*Tested Studies for Laboratory Teaching*
*Proceedings of the Association for Biology Laboratory Education*
Vol. 34, 166-186, 2013

# Measurement of Rates of Aerobic Respiration and Photosynthesis in Terrestrial Plant Leaves Using Oxygen Sensors and Data Loggers

**William V. Glider and Peter Thew**

University of Nebraska-Lincoln, School of Biological Sciences, Lincoln NE 68588-0118 USA
(**wglider1@unl.edu; pthew2002@yahoo.com**)

The relationship between aerobic respiration and photosynthesis in plants is a basic biological concept which is often difficult for students to master. This exercise uses terrestrial plant leaves to measure the rate of photosynthesis at different light intensities and wavelengths as well as the rate of aerobic respiration rates of the leaves in the dark. This makes it possible to calculate gross and net photosynthesis and the light compensation point. The exercise employs the Vernier ™ oxygen sensor, Lab Pro data logger, and Logger Pro 3 software linked to a computer for data collection and analysis.

**Keywords**: plant, respiration, aerobic respiration, photosynthesis, oxygen sensor, leaves, sage leaves

## Introduction

The relationship between aerobic respiration and photosynthesis in plants (specifically angiosperms) is a concept which is often difficult for students to master. This exercise uses terrestrial plant leaves to measure the (1) net rate of photosynthesis at different light intensities and wavelengths and (2) rate of aerobic respiration in the dark. These data are then used to calculate gross photosynthetic rates and the light compensation point.

Traditionally, rates of photosynthesis and aerobic respiration in angiosperm leaves have involved a variety of indirect methods including counting the number of bubbles (assumed to be oxygen) released from the stems of the aquatic plant *Elodea* (Morholt, et.al. 1972), measuring changes in the amount of oxygen in the intracellular spaces of leaf disks in the light and dark (Pitkin 2004), or by measuring changes in carbon dioxide concentration associated with respiration and photosynthesis in *Elodea* using bromthymol blue as an indicator for the presence of carbon dioxide (Ecklund and Flerlage 2008). More sophisticated equipment for precise measurement of photosynthetic rates can be purchased from Qubit Systems Inc. (Qubit 2013). However, this apparatus is far more expensive than the Vernier oxygen sensors.

This exercise employs the Vernier™ oxygen sensor, Lab Pro data logger, and Logger Pro 3 software linked to a computer to directly quantify the net rate of oxygen evolution by a plant leaf in the light and oxygen consumption by the leaf in the dark. The lab focuses on hypothesis formulation, data collection, and data analysis. The exercise introduces the use of the slope of lines as a measure of rate, and provides the opportunity to discuss the difficulty in measuring photosynthetic rates in living leaves.

This lab exercise employs a directed investigative approach and has been used in both majors and mixed majors/non-majors, large enrollment, introductory biology courses. As written, it can be completed in a three hour lab session but can easily be extended to two lab sessions by using a more investigative approach in which students can test a variety of hypotheses related to other factors affecting rates of photosynthesis and respiration as well as the inclusion of a short exercise involving pigment separation and analysis.

# Student Outline

## Introduction

Certain organisms have the capacity to trap **light energy** directly, and utilize this energy to synthesize high energy monosaccharide sugars from low energy inorganic compounds such as carbon dioxide and water. These simple sugars can then be converted into all other organic nutrients (e.g. complex carbohydrates, lipids, proteins, etc.) required by the organisms to carry out their life processes. These organic compounds can then be oxidized within the cells of these organisms to produce ATP during the process of cellular respiration (aerobic or anaerobic) or used as structural components of cells. Such organisms are classified as **photoautotrophs** and the biochemical process by which they produce the simple sugars is called **photosynthesis**.

Photoautotrophs can be classified into two groups depending on whether or not they produce oxygen gas as a by-product of the photosynthetic reactions.

- Certain species of photosynthetic bacteria (e.g. purple sulfur bacteria) use special photosynthetic pigments (bacteriochlorophylls) to trap light energy. During the photosynthetic process **a substance other than water (often H2S) is oxidized** (loses electrons) with no oxygen being produced as a by-product.
- **Cyanobacteria, plants**, and most **algae** (plant-like eukaryotic organisms including the seaweeds traditionally classified in the Kingdom Protista) use the primary photosynthetic pigment **chlorophyll a** and a variety of **accessory pigments** to trap light energy. During the photosynthetic process **water is oxidized** (loses electrons) with the resulting production of **oxygen**. In **plants and algae** the reactions of photosynthesis take place in the **chloroplasts** whereas in the cyanobacteria the reactions take place on the **thylakoid membranes** in the cytoplasm. The following equation summarizes the process by which energy from light becomes converted to chemical potential energy in the bonds of the monosaccharide glyceraldehyde 3- phosphate (**G3P**), which is often converted directly into glucose (**$C_6H_{12}O_6$**):

$$12H_2O + 6CO_2 \xrightarrow{\text{light}} G_3P \longrightarrow C_6H_{12}O_6 + 6O_2 + 6H_2O$$

In **plants** the process of photosynthesis takes place in two successive steps called the **Light Dependent Reactions** (light reactions) and the **Light Independent Reactions** (dark reactions). Each of these two major steps is composed of a series of enzyme-catalyzed biochemical reactions.

The **light-dependent reactions**, also referred to as the light reactions, can take place only in the presence of light. During this phase of photosynthesis, light is absorbed by pigment molecules located in the thylakoids of the chloroplasts. This initiates a series of reactions that result in the conversion of some of the light energy to chemical energy. In the process,

- Water molecules are split apart, producing hydrogen ions, electrons, and oxygen gas. Some of the oxygen is used by the plant for cellular respiration, but most of it is released into the atmosphere
- Energy storing ATP molecules are created.
- The hydrogen ions resulting from the splitting of water are transferred to the hydrogen carrier, NADP, resulting in the formation of NADPH.

Thus, in the light-dependent reactions the energy from sunlight is used to make ATP and to reduce NADP+ to NADPH. Some of the captured energy is temporarily stored within these two compounds. Both ATP and NADPH are used in the light-independent reactions.

The **light independent reactions**, often referred to as the dark reactions or the Calvin Cycle, take place in the stroma of the chloroplast regardless of whether or not light is present. In this series of reactions, the reactions in the stroma use the ATP and NADPH produced in the light-dependent reactions to build simple sugars from $CO_2$. The light independent reactions can proceed only as long as ATP and NADPH are available. Because ATP and NADPH normally are produced only in the light, the light independent reactions normally stop within a few minutes of the onset of darkness.

Note that the light dependent reactions "drive" the light independent reactions, i.e., the products of the light dependent reactions, namely ATP and NADPH, are required for the light independent reactions to proceed (Fig. 1).

## Photosynthesis vs. Aerobic Respiration in Plants

It is important to remember that plants **respire continuously twenty-four hours per day** (taking in $O_2$ and releasing $CO_2$), as all aerobic organisms must do in order to obtain energy to carry on their life activities. A part of the oxygen released during photosynthesis is required for normal respiration, and when the plant is not carrying out photosynthesis (i.e., in the dark) it must obtain respiratory oxygen from the environment. **The consumption of oxygen in the dark may be used as a measure of respiration. In the light, with both photosynthesis and respiration proceeding simultaneously, the oxygen evolved is the difference between these opposing reactions.** Under optimal conditions, the rate of photosynthesis is twenty times greater than the rate of oxygen consumption by respiration. Hence, under such conditions, the net oxygen measured during photosynthesis represents 90% to 95% of the total oxygen produced.

