism on your slide and make a sketch of this organism in your lab notebook. Try to make a tentative identification using one of the identification guides on your lab bench.

10. On your sketch, include a note about the magnification of the specimen (what is the magnification when using the 40x objective?).

11. Finally, include a small (2-5 cm) reference scale on your sketch. It is like the mile reference scale on a map – it indicates to your reader what are the actual dimensions of the sketch. The scale should be in real units, such as µm. You can determine the scale of your specimen using the ocular micrometer on your microscope – this is the tiny ruler that you can see through one of the microscope ocular lenses. This micrometer corresponds to different real distances for each microscope objective – see the note on the microscope or the lab board for the appropriate conversion. To determine the size of your specimen, multiply the number of ocular units by the calibration factor.

E. Putting away the microscope

Be sure to check all of the following points before leaving your microscope for the next student to use.

- Slide is removed from stage and returned to proper place.
- Objective nosepiece is rotated so 4X objective is in place.
- Lamp on/off switch is switched off.
- Microscope is covered.

F. Microscope Rules

Here are few reminders about the care and use of compound microscopes in lab.

- Always start focusing a specimen with the low power objective.
- When increasing magnification, move sequentially from one objective to the next higher power. Don’t skip objectives – i.e., don’t change directly from 4x to 40x. This is like shifting from 1st to 3rd gear in a manual shift car – this is bad for the car and the microscope.
- When using high power (40X) and oil immersion lenses (100X), USE ONLY THE FINE ADJUSTMENT KNOB! Using the coarse adjustment can result in damage to the slide and objective lens (this is because you will most likely miss the appropriate focusing plane and push the objective into the slide).
- The 100x objective is only to be used with oil – we’ll do this later in the term.
- Clean the glass surface gently and use only kimwipes.
- Keep the microscope dry and clean. Immediately wipe up any liquid spills from the stage, base, or lenses.
- Always carry the microscope with two hands, one hand grasping the microscope arm and the other hand under the microscope base.
III. PROTEIN ASSAY EXPERIMENT

In this lab exercise, we will practice two important and use lab techniques: using a spectrophotometer and micropipetting. In the exercise, you will use these methods for a common type of assay in biochemistry - determining the protein concentration of an unknown sample.

The determination of protein concentration is frequently required in biochemical work. For instance, if we are comparing the activity of 2 different enzyme preparations, and find one to display substantially more activity than other, we really can't conclude anything until we have ascertained how much of each enzyme is present (remember that enzymes are proteins).

A. Spectrophotometry

Spectrophotometric techniques are techniques based on the differential absorption of light by different chemicals. These techniques serve a wide array of functions in biology. They can be used to determine the concentration of many compounds, such as DNA and proteins, and they can be used to measure enzyme activity.

![Diagram of interior workings of a spectrophotometer. The collimator, or focusing device, focuses the light into an intense beam. The prism separates the light into component wavelengths, and the wavelength (λ) selector selects the particular wavelength at which your sample absorbs.](image)

The machine we'll be using is a spectrophotometer. It measures absorbance, the amount of light of a given wavelength (λ) absorbed by a substance. It does this by measuring the intensity of light after it has passed through the substance. The essential components of the instrument are shown below in Figure 2.2.

We make use of the phenomenon that the absorption of light at a given wavelength is related to the concentration of the absorbing chemical. For instance, if I₀ is the intensity of the incident light (the light entering the sample) and I is the intensity of the transmitted light (the light leaving the sample), then the absorbance is the relative amount of light that is absorbed by the sample:

\[ A = \log \frac{I_0}{I} \]

Thus, if the intensity of light coming out of the sample is the same as the light going in, then no light has been absorbed and \( A = \log (1) = 0 \). On the other hand, if the absorbance is high, this means that very little light passed through the solution. In the visible spectrum, you can think of water as having an absorbance close to 0, while milk has a large absorbance. Note that the relationship between absorbance and proportion of light transmitted is logarithmic, so a 10 times reduction in transmitted light results in an increase in absorbance (A) of only 1.

