Advanced Biology
with Vernier

Contents

Experiments

Appendixes

Sensors Used in Experiments

		Conductivity	Gas Pressure	O_2	Colorimeter or Spectrometer	CO_2	Blood Pressure	Heart Rate	Dissolved Oxygen	Temperature	White or Blue Digital Bioimaging System	ProScope
1A	Diffusion through Membranes	1										
1B	Osmosis		1									
2	Enzyme Action (Method 1)			1								
	Enzyme Action (Method 2)		1									
3	Mitosis and Meiosis	No sensor										
4A	Plant Pigment Chromatography	No sensor										
4B	Photosynthesis				1							
5	Cell Respiration (Method 1)			1		1						
	Cell Respiration (Method 2)					1						
	Cell Respiration (Method 3)			1								
	Cell Respiration (Method 4)		2									
6A	pGLO™ Bacterial Transformation	No sensor										
6B	Analysis of Precut Lambda DNA (Option 1)										1	1*
	Forensic DNA Fingerprinting (Option 2)										1	1*
7	Genetics of Drosophila	No sensor										
8	Population Genetics and Evolution	No sensor										
9	Transpiration		1									O
10A	Blood Pressure as a Vital Sign						1					
10B	Heart Rate and Physical Fitness							1				
11	Animal Behavior	No sensor										
12A	Dissolved Oxygen in Water								1	1		
12B	Primary Productivity								1			
13	The Visible Spectra of Plant Pigments				1+							
14	Determination of Chlorophyll in Olive Oil				1+							
15	Enzyme Analysis using Tyrosinase				1							
16	Introduction to Neurotransmitters using ACHe				1							
17	Macromolecules: Experiments with Protein				1							

* - Included in the Blue or White Digital Bioimaging System

O - Optional

\+ - Spectrometer only

Transpiration

Water is transported in plants, from the roots to the leaves, following a decreasing water potential gradient. *Transpiration*, or loss of water from the leaves, helps to create a lower osmotic potential in the leaf. The resulting transpirational pull is responsible for the movement of water from the xylem to the mesophyll cells into the air spaces in the leaves. The rate of evaporation of water from the air spaces of the leaf to the outside air depends on the water potential gradient between the leaf and the outside air.

Various environmental factors, including those conditions which directly influence the opening and closing of the stomata, will affect a plant's transpiration rate. This experiment will measure transpiration rates under different conditions of light, humidity, temperature, and air movement. The data will be collected by measuring pressure changes as the plant takes up water into the stem.

OBJECTIVES

In this experiment, you will

- Observe how transpiration relates to the overall process of water transport in plants.
- Use a handheld interface and a Gas Pressure Sensor to measure the rate of transpiration.
- Determine the effect of light intensity, humidity, wind, and temperature on the rate of transpiration of a plant cutting.

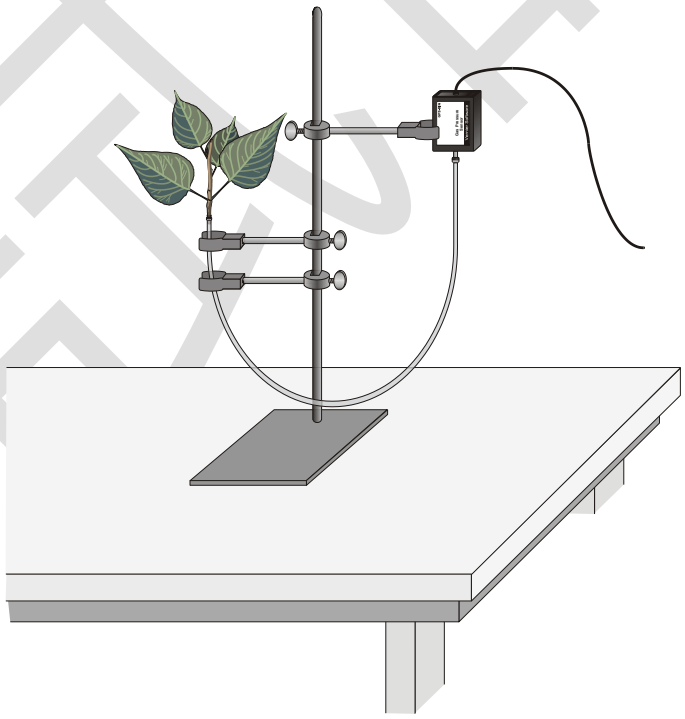

Figure 1

MATERIALS

LabQuest
LabQuest App
Vernier Gas Pressure Sensor
utility clamps
ring stand
plant cuttings
plastic tubing clamps
dropper or Beral pipette
razor blade or scalpel
100 watt light source
metric ruler
masking tape
plastic gallon size bag with twist tie
heater, small electric
fan with slow speed
aerosol spray container or plant mister
plastic syringe
graph paper or Logger *Pro* program
ProScope (optional)