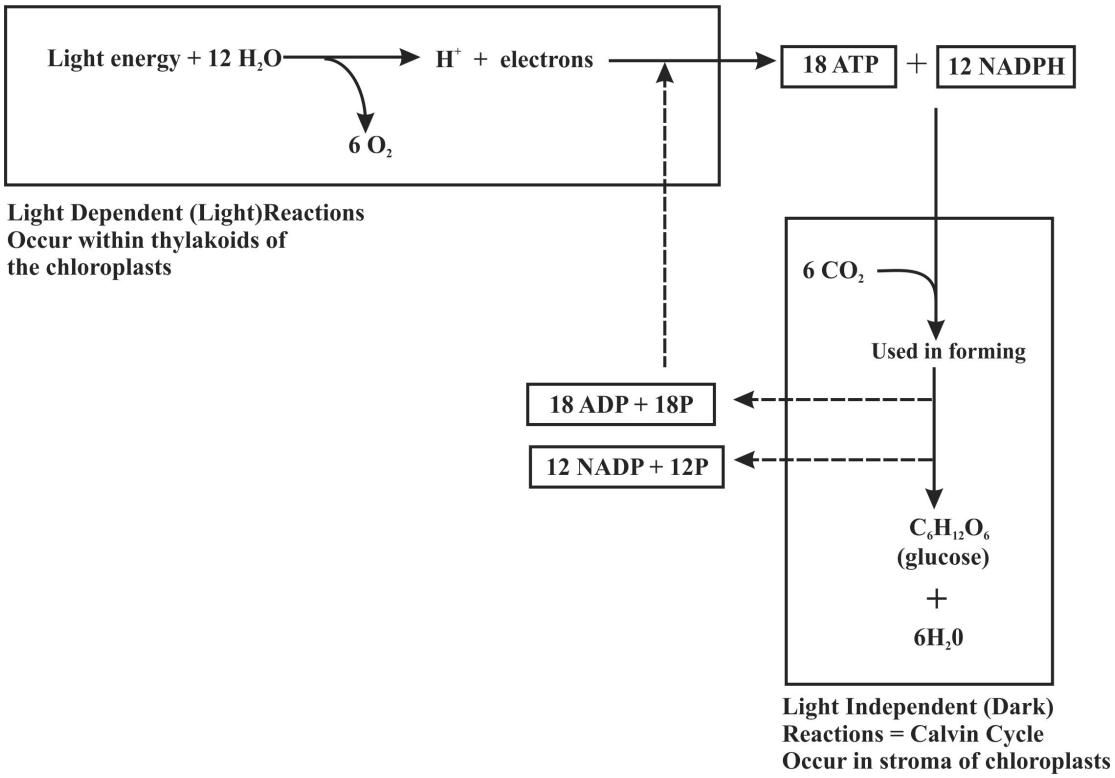



**Figure 1.** Relationship between Light Dependent and Light Independent Reactions of Photosynthesis

**General Procedure**

1. Each pair of students will carry out the following three experiments:
   a. Effect of Light Intensity on Net Photosynthetic Rate of a Leaf
   b. Effect of Light Wavelength on Net Photosynthetic Rate of a Leaf
   c. Rate of Oxygen Consumption of a Leaf in the Dark
2. This lab will require the use of a data logger (an electronic data collector) connected to a computer and an oxygen sensor (detector) for the collection of data. The oxygen sensor detects changes in the oxygen concentration in a sealed test tube containing a leaf.

   a. When using the Logger Pro program, only use those commands given in the procedure. Do not randomly click on menu items. If you mistakenly click on a menu item which takes you to a part of the program that is not called for in the lab exercise, notify your instructor so that he/she can help you return to the required part of the program.

   **b. The oxygen sensor must be kept in an upward position at all times. Therefore, when the sensor is not in use it should be stored upright in the container provided.**

   **c. When moving the sensor, grasp it by the upper end; not by the cord! Do not pull on the sensor cord.**

   **d. It is extremely important that the sensor is not jostled during data collection since it is likely to result in erroneous data being collected. To limit the possibility of the sensor being hit during data collection, work with the apparatus on your lab desk so that it is located close to the computer rather than being stretched out across the desk.**
3. Computer set-up prior to running the experiments:
   a. You will use a software program called **Logger Pro** to collect, analyze and display your data.
   b. Double click on the **Photosynthesis** Icon on the Windows Desktop.
   c. Figure 2 is a screen capture of the Logger Pro program as it will appear at the end of data collection.

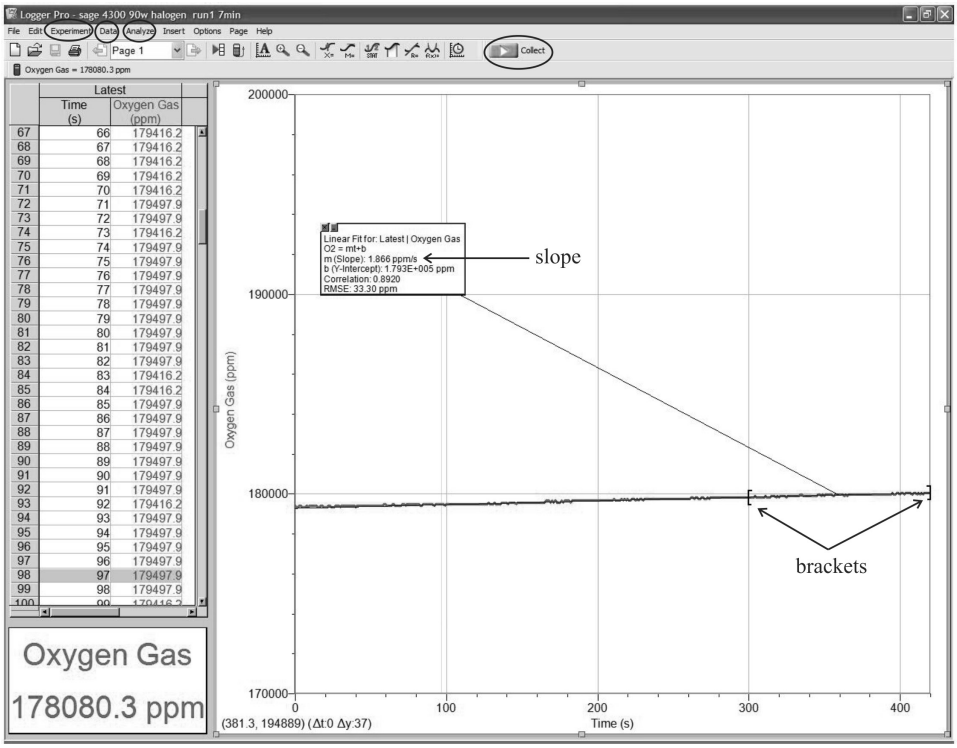



**Figure 2.** Screen Shot of Logger Pro Set-Up

- **Collect Button:** Click on this button to start data collection. Data collection will stop at the end of the 7 minute data collection period.
- **Analyze Button:** Use the drop down menu to display the slope (rate of oxygen production or consumption).
- **Data Button:** Use the drop down menu to Clear All Data after each data collection.
- **File:** Use the drop down menu to save your data to the desktop or flash drive.
- **Ctrl D:** Use for selecting length and rate of data collection.
- **Right Mouse Click:** Use for setting graph options.

**Setting Light Intensities**

1. Read the instructions in Appendix A describing the operation of the light meter. Consult your instructor if you have questions on its operation.
2. Hold the light meter sensor on the back of the heat sink so that the white plastic photocell is **facing** the flood light. Make sure the **photocell is held against the heat sink** while taking the readings.
   - To insure consistent light measurements at each intensity it will be necessary to place the light sensor head in the exact same position on the heat sink. To insure this is the case, trace an outline of the sensor head on the heat sink with a water soluble marker (vis á vis) pen. Each time you make a light measurement place the sensor head in the traced area.
   - It may be necessary to elevate the base of the light source to insure the light beam irradiates the leaf at a 90 degree angle. Styrofoam blocks are provided for this purpose.
   - To insure that the base of the light source remains in a straight line when moved toward or away from the experimental apparatus, draw lines on the plastic template which represent the sides of the lamp base.
3. By moving the light source base to different distances from the heat sink, identify and mark on the template the following light intensities: **800, 1000, 1500** and **3000** and **7000** fc (footcandles).

**Leaf Preparation**

1. Fill the plastic water bath with 23$^{o}$C water and place it on the appropriately marked area on the template.
2. Make sure the inside surfaces of the test tube are dry.

3. Using a graduated plastic pipet, add **2 ml** of the saturated sodium bicarbonate solution to the test tube while keeping the sides of the tube dry. Sodium bicarbonate serves as a carbon dioxide source according to the following equation:

$$NaHCO_3 \longrightarrow NaOH + CO_2$$

4. Using a pasteur pipet, completely fill the micro-centrifuge tube (inserted in a clear tubing base) with tap water. Place a small piece of aluminum foil over the top of the micro-centrifuge tube. Using a push-pin, make a small hole in the center of the aluminum foil.
5. Using a sharp razor blade, excise a leaf from the plant at the base of the petiole. If you are using a pre-harvested leaves (e.g. spinach) cut off the **tip** of the petiole.
6. Insert the petiole through the foil cover and into the micro-centrifuge tube containing water (Fig. 3).
7. Using the long forceps, place the micro-centrifuge tube containing the leaf into the test tube containing the sodium bicarbonate solution. The leaf may have to be curled slightly in order for it to fit inside the tube. **Make sure that the underside of the leaf (lighter in color) does not come in contact with the walls of the tube** since this is the side where the majority of the gas exchange takes place via the stomata.
8. Insert the oxygen sensor into the opening of the test tube so that it is **tightly sealed**. To insure there are no air leaks, wrap a piece of **parafilm** tightly around the junction of the test tube with the sensor. The final set-up is shown in the figure 4.