The conversion of absorbance to concentration of absorbing substance is straightforward:

\[ A = \varepsilon c \ell \quad \text{Beer-Lambert Law} \]

where A is absorbance, \( \varepsilon \) is the extinction coefficient (a property of the compound that is doing the absorbing), c is the concentration of the absorbing material, and \( \ell \) is the length of the light path, usually 1 cm. This little equation is so important that it has been made into a law -- the Beer-Lambert Law or sometimes Beer's Law.
B. Use of Micropipettors

Throughout this term, you will be required to pipette accurately and reproducibly. This is not as easy as it sounds. In fact, next to failing to read and think about the lab directions, pipetting mistakes are the greatest source of heartache in the laboratory.

To facilitate this important task, we will be using highly accurate and sophisticated (and expensive) automatic pipettors. There are several brands available, but we will be using the Pipetman. You will need to make use of 3 different micropipettors:

1) the **P1000**, which measures 200 to 1000 µl (microliters);
2) the **P200**, which measures 20 to 200 µl;
3) and the **P20**, which measures 1 to 20 µl.

Fill out the following chart:

1 µl = ____ml; measure it with a P____
10 µl = ____ml; measure it with a P____
100 µl = ____ml; measure it with a P____
1000 µl = ____ml; measure it with a P____

Figure 2.3. All of the micropipettors operate in the same fashion. To choose the volume, hold the micropipettor body in one hand and turn the volume adjustment knob until the correct volume shows on the digital indicator. The volumes are read from the top down.

Setting volumes on the pipetmen:

To use the pipettors, first set the volume to pipet using the dial on the pipet. See the table below for example settings on the pipettors.

For the **P1000**:

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equals 1000 µl or 1 ml
equals 500 µl or 0.5 ml
equals 250 µl or 0.25 ml

For the **P200**:

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<td>0</td>
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equals 200 µl or 0.2 ml
equals 85 µl or 0.085 ml
equals 50 µl or 0.05 ml

For the **P20**:

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<td>2</td>
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equals 20 µl or 0.02 ml
equals 10.5 µl or 0.0105 ml
equals 5.5 µl or 0.0055 ml
Using the pipettes:

After you have dialed in the appropriate volume, attach a disposable tip to the shaft. The P1000 uses the large tips (usually blue or clear) and the P200 and P20 use the smaller size (usually yellow). Next, depress the plunger to the first stop. This part of the stroke is the calibrated volume displayed on the dial. Immerse the tip into the sample liquid to a depth of several mm. Allow the plunger to return slowly to the up position. Never let it snap up. (Why?) Withdraw the tip from the liquid and remove any adhering liquid by touching to the inside of the tube holding the sample liquid.

To dispense the sample, place the tip end against the side wall of the receiving tube and depress the plunger slowly to the first stop. Wait a second and then depress the plunger to the second stop. With the plunger depressed, remove the tip from the tube, allow the plunger to return to the top position and discard the tip.

The **most common mistake** in pipetting is using the second plunger stop to fill the pipet – don't do this! You should use the first stop for filling the pipet, and the second stop for emptying it.

Rules for pipetting

It is always good to have a list of rules. Here is a list of rules for pipetting:

- Never rotate the volume adjuster beyond the upper or lower range of the pipettor.
- Never force the volume adjuster. If force is required, you are doing something wrong or the pipettor is broken. In either case, see your instructor. In general, in laboratories as well as life, if force is required, back off and think about it.
- Never use the pipettor without a tip (No duh!).
- Never lay down the pipettor with liquid in the tip.
- Never let the plunger snap back after withdrawing or ejecting sample.
- Never immerse the barrel into solution.

Follow these rules throughout your life and you will be a great success in any molecular or biochemistry lab.
C. Making a Standard Curve

For this lab, we'll determine protein concentration of an unknown sample using a standard curve. We will first measure the absorbance of several samples of known protein concentration. We can then draw a graph of the relationship between absorbance and protein concentration. Once we have this standard curve, we can measure the absorbance of an unknown sample and read its corresponding protein concentration from the graph. Without an accurate standard curve, well, there is just not much point in going on.