PROCEDURE

1. Position the ring stand, utility clamps, and Gas Pressure Sensor as shown in Figure 1.

2. Prepare the plastic tubing.
   a. Connect the plastic syringe to one end of a 36–42 cm piece of plastic tubing.
   b. Place the other end of the tubing into water and use the syringe to draw water up into the tubing until it is full. Tap the tubing to expel any air bubbles that form inside the tube.
   c. Slip a plastic tubing clamp onto the tubing as shown in Figure 2.
   d. Bend the tubing into a U shape with both ends up. Remove the syringe, leaving the tubing full of water.

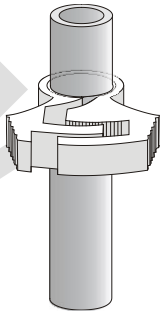

Figure 2

3. Select a plant which has a stem roughly the same diameter as the opening of the plastic tubing. Using a scalpel or razor blade, carefully cut the plant one inch above the soil. Place the plant under water against a hard surface and make a new cut at a 45° angle near the base of the stem.

4. Connect the plant to the tubing.
   a. The plastic tubing has a white plastic connector at one end that allows you to connect it to the valve on the Gas Pressure Sensor. Raise the end of the tubing with the connector until you see water beginning to drip out of the other end.
   b. Carefully push the cut stem of the plant down into the end of the tubing where the water is dripping out. Be careful not to allow any air bubbles to form between the cut portion of the stem and the water in the tube.
   c. Push the plant down as far as it will go without damaging the plant. At least one centimeter of the plant stem should fit into the tubing. If the stem is too large for the tubing, cut the stem at a higher point where it is smaller.
   d. Squeeze the tubing clamp shut as tight as possible as shown in Figure 3.

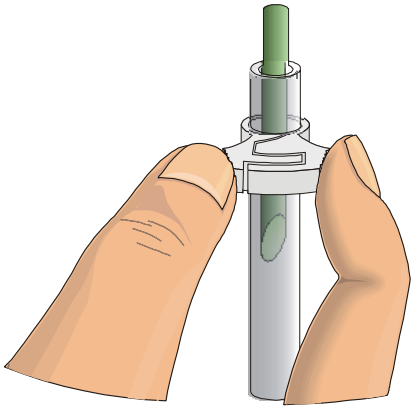

Figure 3

5. When the tubing clamp is shut tight, invert your plant cutting to check for any leaks. If water does leak out, turn the plant right-side up and try tightening the clamp further.

   Important: Be sure the tubing is filled completely with water. The water column must be flush with the stem. There should be no air visible at the base of the stem. If water moves down the tube away from the stem after it has been inserted, check for a leak in the system.

6. Connect the plastic tubing to the sensor valve. **Caution**: Do not allow water to enter the valve of the Gas Pressure Sensor.

7. Secure the plant in an upright position with the utility clamps as shown in Figure 1. It should be positioned so that the cut stem is about 8 cm below the water level at the other end of the tubing, as shown in Figure 1.

8. Place a mark on the tube at the starting water level to allow you to refill the tube to the proper level when you repeat data collection.

9. Place your plant setup in an area where the wind, humidity, and temperature are reasonably constant. This will be your control setup.

10. Allow the system 5 minutes to adjust to the environment. Proceed to the next step to set up the sensor and LabQuest while the system is adjusting.

11. Connect the Gas Pressure Sensor to LabQuest and choose New from the File menu. If you have an older sensor that does not auto-ID, manually set up the sensor.

12. On the Meter screen, tap Length. Change the data-collection length to 900 seconds. Select OK.

13. Check the base of the plant stem in the water tube to make sure that no air bubbles or air pockets have formed that will prevent the plant from taking up water. If an air pocket has formed, refit the plant in the tubing before initiating data collection in Step 13.

14. After the plant has equilibrated for 5 minutes, start data collection. Data will be collected for 15 minutes and then a graph of pressure *vs.* time will be displayed. If it is necessary to quit data collection early, you can stop data collection to view a graph of pressure *vs.* time.