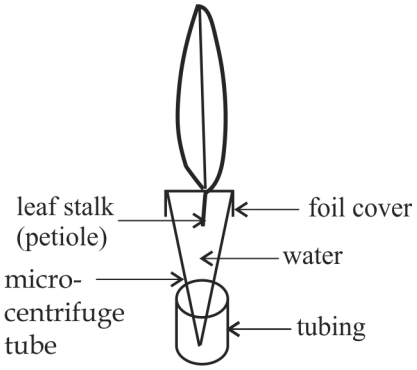



**Figure 3.** Leaf Set-up

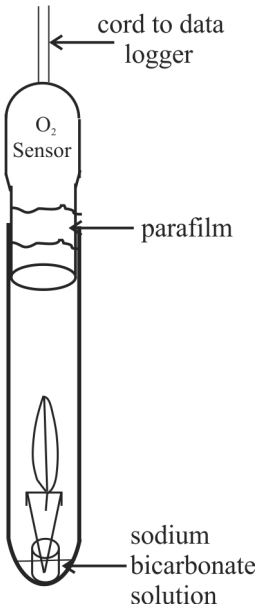



**Figure 4.** Leaf and Oxygen Sensor Set-up

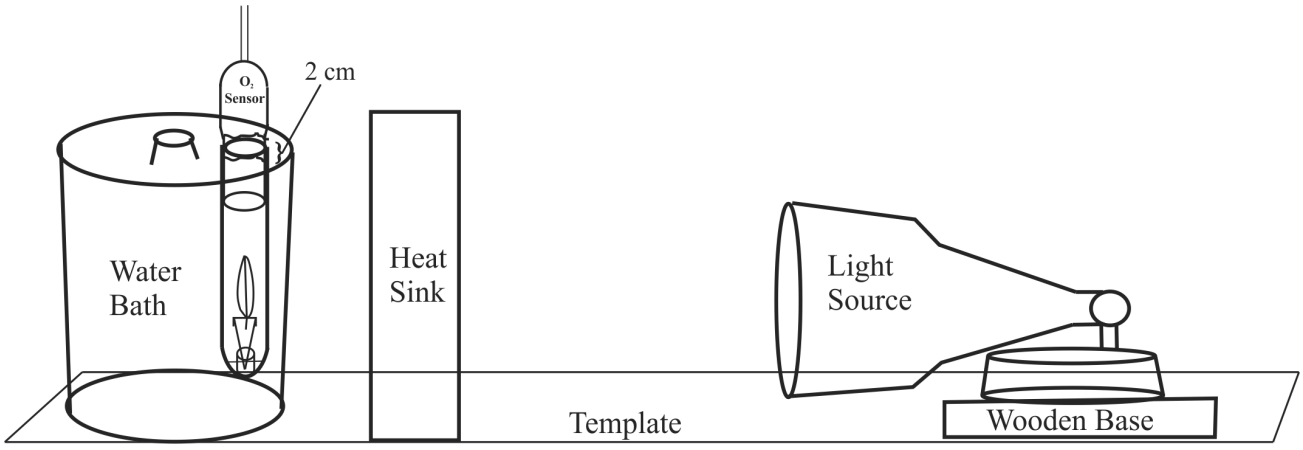



**Figure 5.** Side View of Experimental Set-up

**Apparatus assembly using template (see Figure 5)**

1. Fill the plastic **heat sink** to the top with **cold** water and place it on the template in the space marked heat sink. Note that the heat sink does not touch the water bath.
2. Insert the test tube containing the leaf into the hole in the water bath cover so that about **2 cm** of the tube is sticking out of the top of the cover.
3. Place **the wooden base of the light source** at the 800 fc mark. Your apparatus should now look like Figure 5.

**Exercise A: Effect of Light Intensity on the Net Photosynthetic Rate of a Leaf**

**Procedure**

1. In this experiment you will be measuring the photosynthetic rate¬ of a leaf at the following light intensities: 1000, 1500, 3000, and 7000 fc.
2. Hypothesis Formulation

   Based on your knowledge of photosynthesis, formulate a hypothesis concerning the effect of light intensity on photosynthetic rate in leaf cells. **Write down the hypothesis before starting the laboratory experiments**.

3. Pre-Illumination of the leaf at **800 fc**

   To stimulate the light dependent reactions of photosynthesis to produce measurable levels of oxygen, it is necessary to illuminate the leaf at 800 fc before taking photosynthetic rate measurements.

   a. Place the wooden base of the light source at the 800 fc mark on the template and **make sure the face of the bulb is parallel to the face of the heat sink**. Turn on the light for 7 minutes. Do not collect photosynthetic rate data at this light intensity.

4. After equilibrating the leaf at 800 fc for 7 minutes, move the base of the light source to the **1000 fc** mark on the template.

   a. Begin collecting data by clicking on the **Collect** button on the **Tool Bar.** The data logger will collect data for 7 minutes.

   b. As the data is being collected periodically check the following:

      - The current ppm oxygen reading appears in the Meter Window.
      - A graph of the results will appear in the Graph Window. The Y-axis is generally set between 180,000 - 190,000 ppm (parts per million) oxygen. However, the initial oxygen reading may lie outside this range. To find where the current ppm oxygen begins, scroll along the Y- axis by clicking on the up or down arrows next to the Y-axis. More precise interval settings for the graph axes can be made by right clicking the mouse and selecting **Graph Options** from the drop-down menu.

5. After 7 minutes, the data logger will automatically stop collecting data.
6. Obtain the net photosynthetic rate (**= slope of the line**) for the last 2 minutes of the collection period by doing the following:

   a. Click the **Analyze** button on the **Menu Bar.**

   b. Select **Linear Fit** on the drop-down menu.

   c. A box giving the slope of the entire line will appear on screen.

   d. Move the first bracket symbol to the 300 sec (5 minutes) mark on the X axis and move the second bracket to the 420 (7 minutes) sec mark.

   e. Record the slope of the line (net rate of oxygen production or consumption) for **the last 2 minutes** of the collection period in **Table 1a for 1000 fc**.

7. **Save the data on your flash drive for further analysis. Once the data is cleared from the computer's memory, it can't be restored! Be sure the data has been recorded before starting to collect data at the next light intensity.**

8. Once you have saved your data for 1000 fc, the data must be cleared from memory so that a new trial can be run. Click on the **Data** button in the menu and select the **Clear All Data** option at the bottom of the drop down menu.
9. You will now repeat the above procedure for light intensities 1500, 3000 and 7000 fc. **Do not remove the oxygen sensor from the tube and do not move the water bath or heat sink.**
10. **At each light intensity** record the **slope of the line (= net rate of $O_2$ evolution or consumption) in Tables 1b – 1d.**

**Exercise B: Effect of Light Wavelength on Net Photosynthetic Rate of a Leaf**

**Introduction**

*What is Light?*

Light is a type of electromagnetic energy (radiation). Electromagnetic radiation travels in waves or packets of light called photons. The distance between the crests of electromagnetic waves is termed the wavelength and is measured in nanometers. The entire range of radiation is known as the electromagnetic spectrum. Each type of radiation in this spectrum has a characteristic wavelength and energy content. These two characteristics are inversely related; i.e. **the longer the wavelength, the smaller the energy content**. The portion of the spectrum which is detected as various colors by the human eye is referred to as visible light and ranges from about 400 - 700 nanometers. W**hite light is composed of all wavelengths (colors) in the visible light spectrum.**

We see colors because objects contain **pigments** that selectively absorb certain wavelengths of visible light and reflect or transmit others. What we recognize as an object's color is composed only of those wavelengths of light that are transmitted or reflected. If a pigment absorbs all wavelengths, it appears black. The diagram below illustrates what is meant by the terms incident light, reflected light, and absorbed light.

*Photosynthetic Pigments in Plants*

Several different photosynthetic pigments, i.e. those pigments which are able to capture various wavelenghts of light, are embedded in the thylakoid membranes of chloroplasts. These include the **primary pigment chlorophyll a** and the **accessory pigments**. **Chlorophyll *a***, which is blue-green in color, is referred to as the primary pigment since it is the only pigment which can participate directly in the light dependent reactions. The **accessory pigments** absorb different wavelengths of light than those absorbed by chlorophyll *a* and transfer the energy to chlorophyll *a*, which then initiates the light dependent reactions. This results in a broadening of the spectrum of colors that can drive photosynthesis. The accessory pigments commonly found embedded in the thylakoid membranes of the chloroplasts are yellow-green **chlorophyll b** and a group of yellow and orange pigments called **carotenoids.** There are two major groups of carotenoid pigments: **carotenes** and **xanthophylls**.