Preparation of the Standard Curve

We will construct a standard curve according to the table below. In light of the discussion above, the standard curve must be linear. If it is not linear, do it again. However, before you redo the curve, graph it and show it to your lab instructor or teaching assistant in order to confirm that you need to do it again. Sometimes you are too hard on yourselves, although sometimes you give yourself too much slack, also.

We will do each tube in duplicate. This is always a good idea. (Why?) In our case, the duplicates must be within 10% of the average of the two readings. (The acceptable difference varies with the nature of the experiment, the type of equipment, and the sophistication of the experimenter.)

The Bradford Assay

The Bradford assay is, more or less, the gold standard of protein concentration determination. It is quick and can detect protein concentrations as low as 1µg/ml. The assay takes advantage of the fact that the color of the dye Coomassie Brilliant Blue G-250 changes when it is bound to proteins. This color change alters the amount of light absorbed by the solution, which in turn can be measured with the spectrophotometer. The Coomassie blue binds primarily to basic and aromatic amino acid residues, especially arginine. This specificity does introduce the problem of varying sensitivity, depending on the amino acid composition of the proteins, but this is compensated for by its ability to detect such small amounts of protein. (Under what circumstances would the specificity of the Coomassie blue binding a potential problem?) The Bradford reaction comes in 2 forms, one which can detect 200 µg/ml to 1200 µg/ml, and a souped up version that can detect 1µg/ml. We will use the less sensitive version.
Directions for using the Spectrophotometer:

1. Turn on the spectrophotometer (the switch is on the back of the machine next to the power cord). When you turn on the Spectronic 20 Genesys instrument ("spec 20"), it performs its automatic power-on sequence (check to be sure that the cell holder is empty and its cover is closed before turning on the instrument). The self-check sequence takes about 2 minutes to complete; do not interrupt it during this sequence. **Allow the instrument to warm up for about 30 minutes** before you use it.

2. The machine should be in "Absorbance mode" – i.e., the display should show some numbers followed by an "A". If not, press 'A/T/C' to select the absorbance mode.

3. Press \( \text{nm} \) or \( \text{nm} \) to set the wavelength to 595 nm. (The Coomassie blue absorbs light in the yellow portion of the spectrum, around 595 nm, leaving only the blue-purple color that you see.) Note: holding either key will cause the wavelength to change more rapidly than pressing many times.

4. Next, set up your standard curve tubes (#1-12), including the five minute incubation, according to the table on the next page.

5. After the machine is warmed up, you need to **blank the spectrophotometer**. To do this, pour the fluid from tube #1 (see the table on the next page) into the cuvette (see illustration below), wipe the cuvette with a kimwipe, and place the cuvette into the spec 20, aligning the mark on the cuvette with the mark on the sample holder. Press the "0 ABS/100% T" button. The display should show an absorbance of 0.000.

You will be reading all your samples in a cuvette (see illustration below). To transfer your samples to the cuvette, pour from your sample tube directly into the cuvette. Each time you put the cuvette into the spec, you should wipe the outside of the cuvette with a **kimwipe** (a lab tissue). Any crud on the outside of your cuvette will give you an erroneous reading and tend to build up inside the spec (a bad thing). After you take a reading, we recommend that you pour your sample back into the tube it came from, just in case you need to take a second reading later. After you get most of the sample back in its original tube, gently tap the cuvette upside down on a Kimwipe to get as much fluid out as possible. Rinse out the cuvette with water from a squirt bottle between samples; again, tap the cuvette on a Kimwipe to remove extra fluid. You do not need to dry the cuvette.

All absorption measurements need to be made relative to your blank (i.e., tube #1). This solution contains all of the components of the experimental tube, except the compound being measured. You can check your blank using tube 2, which should also give a reading of zero absorbance. Save tubes 1 and 2 to re-blank the spec for parts B and C below. Think carefully about why you are using tubes 1 and 2 to blank the spectrophotometer (why not just use water?). Check with your TA if you're not sure.