15. When data collection has stopped, perform a linear regression to calculate the rate of transpiration.

    a. Choose Curve Fit from the Analyze menu.
    b. Select Linear as the Fit Equation. The linear-regression statistics for these two data columns are displayed for the equation in the form

    $$y = mx + b$$

    where x is time, y is pressure, m is the slope, and b is the y-intercept.
    c. Enter the absolute value of the slope, m, as the rate of transpiration in Table 1.
    d. Select OK.

16. Design an experiment to simulate *one* of the following environmental conditions, as assigned by your teacher:
    - the effect of light intensity
    - the effect of the wind blowing on the plant
    - the effect of humidity
    - the effect of temperature
    - the effect of another self-identified environmental variable

    Be sure to address the following questions in your design:
    - What is the essential question being addressed?
    - What assumptions are made about the system being measured?
    - Can those assumptions be easily verified?
    - Will the measurements provide the necessary data to answer the question under study?
17. After checking your procedure with your teacher, obtain the materials needed for the experiment and perform the tests. Record your values in Table 1.

PROCESSING THE DATA

1. Determine the surface area of all the leaves on your plant cutting by using one of the following methods:

Method 1 – Using Logger *Pro* to Determine Leaf Surface Area

This method requires a ruler, a digital camera such as a ProScope HR with the 1–10X lens, and Logger *Pro* software.

a. Carefully remove the leaves from the stem.
b. Select a sample leaf and place it on a flat surface with a ruler along the horizontal axis of the leaf to provide scale. Place a clear Plexiglas plate over the leaf specimen (optional).
c. Select Video Capture from the Insert menu. Adjust the ProScope HR lens making sure that both the leaf and the ruler are in focus. The ProScope handle should be parallel to the table surface to avoid angular distortion.
d. Click Take Photo to capture the leaf image. **Note:** The captured image may be hidden behind the Video Capture window. Close the Video Capture window.

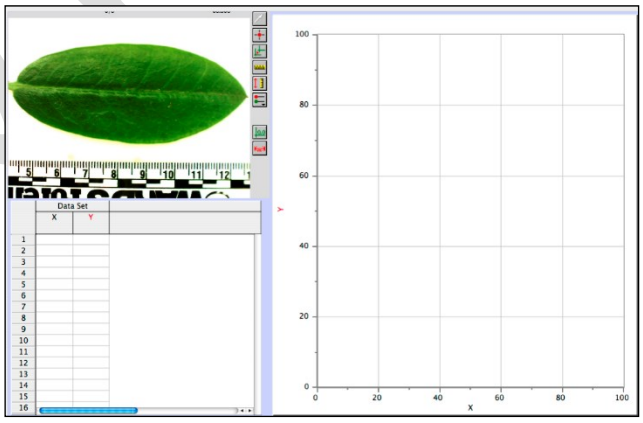

Figure 4

e. Select Auto Arrange from the Page menu. **Note:** If there are no analysis buttons along the right side of the captured image as seen in Figure 4, double-click on the image. Select Standard Analysis from the Picture Analysis options and select OK.

f. Set the scale of the image by clicking Set Scale, . Click and drag the cursor between two distinct points on the ruler separated by several centimeters. Enter the distance between the points and the units. Select OK.

g. Click Add Point, . Move your cursor to the edge of the leaf and click to add a point. Circumscribing the leaf with points in a sequential clockwise manner as shown in Figure 5. Points need to be close enough to register all directional changes along the leaf edge. Place the final point directly on top of the starting point.

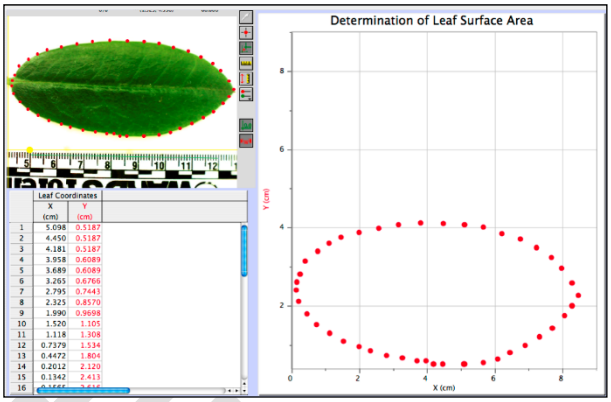

Figure 5

h. Click the Integral button, . The displayed integral value is equal to the surface area of the leaf. Record this value in the Individual Leaf Surface Areas table below.

i. Repeat Steps 1a–h for each leaf.

j. Add the surface areas of all the leaves and record the total surface area in Table 1.