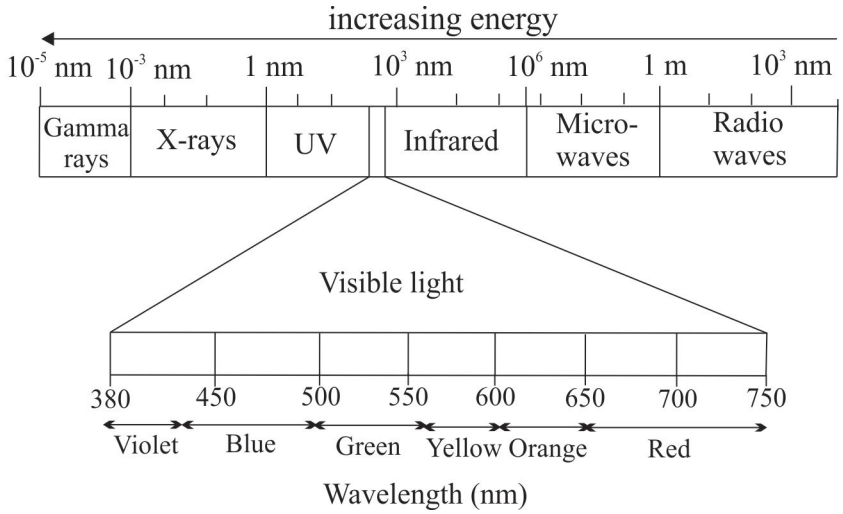



**Figure 6.** Electromagnetic Radiation Spectrum

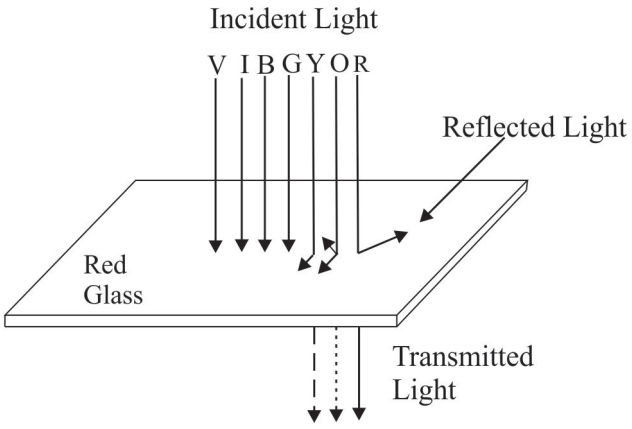



**Figure 7.** Reflected, Transmitted, and Absorbed Light Wavelengths

**Procedure**

1. In this experiment you will determine if all wavelengths (colors) of light are equally effective in driving the photosynthetic reactions in leaves. You will measure net photosynthetic rate (ppm $O_2$/s) of leaves when illuminated with blue, red, and green light at a light intensity of 1500 fc.
2. **Based on your knowledge of photosynthesis**, formulate a hypothesis concerning the change in rate of photosynthesis in leaf cells when exposed to red, green, and blue light. **Write down the hypothesis before starting the laboratory experiments**.
3. Place the **red** filter **between the heat sink and the water bath**. Place the light source at distance which results in a final light intensity of **1500 fc**. This light intensity generally can be obtained when the light source is moved to a position very close to the heat sink.

   Start collecting data by clicking on the Collect button on the Windows Tool Bar. The data logger will collect data for seven minutes.

   After 7 minutes, the data logger will automatically stop collecting data.

4. Obtain the rate of oxygen production (= slope of the line) for the last 2 minutes of thecollection period by doing the following:
   a. Click on the Analyze button on the Menu Bar. Select Linear Fit on the drop-down menu
   b. A box giving the slope of the entire line will appear on screen.
   c. Move the first bracket symbol to the 300 sec (5 minutes) mark on the X axis and the second bracket to the 420 sec (7 minute mark).
   d. Record the slope (rate of oxygen production/s) for the last 2 minutes of the collection period in **Table 3a**.
5. **Save the data on your flash drive for further analysis. Once the data is cleared from the computer's memory, it can't be restored! Be sure the data has been recorded before starting to collect new data.**
6. Once you have saved your data for red wavelength the data must be cleared from memory so that a new trial can be run. Click on the Data button in the menu and select the Clear All Data option at the bottom of the drop down menu.
7. Repeat the above procedure for the blue filter and then the green filter, **in that order.** For each different colored filter record the slope of the line (net photosynthetic rate/s) in **Tables 3b – 3c**. You may obtain negative values at certain wavelengths? What do negative values mean?

**Exercise C: Rate of Oxygen Consumption (= rate of aerobic respiration) of a Leaf in the Dark**

**Procedure**

1. Completely cover the water bath with the black plastic and secure it with rubber band **so that no light reaches the leaf in the tube.**
2. Begin collecting data by clicking on the **Collect** button on the **Tool Bar.** The data logger will collect data for 7 minutes.
3. After 7 minutes, the data logger will automatically stop collecting data..
4. Obtain the rate of oxygen consumption **(= slope of the line**) for the last 2 minutes of the collection period by doing the following:
    a. Click on the **Analyze** button on the **Menu Bar**. Select **Linear Fit** on the drop-down menu
    b. A box giving the slope of the entire line will appear on screen.
    c. Move the first bracket symbol to the 300 sec (5 minutes) mark on the X axis and the second bracket to the 420 sec mark.
    d. Record the slope (rate of oxygen consumption) for the last 2 minutes of the collection period in **Table 5**.
5. **Save the data on your flash drive for further analysis. Once the data is cleared from the computer's memory, it can't be restored! Be sure the data has been recorded before starting to collect new data.**
6. Once you have saved your data it must be cleared from memory so that a new trial can be run. Click on the **Data** button in the menu bar and select the **Clear All Data** option at the end of the drop down menu.

*Calculation of Leaf Surface Area*

1. Remove the leaf from the test tube.
2. Place the plastic grid over the leaf.
3. Using a vis á vis marker, trace the outline of the leaf on the plastic grid.
4. Count how many squares are covered by the leaf. Estimate partial squares. Each square is equal to 0.25 $cm^2$. The total surface area of the leaf ($cm^2$) = # squares x 0.25 $cm^2$. Record the surface area in **Table 7.**

*Lab Clean-up*

1. Dispose of the leaf in the trash.
2. Pour the sodium bicarbonate solution down the sink drain, rinse the tube with water (no soap) and dry the tube out using a paper towel. Place the tube in the metal pan at your desk.
3. Discard the water from the micro-centrifuge tube and return it to the metal pan at your desk.

**Data Analysis and Questions**

**Exercise A: Effect of Light Intensity on Photosynthetic Rate**

*Data Analysis*

Perform the following calculations at **each** of the light intensities. Report all values to the nearest 0.1

1. Individual Student Pair Data (Tables 1a - 1c)
    a. First calculate the net rate of photosynthesis per min (ppm $O_2$/**min**) by multiplying the net rate per sec (ppm $O_2$/**s**) by 60.
    b. Next calculate the net rate of photosynthesis/min/leaf surface area (ppm $O_2$/min/$cm^2$ ) by dividing the net photosynthetic rate/min by the leaf surface area ($cm^1$)
    c. Finally, calculate the **gross photosynthetic rate** which is equal to the net photosynthetic rate **plus** the **absolute value** of the aerobic respiration rate (ppm $O_2$/min/$cm^1$) recorded in Table 5.

2. In the Class Data Table (Table 2), record the **net** and **gross** photosynthetic rates (ppm $O_2$/min/cm$^2$) at each light intensity. Next, calculate the mean (average) net and gross photosynthetic rate (ppm $O_2$/min/cm$^2$ and record these values in the last row of Table 2.