Follow the protocol below. You should display confidence. By the way, BSA stands for bovine serum albumin, a blood protein from cows. You have a very similar protein in your blood. It is essential for maintaining proper osmotic conditions and is important in carrying fatty acids, which are a common cellular fuel.
**The Standard Curve**

Note: All volumes are in ml

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<tr>
<td>BSA (1.0 mg/ml)</td>
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<td>0.02</td>
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<td>0.04</td>
<td>0.06</td>
<td>0.06</td>
<td>0.08</td>
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<td>0.1</td>
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<td>Water</td>
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<td>0.08</td>
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Mix each solution by gently shaking the tube, then add the Bradford Reagent.

Bradford Reagent | 5.0 | 5.0 | 5.0 | 5.0 | 5.0 | 5.0 | 5.0 | 5.0 | 5.0 | 5.0 | 5.0 |

Cover the tube with a small square of parafilm, invert several times to mix and then let stand at room temperature for at least 5 min. Insert tube #1 into the spec and zero the machine as described on the previous page. Then measure the absorbance at 595 nm for each of the other tubes and record these values in the table below. It is good form to re-blank the machine a couple of times (with tube #1) during these your runs.

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<td>$A_{595}$ (Reading from the Spec 20)</td>
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Now, calculate how many micrograms of the standard protein were present **in 0.1 ml of sample** (that is, before you added the Bradford Reagent volume) and record that number below. (Hint: you can do this before coming to lab.)

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<td>µg of protein added:</td>
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**Graphing a Standard Curve**

Using the graph paper included at the end of this lab, plot the absorbance readings on the ordinate of a graph (y-axis) and the amount of protein in µg on the abscissa (x-axis). Now show this graph to your lab instructor or teaching assistant in order to confirm that your curve is linear. If it is not, the most likely cause is pipetting errors.

Now enter your data into a Microsoft Excel spreadsheet using a computer in the adjacent computer lab (Hulings 106) – enter the absorbance for each tube separately (don't average them) so you can tell if one of the values is abnormal. Your graph should produce a straight line. Fit a line to the curve and record the equation for the best-fit line.

With this standard curve, you can determine the protein concentration of an unknown sample by recording its absorbance in the Bradford Assay, and then reading the corresponding amount of protein off of the standard curve.
D. Determining the concentration of protein in an unknown sample

Preparation of the Unknown Sample of Bovine Serum Albumin (BSA)

It is essential that the absorbance for the unknown falls within the parameters of the standard curve. (Make sure that you understand why this is so.) But, because it is an unknown, we don’t know for sure whether this will be the case. One way around this difficulty is to test the unknown and if it is off of the standard curve, do it again at a lower or higher concentration, depending on which end of the standard curve the unknown fell off. Another way is to dilute the sample before we run the test. This is the approach we will take.

You need to make a 1:10 and a 1:100 dilution of your unknown. Once you make up these dilutions, in smaller tubes, you can take samples from these to use in the Bradford assay.

1:10 Dilution:

Into a microfuge tube (these are small, plastic, colored tubes found in a jar or beaker at your bench), pipette 0.27 ml of water. To this, add 30 µl of your unknown. Mix carefully--one common way in which biologists mix small volumes is by pipetting up and down. When you add the last component (in this case the 30µl of unknown) to your microfuge tube, expel the fluid into the solution by putting the tip directly into the solution (rather than on the side of the microfuge tube above the solution). Then suck some of the solution back up into the pipet tip; expel this back into the microfuge tube and repeat several times. If you use this technique, you must be careful not to get fluid up into the pipettor, especially when the volume measured is close to the capacity of the pipettor! It is very easy to suck up too much fluid or air bubbles, which will cause fluid to rise into the barrel of the pipettor--this is very bad, since the pipettor then will not work properly. Watch closely when you pipet small volumes.

Make sure you label your microfuge tube. You will use 0.1 ml of this (in tubes 15 and 16) for the Bradford assay below.

1:100 Dilution:

Add 0.297 ml of water to a clean, labeled microfuge tube. To this, add 3 µl of your unknown. Alternatively, you can make a 1:10 dilution of your 1:10 dilution. Mix carefully (see note above).