Individual Leaf Surface Areas							
Leaf Number	Surface Area (cm^2)	Leaf Number	Surface Area (cm^2)	Leaf Number	Surface Area (cm^2)	Leaf Number	Surface Area (cm^2)

Method 2 – Using Mass to Determine Leaf Surface Area

This method requires a balance.

a. Cut all the leaves (not stems) off your plant and determine their mass using a balance.

b. Estimate the total leaf surface area in cm^2 for your plant by cutting out a section of leaf 5 cm × 5 cm.

c. Determine the mass for this leaf section and divide by 25 cm^2 to find the mass of 1 cm^2 of leaf.

d. Divide the total mass of the leaves by the mass of 1 cm^2 to find the total leaf surface area.

e. Record the calculated surface area in Table 1.

2. Calculate the rate of transpiration/surface area. To do this, divide the rate of transpiration by the surface area for each plant. These rate values can be expressed as kPa/min/cm^2. Record the rate/area in Table 1.

3. Subtract the control (rate/area) value from the experimental value. Record this adjusted rate in the last column of Table 1.

4. Record the adjusted rate for your experimental test on the board to share with the class. Record the class results in Table 2 for each of the environmental conditions tested. If a condition was tested by more than one group, take the average of the values and record in Table 2.

5. Make a bar graph that shows the effect of different environmental conditions on the transpiration of water in plant cuttings. Using the data in Table 2, plot the adjusted rate for each test on the y-axis and the test label on the x-axis.

DATA

Table 1				
Test	Slope (kPa/s)	Surface area (cm^2)	Rate/area ($kPa/s/cm^2$)	Adjusted rate ($kPa/s/cm^2$)
Experimental _____				
Control				

Table 2: Class Data	
Test	Adjusted rate ($kPa/s/cm^2$)
Light	
Humidity	
Wind	
Temperature	

QUESTIONS

1. How was the rate of transpiration affected in each of the experimental situations as compared to the control?

2. Which variable resulted in the greatest rate of water loss? Explain why this factor might increase water loss when compared to the others.

3. What adaptations enable plants to increase or decrease water loss? How might each affect transpiration?

EXTENSIONS

1. Using a compound microscope, identify the vascular tissues of a plant stem. Describe the function of each tissue type identified.
   a. Obtain a section of stem from the plant you used during the transpiration experiment.
   b. Using a nut-and-bolt microtome, carefully cut 6 cross sections of the plant stem. The cross sections should be cut as thin as possible.
   c. Place each of the cross sections in a dish or cup of 50% ethanol solution for 5 minutes.
   d. Remove the cross sections from the alcohol and place them in a dish containing toludine blue O stain for 5 minutes.
   e. Rinse the cross sections with distilled water and mount them on a microscope slide with a drop of 50% glycerin. Place a cover slip on the slide and examine the cross sections using a compound microscope.
   f. On a separate sheet of paper, make a drawing of the cross sections. Identify and label the cell and tissue types described by your teacher.
2. Test cuttings from a variety of different plant species. How does each compare?
3. Count the number of stoma/cm^2 for each of the plants in Extension 1. How does this relate to the plant's ability to transpire water?
4. Design an experiment to test for the variables in Question 3.

Determination of Chlorophyll in Olive Oil

Olive oil is made by pressing or extracting the rich oil from the olive fruit. It seems like a simple matter to press the olives and collect the oil, but many oil extraction processes exist for the many different types of olives grown around the world. To complicate things further, there are also various grades of olive oil, and carefully selected groups of officials meet to define and redefine the grading of olive oil. To help make our experiment a more scientific and less political exercise, we will winnow our investigation of olive oil down to a manageable few variables.

After processing, olive oil comes in three common grades: extra virgin, regular, and light. Extra virgin olive oil is considered the highest quality. It is the first pressing from freshly prepared olives. It has a greenish-yellow tint and a distinctively fruity aroma because of the high levels of volatile materials extracted from the fruit. Regular olive oil is collected with the help of a warm water slurry to increase yield, squeezing every last drop of oil out of the olives. It is pale yellow in color, with a slight aroma, because it contains fewer volatile compounds. Light olive oil is very light in color and has virtually no aroma because it has been processed under pressure. This removes most of the chlorophyll and volatile compounds. Light olive oil is commonly used for frying because it does not affect the taste of fried foods, and it is relatively inexpensive.