*Results and Questions*

1. Construct a line graph using the **class data**. Plot the **net photosynthetic** rate on the Y-axis and the light intensity on the X-axis as shown in Fig 8:

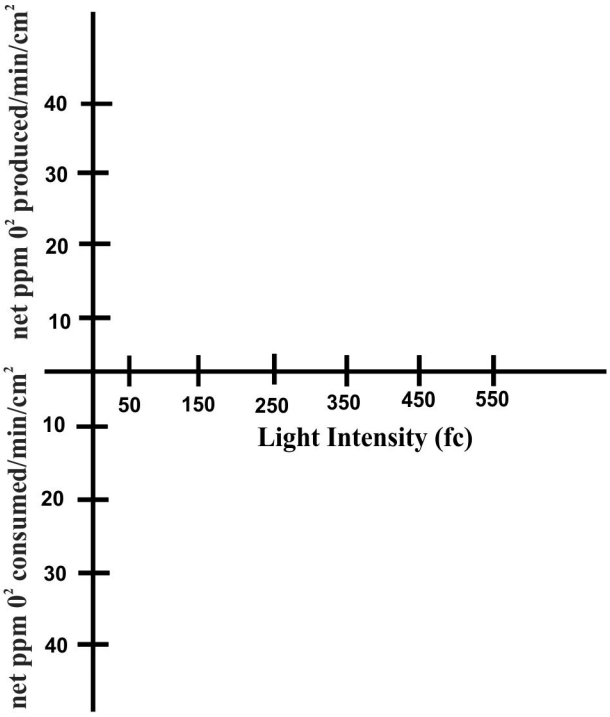



**Figure 8.** Light Intensity vs. rates of $O_2$ Consumption and $O_2$ Production

2. From your graph, what can you conclude about the effect of light intensity on the rate of photosynthesis, both gross and net?
3. Analyze your graphs (pair data) generated by the Logger Pro software at each light intensity. These graphs are called **light response curves.**
    a. You will note that there is a time period (called the **lag period)** between the time the lights are turned on and when photosynthetic rates reach steady state. Why do these light response curves display a lag period?
    b. Light saturation point
4. The **Light Compensation Point** is the **light intensity** (measured in foot candles) at which the rate of oxygen **production** equals the rate of oxygen **consumption** by a plant. The light compensation point for your leaf can be determined from your graph by extending the line from the last data point (i.e. lowest light intensity measured) through the x-axis. The point on your graph where this line **intersects (crosses) the x-axis** is the light compensation point, measured in foot candles. Note that the value on the Y axis below the 0 point represents the aerobic respiration rate of the plant in the dark (see Table 6 for the mean value).
    a. What is the light compensation point, in foot-candles, of your leaf? **Draw an arrow to this point on your graph.**
    b. At the light compensation point what metabolic process in the cells is producing oxygen and what metabolic process is consuming oxygen ?
    c. If all plants were kept at light levels close to the compensation point, what possible effects would this have on the (a) total biomass of plants on earth, (b) oxygen concentration in the atmosphere?

5. Global warming is the slow but steady rise in the temperature of the Earth's surface as a result of the buildup of greenhouse gases (e.g. carbon dioxide, methane, and CFCs) in the atmosphere.
    a. Based on your knowledge of photosynthesis, explain why planting trees might help counteract global warming.
    b. Explain why a few degrees increase in the Earth's temperature might increase rates of photosynthesis in plants significantly. Hint: The biochemical reactions of photosynthesis require enzymes.
6. What factors other than light intensity and light wavelength affect the rate of photosynthesis?

**Exercise B: Effect of Wavelength of Light on Photosynthetic Rate**

*Data Analysis*

Perform the following calculations **at each wavelength** (color). Report all values to the nearest 0.1.

1. Individual Student Pair Data (Tables 3a - 3c)
    a. First calculate the net rate of photosynthesis per min (ppm $O_2$/**min**) by multiplying the net rate per sec (ppm $O_2$/**s**) by 60.
    b. Next calculate the net rate of photosynthesis/min/leaf surface area (ppm $O_2$/min/ $cm^2$ ) by dividing the net photosynthetic rate/min by the leaf surface area ($cm^2$)
    c. Finally, calculate the **gross photosynthetic rate** which is equal to the net photosynthetic rate **plus** the **absolute value** of the aerobic respiration rate (ppm $O_2$/min/$cm^2$) which was recorded in Table 5.
2. Class Data (Table 4)
    a. In the Class Data Table 4, record the **net** and **gross** photosynthetic rates (ppm $O_2$/min/$cm^2$) at each wavelength. Next, calculate the mean (average) net and gross photosynthetic rate (ppm $O_2$/min/$cm^2$ and record these values in the last row of Table 4.

*Results and Questions*

1. Construct the following **bar graph** using **Class Data** (Table 4)
    a. On the same graph plot both the mean net photosynthetic rate (ppm $O_2$ /min/$cm^2$) and mean gross photosynthetic rate on the y-axis vs wavelength (color) on the x-axis. These data are in the last row of Table 4. Use the entire sheet of graph paper when constructing your graphs. Be sure to construct bar graphs; not line graphs!
2. Summarize and explain the results of this experiment.
    a. Which wavelengths (colors) of light tested are **most** effective in promoting photosynthesis in leaves?
    b. Which wavelengths (colors) of light tested are **least** effective in promoting photosynthesis in leaves?
    c. Which wavelengths of light tested are probably absorbed most efficiently by the chloroplast pigments in leaves? How did your experiment lead you to this conclusion?
    d. Which wavelengths of light tested are probably absorbed least efficiently by the chloroplast pigments in leaves? How did your experiment lead you to this conclusion?
    e. Are the photosynthetic rates for the filtered light lower than the rates for white light? Explain why.
    f. Based on the properties of visible light, why do green plants appear green to us? (Hint: How do we recognize an object's color?)
3. In which reactions, the light dependent or light independent reactions, does the absorption of light by chlorophyll and accessory pigments occur?
4. If you were to design the lighting in a greenhouse, what color of bulbs would you most likely use for illumination? Why?
5. A student obtained a negative photosynthetic rate (ppm $O_2$/min/$cm^2$) when the leaf was illuminated with green light for ten minutes. The student concludes that the leaf does not photosynthesize in green light. Is this a valid conclusion? Explain why or why not? Hint: How could knowing the aerobic respiration rate of the leaf in the dark help the student answer this question?

**Aerobic Respiration Rates of Leaf in the Dark**

*Data Analysis*

Perform the following calculations for the leaf in the dark. Report all values to the nearest 0.1:

1. Individual Student Pair Data (Table 5)
   a. First calculate the rate of respiration per min (ppm $O_2$/min) by multiplying the net rate per sec (ppm $O_2$/s) by 60.
   b. Next calculate the by dividing the respiration rate/min by the leaf surface area ($cm^2$)
2. Class Data (Table 6)
   a. Record the rate of respiration/min/leaf surface area (ppm $O_2$/min/ $cm^2$ ) in Table 6. Next, calculate the **mean** respiration rate and record these values in the last row of Table 6.

**Table 1.** Photosynthetic Rates vs Light Intensity of a Sage leaf.

| **Table 1a. Light Intensity = 1000 fc** | | | |
|---|---|---|---|
| Net photosynthetic rate (ppm $O_2$/s) | Net photosynthetic rate (ppm $O_2$/min) | Net photosynthetic rate (ppm $O_2$/min/$cm^2$) | Gross photosynthetic rate (ppm $O_2$/min/$cm^2$) |
| | | | |

| **Table 1b. Light Intensity = 1500 fc** | | | |
|---|---|---|---|
| Net photosynthetic rate (ppm $O_2$/s) | Net photosynthetic rate (ppm $O_2$/min) | Net photosynthetic rate (ppm $O_2$/min/$cm^2$) | Gross photosynthetic rate (ppm $O_2$/min/$cm^2$) |
| | | | |

| **Table 1c. Light Intensity = 3000 fc** | | | |
|---|---|---|---|
| Net photosynthetic rate (ppm $O_2$/s) | Net photosynthetic rate (ppm $O_2$/min) | Net photosynthetic rate (ppm $O_2$/min/$cm^2$) | Gross photosynthetic rate (ppm $O_2$/min/$cm^2$) |
| | | | |

| **Table 1d. Light Intensity = 7000 fc** | | | |
|---|---|---|---|
| Net photosynthetic rate (ppm $O_2$/s) | Net photosynthetic rate (ppm $O_2$/min) | Net photosynthetic rate (ppm $O_2$/min/$cm^2$) | Gross photosynthetic rate (ppm $O_2$/min/$cm^2$) |
| | | | |

**Table 2.** Effect of Light Intensity on Net Rate of Photosynthesis in Leaves (Class Data)

| Group | Photosynthetic rate (ppm $O_2$/min/$cm^2$) 1000 fc | | Photosynthetic rate (ppm $O_2$/min/$cm^2$) 1500 fc | | Photosynthetic rate (ppm $O_2$/min/$cm^2$) 3000 fc | | Photosynthetic rate (ppm $O_2$/min/$cm^2$) 7000 fc | |
|---|---|---|---|---|---|---|---|---|
| | net | gross | net | gross | net | gross | net | gross |
| 1 | | | | | | | | |
| 2 | | | | | | | | |
| 3 | | | | | | | | |
| 4 | | | | | | | | |
| 5 | | | | | | | | |
| 6 | | | | | | | | |
| 7 | | | | | | | | |
| 8 | | | | | | | | |
| 9 | | | | | | | | |
| 10 | | | | | | | | |
| 11 | | | | | | | | |
| 12 | | | | | | | | |
| Mean | | | | | | | | |
| | | | | | | | | |