You will use 0.1 ml of this (in tubes 17 and 18) for the Bradford assay below.

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1 A note on dilutions: Conventions for expressing dilutions vary from lab to lab, and sometimes from situation to situation. Usually, a 1:10 dilution means a "10-fold" or a "10x" dilution. When people write "1:10 dilution," they tend to mean 1 part of stuff you're diluting in ten parts TOTAL. You're making something ten times weaker than it started out. A more correct way of stating this as a true ratio is to call it a 1:9 dilution (one part stuff to nine parts buffer), and some people do this. One common exception to this is circumstances which require a large dilution (1:200, or 1:500), and the actual amount of stuff isn't critical. In these cases, people will actually use 200 parts of buffer, add one part of stuff, and disregard the extra part. The best thing to do is to ask for clarification when you're in a new situation.
Determination of the Protein Concentration of an Unknown Sample of BSA

Prepare a Bradford Assay according to the table below.

**Note:** All volumes are in ml

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<th>Tube #</th>
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<th>15</th>
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<th>18</th>
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</thead>
<tbody>
<tr>
<td><strong>Additions</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unknown (undiluted)</td>
<td>0.1</td>
<td>0.1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>1:10 Dilution of Unknown</td>
<td>-</td>
<td>-</td>
<td>0.1</td>
<td>0.1</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>1:100 Dilution of Unknown</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.1</td>
<td>0.1</td>
</tr>
</tbody>
</table>

Mix the solutions by gently shaking the tube, then add the Bradford Reagent.

Bradford reagent | 5.0 | 5.0 | 5.0 | 5.0 | 5.0 | 5.0

Cover the tubes with parafilm, invert several times to mix and then let stand at room temperature for at least 5 min. Rezero your machine with tube #1. For each of tubes 13-18, pour the contents into a cuvette and measure percent absorbance at 595 nm. Record your readings in the table below.

<table>
<thead>
<tr>
<th>Tube #</th>
<th>13</th>
<th>14</th>
<th>15</th>
<th>16</th>
<th>17</th>
<th>18</th>
</tr>
</thead>
<tbody>
<tr>
<td>(A_{595}) (Reading from the Spec 20)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Now, using the absorbance values of the unknown that you have just recorded and the equation for standard curve from above, determine how much protein was present in 0.1 ml (100 µl) in each of the experimental tubes (how much protein sample was added to each tube?). Enter these values into the table below.

<table>
<thead>
<tr>
<th>Tube #</th>
<th>13</th>
<th>14</th>
<th>15</th>
<th>16</th>
<th>17</th>
<th>18</th>
</tr>
</thead>
<tbody>
<tr>
<td>(\mu g) protein in 0.1 ml sample: (From Standard Curve)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Now calculate the concentration of your undiluted unknown in terms of mg/ml. Here is one way to do this: For each of the three dilutions, average the values for the duplicate samples. Then convert these averages from µg to mg by dividing by 1000 (which is the number of µg per mg). This will give you values in terms of mg per 0.1 ml, so to get to mg/ml, you’ll need to divide by 0.1. Finally, for the 1:10 dilution, multiply the resulting value by 10, and for the 1:100 dilution, multiply the resulting value by 100. These results will give you estimates of the concentration of your undiluted unknown in mg/ml.

Did all three dilutions of your unknown yield the same final value of protein concentration? If not, why not? What values do you trust most? Why?
IV. LABORATORY WRITE-UP

Due date: in lab next week

Include only the following items in this week’s lab write-up. You are not responsible for turning in answers to the questions scattered throughout the manual. And this is not a group assignment; you should turn in your own work.

1) A copy of your sketch of an organism from the pondwater sample. Make sure to include the scale reference and a note of the magnification.

2) The BSA standard curve, including all components of a good graph. This should include a caption below your figure and clearly labeled axes.

3) A detailed calculation of the protein concentration (in mg/ml) of the unknown solution of BSA; include a calculation for each dilution.

4) State which dilution you have the most confidence in, and why.