The visible light absorbance spectrum of chlorophyll gives interesting results. The chemistry of chlorophyll (some references site four types: a, b, c, and d) creates absorbance peaks in the 400–500 nm range and in the 600–700 nm range. The combination of visible light that is not absorbed appears green to the human eye, but different sources of chlorophylls will have different ratios of these peaks, which create various shades of green. The ability of chlorophyll to soak up light energy across a wide swath of the visible range helps power photosynthesis at optimum efficiency in plants.

In this experiment, you will have two primary goals. First, you will analyze the various grades of olive oil to determine the absorbance peaks that are present and the relative amount of chlorophyll found in each grade. You will use a Spectrometer to measure the absorbance of the olive oil samples over the visible light spectrum. You will then test an unknown sample of olive oil and grade it as extra virgin, regular, or light.

OBJECTIVES

In this experiment, you will

- Measure and analyze the visible light absorbance spectra of three standard olive oils: extra virgin, regular, and light.
- Measure the absorbance spectrum of an “unknown” olive oil sample.
- Identify the unknown olive oil as one of the three standard types.

MATERIALS

LabQuest
LabQuest App
Spectrometer
samples of three olive oil standards:
extra virgin, regular and light
olive oil of unknown grade
five cuvettes and lids
plastic Beral pipets
distilled water
isopropyl alcohol

PROCEDURE

1. Obtain and wear goggles.
2. Connect the Spectrometer to LabQuest and choose New from the File menu.
3. Obtain small volumes of the three standard and one unknown olive oils. Transfer enough of one olive oil sample to fill a cuvette 3/4 full. Place a lid on the cuvette and mark the lid. Prepare all of your samples in this way so that you have four cuvettes of olive oil with labeled lids.

Part I Comparing Three Grades Of Olive Oil and Identifying an Unknown

For Part I of this experiment, you will calibrate the Spectrometer with distilled water. Your goals are: (1) to compare the absorbance spectra of the different grades of olive oil; and (2) to identify the grade of an unknown sample of olive oil.

4. Calibrate the Spectrometer.
   a. Prepare a blank by filling an empty cuvette 3/4 full with distilled water.
   b. Choose Calibrate from the Sensors menu.
   c. When the warmup period is complete, place the blank in the Spectrometer. Make sure to align the cuvette so that the clear sides are facing the light source of the Spectrometer.
   d. Tap Finish Calibration, and then select OK.
5. Conduct a full spectrum analysis of an olive oil sample.
   a. Place one of the olive oil samples in the Spectrometer.
   b. Start data collection. A full spectrum graph of the olive oil will be displayed.
   c. Stop data collection.
   d. Review the graph to identify the peak absorbance values.
6. Tap the File Cabinet icon to store your data.
7. Repeat Steps 5–6 with the remaining olive oil standard samples.
8. Repeat Step 5 with the unknown. **Note:** Do not store the last run.
9. Examine the plots of the olive oil samples. Before continuing with data collection, answer the Part I Data Analysis questions. Sketch, print, or save your experiment file, as instructed.
10. Rinse and clean the cuvettes and other oil-bearing containers with isopropyl alcohol.

Part II Comparing the Chlorophyll Concentration of Regular and Extra Virgin Olive Oil

In Part II, you will use the light grade of olive oil to calibrate the Spectrometer and presume that light olive oil contains no chlorophyll. Next, you will compare the chlorophyll content of the regular grade with the extra virgin grade.

11. Set up a new file and calibrate the Spectrometer using light olive oil.
    a. Choose New from the File menu.
    b. Prepare a blank by filling an empty cuvette 3/4 full with light olive oil.
    c. Choose Calibrate from the Sensors menu.
    d. When the warmup period is complete, place the light olive oil blank in the Spectrometer. Make sure to align the cuvette so that the clear sides are facing the light source of the Spectrometer
    e. Tap Finish Calibration, and then select OK.
12. Measure the absorbance spectrum of regular and extra virgin olive oil.
    a. Remove the cuvette of light olive oil from the Spectrometer and replace it with the cuvette of regular olive oil.
    b. Start data collection. A full spectrum graph of the regular olive oil will be displayed. Note the slight difference in the plot as a result of using the light olive oil as the calibration blank.
    c. Stop data collection.
    d. Tap the File Cabinet icon to store your data
    e. Repeat data collection to measure the absorbance spectrum of the extra virgin grade.
13. Sketch, print, or save your data, if instructed to do so.