**Tables 3a - 3c.** Net Photosynthetic Rates vs. Wavelength of Light of a Sage Leaf at 1500 fc

| Table 3a. Wavelength (color) = Red. | | | | |
|---|---|---|---|---|
| Net photosynthetic rate (ppm $O_2$/s) | Net photosynthetic rate (ppm $O_2$/min) | Net photosynthetic rate (ppm $O_2$/min/$cm^2$) | Gross photosynthetic rate (ppm $O_2$/min/$cm^{2)}$ | Light Intensity (fc) |
| | | | | |

| Table 3b. Wavelength (color) = Blue | | | | |
|---|---|---|---|---|
| Net photosynthetic rate (ppm $O_2$/s) | Net photosynthetic rate (ppm $O_2$/min) | Net photosynthetic rate (ppm $O_2$/min/$cm^2$) | Gross photosynthetic rate (ppm $O_2$/min/$cm^{2)}$ | Light Intensity (fc) |
| | | | | |

| Table 3c. Wavelength (color) = Green | | | | |
|---|---|---|---|---|
| Net photosynthetic rate (ppm $O_2$/s) | Net photosynthetic rate (ppm $O_2$/min) | Net photosynthetic rate (ppm $O_2$/min/$cm^2$) | Gross photosynthetic rate (ppm $O_2$/min/$cm^{2)}$ | Light Intensity (fc) |
| | | | | |

**Table 4.** Effect of Light Wavelength on Net Rate of Photosynthesis in Sage Leaves at 1500 fc (Class Data).

| Group | Photosynthetic rate ($ppmO_2/min/cm^2$) red light | | Photosynthetic rate ($ppmO_2/min/cm^2$) blue light | | Photosynthetic rate ($ppmO_2/min/cm^2$) green light | |
|---|---|---|---|---|---|---|
| | net | gross | net | gross | net | gross |
| 1 | | | | | | |
| 2 | | | | | | |
| 3 | | | | | | |
| 4 | | | | | | |
| 5 | | | | | | |
| 6 | | | | | | |
| 7 | | | | | | |
| 8 | | | | | | |
| 9 | | | | | | |
| 10 | | | | | | |
| 11 | | | | | | |
| 12 | | | | | | |
| Mean | | | | | | |

**Table 5.** Rate of Oxygen Consumption by Sage Leaves in the Dark.

| Respiration Rate (ppm $O_2$/s consumed) | Respiration rate (ppm $O_2$/min consumed) | Respiration rate (ppm $O_2$/min/$cm^2$ consumed) |
|---|---|---|
| | | |

**Table 6.** Rates of Oxygen Consumption by Sage Leaves in the Dark (Class Data).

| Group | ($ppmO_2$/ $min/cm^2$) |
|---|---|
| 1 | |
| 2 | |
| 3 | |
| 4 | |
| 5 | |
| 6 | |
| 7 | |
| 8 | |
| 9 | |
| 10 | |
| 11 | |
| 12 | |
| Mean | |

**Table 7.** Surface Area of Sage Leaf

| Surface Area= | |
|---|---|

## Materials

1. Vernier ™ Equipment

   - LabPro Data Loggers ™
   - LoggerPro ™ 3.0 software
   - Oxygen Sensors
     Vernier Software & Technology
     13979 SW Millikan Way
     Beaverton, Oregon 97005-2886
     503-277-2299
     **www.vernier.com**

2. Leaf holders

   - micro-centrifuge tubes with caps removed
   - tygon tubing, 3/8 inch inside diameter, to hold micro-centrifuge tubes

3. Test tubes

   - Plastic or glass with rim, 160 mm length; inside diameter = 28 mm

4. Test tube sealant provides a secure seal between oxygen probe and test tube to prevent air leaks.

   - Multi-Purpose Rubber Coating: Plasti Dip™ Performix Brand

5. Water baths

   - 1 gal plastic aquaria with plastic lids: Carolina Biological Supply Company, Cat. #670380
   - Use a drill press (1¼ in) to drill holes in the plastic lids.

6. Heat sinks

   - fabricated by a local plastics company using 1/4 inch plexiglass
   - dimensions: base = 4 x 4.5 inches; sides = 12 x 12 x 1.5 inches

7. Leaves

Most any C3 leaves can be used as long as they fit into the test tube without coming in contact with the oxygen sensor. Some leaves that have proven successful include:

- Leaves from potted Sage (*Salvia officinalis*) plants, the culinary herb. We generally use Sage leaves in this laboratory exercise.
- Leaves from 2 week old sunflower (*Helianthus sp.*) seedlings grown in a greenhouse.
- Baby spinach (*Spinacia oleracea*) leaves from the supermarket. It is best to use organic spinach leaves packaged in plastic containers rather than in sealed bags.

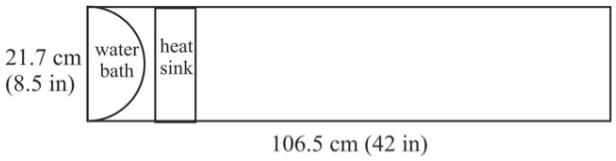
8. Lights and Light Sources

   - Light fixtures fitted with Philips, 90 Watt, 120 volts, **Halogen, Flood**
   - A shop-light fitted with two, 40 Watt, 48 inch, Sylvania, Gro-Lux fluorescent bulbs. These light banks are used in the laboratory to illuminate the greenhouse grown sage plants prior to running the experiments.

9. *Light* Meters

   - VWR Traceable Dual-Range Digital Light Meter, #62344-944

10. Template for Positioning Apparatus




   - Three, legal size pieces of copy paper are taped together and then laminated. The first sheet of paper contains the template for the placement of the water bath and heat sink.

11 Grids for measuring leaf surface area are made by photocopying a piece of graph paper onto a blank acetate sheet (transparency).

12. Colored Filters

   - Theatrical Gels can be purchased from a local sound and lighting store or online at **www.stagespot.com**. The filters have specific spectral characteristics and are number coded.
     - Green: Rosco # 389, Chroma Green
     - Red: Rosco #19, Fire
     - Blue: Rosco # 69, Brilliant Blue

13. Miscellaneous

   - Vis á Vis water soluble markers
   - alcohol thermometers
   - saturated sodium bicarbonate solution - add sodium bicarbonate until a precipitate is formed
   - parafilm

## Notes for the Instructor

### Technical Notes

*Troubleshooting Data Loggers and Oxygen Sensors*

1. **Before lab starts,** press the **start/stop** button on the LabPro interface. If everything is functioning properly the green light on the data logger will be flashing. If

the light is not flashing, unplug the USB cable from the computer, wait a few seconds and then reinsert it into the USB port of the computer.

2. **The oxygen sensor must be kept in an upward position at all times. Therefore, when the sensor is not in use it should be stored upright in a beaker or other container.**
3. **When moving the sensor, grasp it by the upper end; not by the cord! Do not pull on the sensor cord.**
4. **It is extremely important that the sensor is not jostled during data collection since it is likely to result in erroneous data being collected.** To limit the possibility of the sensor being hit during data collection, have students work with the apparatus on their lab desk so that it is located close to the computer rather than being stretched out across the desk.

*Sage culture*

Sage is a perennial herb. It is commonly available from garden stores and can be easily grown and propagated in a greenhouse. Several days prior to their use, the potted plants are removed from the greenhouse and placed under a light bank fitted with grow-lux lights. If kept well watered the plants can be maintained for several weeks in the laboratory.

*Light Intensity Experiments*

1. It is extremely important that the light source is focused directly on the leaf, both vertically and horizontally (see the procedure in the student outline). Blocks of Styrofoam can be used to raise the height of the light source.
2. We have found that pre-illuminating the sage leaf at 800 fc for 7 minutes prior to starting the light intensity series results in more consistent data as you increase the light intensity. The photosynthetic rates at 800 fc are generally highly variable; often much higher than those at 1000 fc. Therefore students do not include these values in their data collection.
3. It is assumed that this pre-illumination initiates the photosynthetic reactions at a high enough level for the sensor to detect significant amounts of oxygen in the chamber.
4. There appears to be a lag time between when the light reactions start and when the sensor is able to detect a stable rate of net oxygen evolution.
5. The light intensity experiments should be run in random order to insure the best experimental design. However, it has been our experience that the most consistent data results when you start at the low light intensities and progress to the high light intensities.
6. Light intensities other than those stipulated in the lab exercise can be used. It has been our experience that the photosynthetic rates at low light intensities are highly variable. The values obtained at very low light intensities (e.g., 200 fc or below) are often negative, indicating rates of oxygen consumption by aerobic respiration are greater than net rates of oxygen production by photosynthesis.

*Light Wavelength and Dark Experiments*

The light wavelength experiments should be run in random order to insure the best experimental design. However, it has been our experience that the most consistent data results by testing green light last followed by dark. Since the photosynthetic rates under green light are greatly reduced, the plant's oxygen evolution is markedly reduced whereas oxygen consumption by aerobic respiration remains constant. Therefore, when the leaf is placed in the dark after green illumination, there is less lag time in obtaining stable oxygen consumption readings in the dark.

*Factors That Can Lead to Variability in Data*

It should be noted that measuring photosynthetic rates in excised leaves with oxygen sensors can, at times, produce inconsistent data. This is most likely due to the fact that stomata opening and closing are affected by the environmental conditions in the leaf chamber. Stomata opening is affected by the temperature, relative humidity, and carbon dioxide levels in the chamber. In addition, efficient water flow to the leaf may be decreased as a result of cutting the petiole.

**Extensions to the Lab Exercise and Investigative Activities**

*Extensions*

1. Statistical analysis of mean rates of photosynthesis, both net and gross, could be analyzed using the student T-test.
2. Extraction and separation of the photosynthetic pigments from the chloroplasts of the Sage leaves using Thin Layer Chromotography (Motten 2004).

*Investigative Activities*

1. Compare photosynthetic rates between sun and shade leaves of the same plant or leaves from sun and shade plants of different species.
2. Compare photosynthetic rates between a C3 and a C4 plants.
3. Investigate the affect of temperature on photosynthetic rate.

4. Determine the light saturation point of the leaves from sun and shade plants.
5. Compare photosynthetic rates of leaves from different plant species.

**Pre-Lab Introduction**

*Background Information*

1. Review the difference between autotrophs and heterotrophs and distinguish between photoautotrophs and chemoautotrophs. Stress the importance of photosynthesis to all organisms. **If photosynthesis suddenly ceased on earth, the majority of living things would eventually die.** Note that some chemoautorophic organisms would survive for short time. However, since most chemoautotrophs are marine prokaryotes which carry out aerobic respiration, they too would die when the dissolved oxygen in the oceans was used up.
2. **Stress that green plants carry on aerobic respiration 24 hours per day but only carry on photosynthesis when in the light.**
    a. Discuss the balanced equation for photosynthesis with emphasis on how you ***could*** measure rates of photosynthesis.
        - In these experiments rates of **oxygen evolution** will be used as a measure of photosynthetic rate.
    b. Discuss the balanced equation for aerobic respiration with emphasis on how you ***could*** measure rates of photosynthesis.
        - In these experiments rates of **oxygen consumption by the leaf in the dark** will be used as a measure of aerobioc respiration rate.
    c. Explain the difference between **net** and **gross** oxygen evolution during photosynthesis. Explain why these experiments measure the **net** oxygen produced by the leaves during photosynthesis (total oxygen released during photosynthesis **minus** the oxygen used for respiration).
    d. Note that the rate of oxygen consumption by the leaf in the dark is the aerobic respiration rate. **The value will be negative and should be reported as such in the tables.**
    e. The **gross** photosynthetic rate is equal to the net rate of photosynthesis plus the ***absolute value*** of the rate of respiration in the dark.
    f. Explain the concept of the **light compensation** point.
4. Discuss how flowering land plants (e.g. sage) and aquatic plants (e.g., *Elodea*) carry out **gas exchange** during photosynthesis and aerobic respiration. For land plants, use the wall chart showing the anatomy of a leaf.
5. Briefly discuss light dependent (light) reactions and light independent (dark) reactions, emphasizing the interdependence of these two phases of photosynthesis using the summary diagram. Explain where these reactions occur in the chloroplasts. Don't spend a great deal of time on the biochemical reactions! Use the wall chart and model showing chloroplast structure.
6. Discuss the properties of light and the reason an object appears to be a particular color.
7. Discuss the difference in function between the **primary pigment** (Chlorophyll "a"), and the **Accessory Pigments** ( chlorophyll b and carotenoids); emphasizing that accessory pigments broaden the range of light that can be used in photosynthesis.

**General Reminders to Students Dealing with Apparatus Set-Up**

1. Remind the students that **each pair** of students at each lab table will run **all** of the experiments and that all students are responsible for the class data.
2. **Run the experiments with the lights off.**
3. **Pieces of cardboard can be provided to help prevent interference of light sources.**
4. Demonstrate to students how to set up the experimental tube.
    a. Show the students how to add the 2 ml bicarbonate solution using the plastic pipets. **Emphasize why the sodium bicarbonate solution is added to the test tube.**
    b. Show the students how to insert the leaf into the micro-centrifuge tube and how to use the forceps to place the micro-centrifuge tube with leaf into the test tube. **Make sure that the underside of the leaf does not come in contact with the walls of the tube** since this is the side where the majority of the gas exchange takes place via the stomata.
5. **Remind students to support the water bath on the bottom with two hands. The water baths break easily if grasped by the top while transporting.**
6. Remind students that the opening of the test tube must be **2 cm above the hole** in the water bath cover.
7. Emphasize that the face of the light bulb must be parallel with the heat sink and aimed below the water line. Make sure students realize why the heat sink is used.

8. The water bath must be filled to the top with 23°C water.
9. Remind students **not to remove the oxygen sensor or the leaf from the tube** during the experiments.
10. Review the operation of the digital light meters. See Appendix A.
11. Emphasize that the rates to be graphed are ppm oxygen/min/cm2. Explain how to calculate leaf surface area after completing all experiments.
12. Effect of Light Intensity on Photosynthetic Rate
    a. The experiments must be run **in the order listed**, starting with lowest light intensity and ending with the highest light intensity.
13. Effect of Light Wavelength on Photosynthetic rate
    a. It is extremely important that the rate of photosynthesis be measured in the following sequence: **red (first), blue, and green (last)**
    b. Remind the students to place the colored filters on the side of the water bath closest to the heat sink; not on the side nearest to the light source.
    c. Generally red light produces slightly higher photosynthetic rates than the blue light. This response is due to the characteristics of the chloroplast pigments (both P680 and P700 absorb in the red wavelength) rather than the energy characteristics of the different wavelengths of light. Students are not expected to explain why the red light produces higher photosynthetic rates than the blue light. They are only expected to explain why the rates of photosynthesis are higher for the blue and red wavelengths in comparison to green.
14. Rate of Oxygen Consumption (aerobic respiration) by the Leaf in the Dark
    a. Note that the rates of oxygen consumption by the leaf in the dark is the aerobic respiration rate. **The value will be negative and should be reported as such in the tables.**
    b. To obtain gross photosynthetic rates at any light intensity = net rate of photosynthesis plus the ***absolute value*** of the rate of respiration in the dark.

**Further Reading**

Percival, D. C., Proctor, J.T.A., and Tsujita, M.J. 1996 Whoeplant Net $CO_2$ Exchange of Raspberry as Influenced by Air and Root-zone Temperature, $CO_2$ Concentration, Irradiation, and Humidity. *Journal of American Society of Horticultural Science* 121(5):838 – 845.

Spilatro, S. R. *Photosynthesis Investigation Study Guide.* **http://www.marietta.edu/~spilatrs/biol103/photolab/index.html**

## Acknowledgements

We would like to thank Bob Hodson, University of Delaware, for suggestions on pre illumination of leaves and how to sequence the collection of data to insure more consistent results.

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Morholt, E., P. Brandwein, and A. Joseph. 1972. *A Sourcebook for the Biological Sciences*. 2nd edition. Harcourt, Brace and World, Inc. 795 pgs.

Motten, A. 2004. Diversity of Photosynthetic Pigments. Pages 159 – 177, in *Tested Studies for Laboratory Teaching*, Volume 25 (M. A. O'Donnell, Editor). Proceedings of the 25th Workshop/Conference of the Association for Biology Laboratory Education (ABLE), 414 pages.

Pitkin, R. B. 2004. Photosynthesis/respiration in leaf disks. Pages 347-351, in *Tested Studies for Laboratory Teaching*, Volume 25 (M. A. O'Donnell, Editor). Proceedings of the 25th Workshop/Conference of the Association for Biology Laboratory Education (ABLE), 414 pages.