DATA ANALYSIS

Part I Comparing Three Grades Of Olive Oil and Identifying an Unknown

1. Describe the graph of each of the standard olive oil solutions. Emphasize the differences between each grade of olive oil, identifying the absorbance peaks and other distinguishing features.
2. Compare the absorbance spectra of the three grades of olive oil with the sample in Figure 1. What evidence is there that regular and extra virgin olive oil contain chlorophyll while the light grade of olive oil does not?
3. Identify your unknown olive oil as extra virgin, regular, or light. Explain your choice.

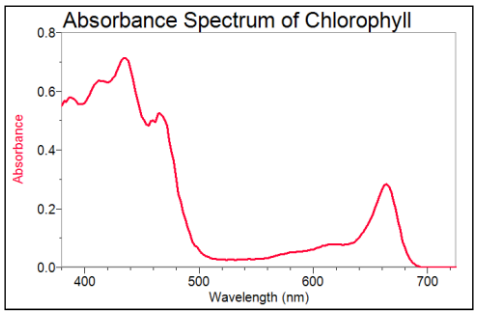

Figure 1

Part II Comparing the Chlorophyll Concentration of Regular and Extra Virgin Olive Oil

4. Which grade of olive oil, regular or extra virgin, contains the greater amount of chlorophyll? Use your absorbance spectrum graphs to speculate about how much more chlorophyll one grade contains compared to the other.

EXTENSIONS

1. Chlorophyll is a fluorescent molecule. Fluorescent molecules can absorb light of one wavelength and then reemit light at a new and longer wavelength of light. As you have seen in this exercise, chlorophyll absorbs light in the violet and blue regions of the spectra. If you were to shine a violet or blue light through a sample of extra virgin olive oil, you would see the oil turn red in color. The intensity of the red color is an indication of how much chlorophyll is in the olive oil. The "Long-Wave UV Pen Light" from Bio-Rad Laboratories, Inc. (Catalog # 166-0530EDU) can be used for this purpose. Shine the light from the Long-Wave UV Pen Light through a cuvette containing extra virgin olive oil. Does the sample that is hit by the light turn red in color? Repeat this test for regular olive oil, light olive oil, and your unknown. Could you use this method to determine if a sample of olive oil is really extra virgin olive oil? Could you use this method to determine the grade of any sample of olive oil?
2. Fluorescence spectroscopy is another method that can be used to determine the quality of olive oil. In fluorescence spectroscopy, a sample can be "excited" with a chosen wavelength of light and the resulting fluorescence from the sample can be measured and quantified. The SpectroVis Plus from Vernier Software & Technology can be used for this purpose. Follow the directions below to measure the fluorescence of all of your olive oil samples using the SpectroVis Plus.
   a. Connect the Spectrometer to LabQuest. Choose New from the File menu.
   b. Place the cuvette containing the extra virgin olive oil into the cuvette slot of the Spectrometer.
   a. Choose Change Units ► Fluorescence 405 nm from the Sensors menu.
   c. Tap Mode. Change the data-collection length to 150 ms. Select OK.
   d. Start data collection. A full spectrum graph of the fluorescence of the oil will be displayed. Note that one area of the graph contains a peak at approximately 675 nm. This peak is from chlorophyll. Stop data collection.
   e. If necessary, adjust the sample time to increase or decrease the size of the fluorescent peak. If the peak intensity is above 1, decrease the sample time by 10 ms and collect a new fluorescent spectrum. Continue to decrease the sample time until the peak is fully visible. If the fluorescent peak is below 0.3, increase the sample time by 10 ms and collect a new fluorescent spectrum. Continue to increase the sample time until the peak fluorescent amplitude for the chlorophyll is above 0.8.
   f. Once you have a nice peak, store your data by taping the File Cabinet icon.
   g. Collect full spectrum graphs from the remaining olive oil samples. Store each run. Do not adjust the sample time.
   h. Compare the fluorescent spectra of the three grades of olive oil. The peak that is visible at approximately 675 nm is from chlorophyll. Which sample has the largest peak in this region?
   i. Using the fluorescence from the known olive oil samples as your standards, determine the quality of your unknown olive oil sample.
   j. Compare your results using fluorescent spectroscopy to your results using traditional spectroscopy. Is one method better than the other? If so, please explain why.
   k. Print or save your experiment file as directed.