Qubit Systems Inc. 2013. *PH1LP Photosynthesis Package*. **http://qubitsystems.com/plant-and-soil/ph1lp-photosynthesis-teachingpackage/.**

# Appendix A

## Operation of the Digital Light Meter

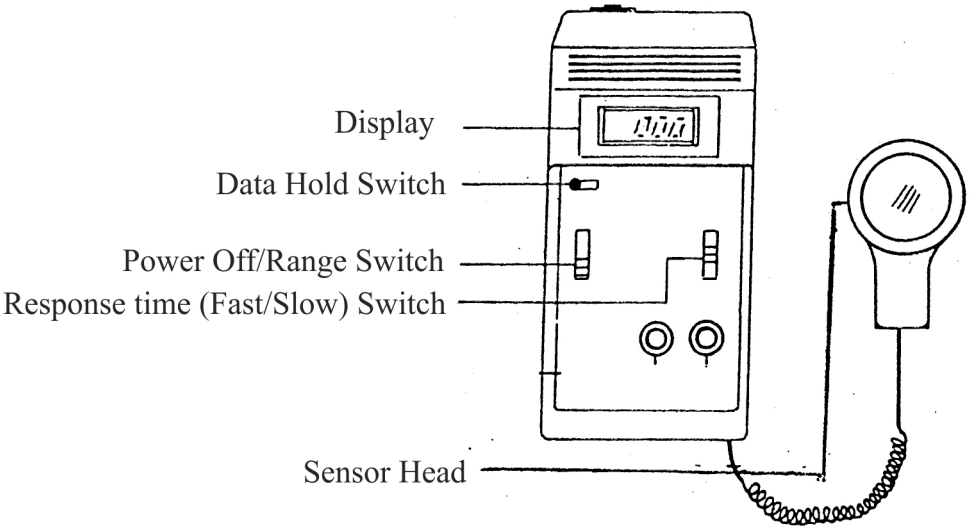


**Figure 9.** Digital Light Meter.

1. Place the sensor head facing the light source.
2. Make sure the response time (fast/slow) switch is set to the FAST position on the gray foot candle scale.
3. Push the power off/range switch downward to the appropriate sensitivity level marked on the gray foot candle scale. The light intensity, in foot candles, will be displayed on the LCD.
    a. In general, set the range switch at the first level for low light intensities (0 - 199.9 fc), the second level for medium light intensities (200 - 2000 fc) and at the third level for the highest light intensities (2000 - 50,000 fc).
    b. **Note that values obtained with the range switch at the third (highest) sensitivity level must be multiplied by 10. For example, if you obtain a reading of 200 on the LCD with the range switch set at the highest (third) level, it would be 200.0 x 10 = 2,000 foot candles.**
    c. Note that the decimal point always lies to the right of the last digit displayed on the LCD. For example, a reading of 0012 = 12.0.
    d. If the number "1" only appears on the LCD, the light intensity is too great to read at the range setting selected. Set the range switch to the next higher level.

# Appendix B

## Sample Student Data

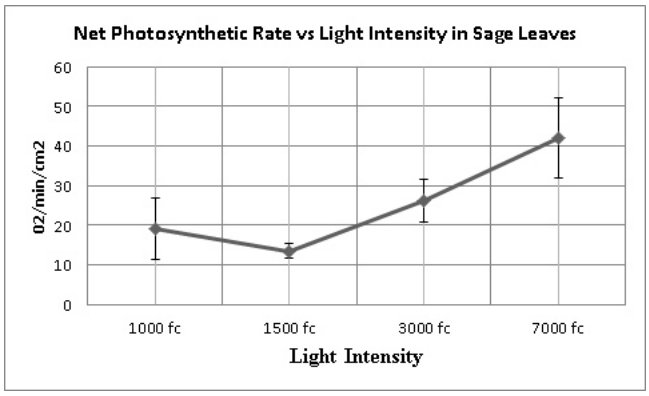


**Figure 10.** Net Photosynthetic Rate vs Light Intensity in Sage Leaves.

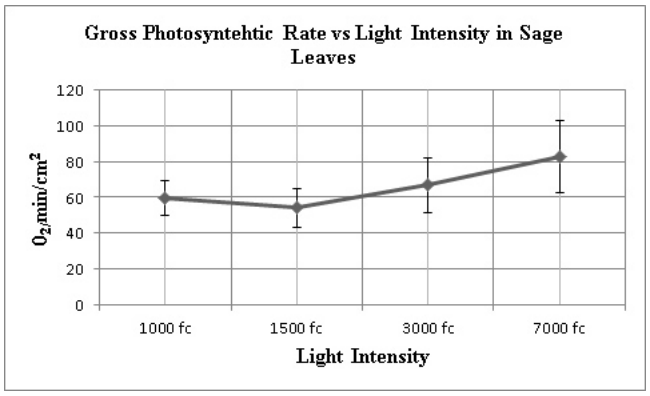


**Figure 11.** Gross Photosynthetic Rate vs Light Intensity in Sage Leaves.

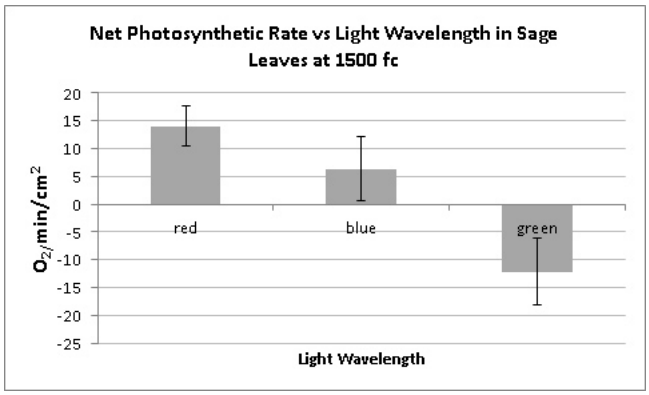


**Figure 12.** Net Photosynthetic Rate vs Light Wavelength in Sage Leaves at 1500 fc.

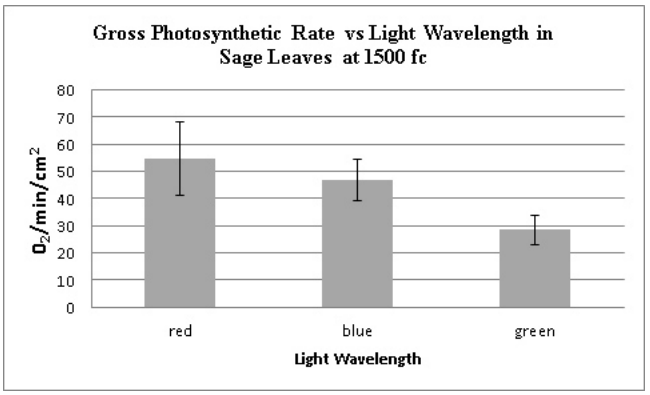


**Figure 13.** Gross Photosynthetic Rate vs Light Wavelength in Sage Leaves at 1500 fc.

## About the Authors

Bill Glider earned his B.S. degree in Secondary Education from Cornell University, his M.S. degree in Botany from the University of Maine and his Ph.D. in Biological Sciences from the University of Nebraska-Lincoln in 1985. He is currently a Full Professor of Practice in the School of Biological Sciences at UNL. He received the Distinguished Teaching Award in 2002 from the College of Arts and Sciences and the 2013 School of Biological Sciences Distinguished Teaching Award. He has spent over 20 years designing laboratory curriculum for the General Biology Program at UNL and lecturing in General Biology. In addition, to General Biology he has taught Botany, Organismal Biology, and field courses at the UNL Cedar Point Biological Station. Currently, he devotes the majority of his time to teaching large lecture sections of General Biology and pursuing a number of collaborative research projects focused on methods of increasing student learning in the biological sciences and designing methods of teaching students with disabilities in both lab and lecture.

Peter Thew earned his B.S. degree in Biological Sciences from the University of Nebraska-Lincoln and is currently completing a Master's Degree in Secondary Science Education.

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## Citing This Article

Glider, W.V. and P. Thew. 2013. Measurement of Rates of Aerobic Respiration and Photosynthesis in Terrestrial Plant Leaves Using Oxygen Sensors and Data Loggers. Pages 166-186, in *Tested Studies for Laboratory Teaching,* Volume 34 (K. McMahon, Editor). Proceedings of the 34th Conference of the Association for Biology Laboratory Education (ABLE), 499 pages. http://www.ableweb.org/volumes/vol-34/?art=7