

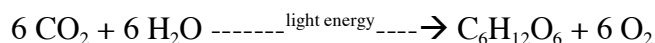
Photosynthesis

Two options for testing photosynthetic rate are given here:

1. CO₂ sensors may be used to look at the rate CO₂ is consumed under various light conditions
2. Floating disks are used to determine photosynthetic rate under various light conditions

Background

Photosynthesis is the metabolic process by which visible light energy is trapped and used to convert inorganic carbon (in the form of carbon dioxide – CO₂) and water into organic carbon (glucose) and molecular oxygen (O₂). Photosynthesis is initiated by the absorption of photons by pigments in the chloroplasts of plants, algae, and some bacteria. Glucose is converted to transport and storage molecules (such as sucrose and starch). The O₂ produced is utilized for aerobic respiration in the cells of those plants and other organisms.



The rate of photosynthesis is affected by numerous factors including the amount and the wavelength of light available. One tends to think of sunlight as a single “white” wavelength, but in reality it is a continuum of wavelengths - each wavelength representing a different color of light (fig. 1).

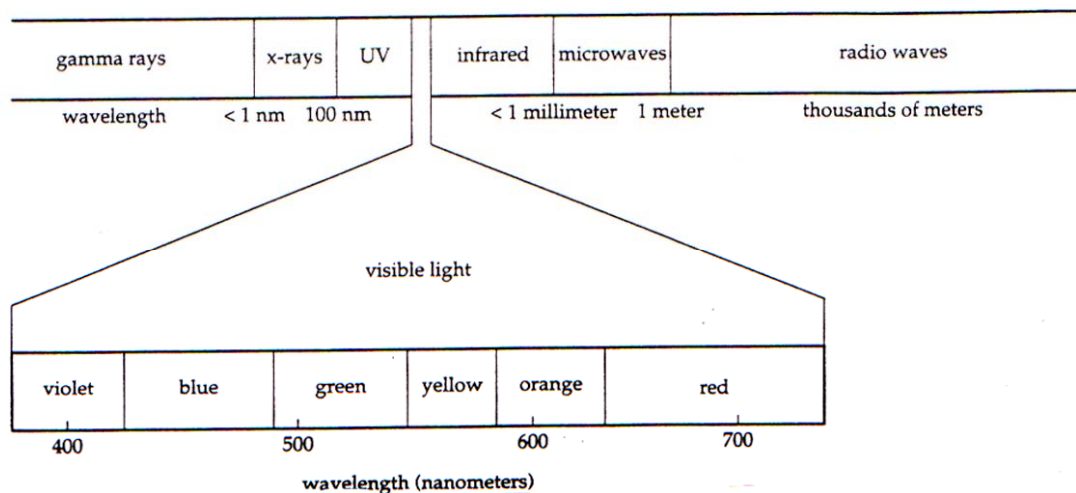


Figure 1. The electromagnetic spectrum showing wavelengths of visible light

When light hits a pigmented surface (plant or otherwise) some of the wavelengths are absorbed while others are reflected or transmitted. An object appears “green” in color

because it absorbs all wavelengths of light *except* green; green light is therefore transmitted/reflected back to our eye.

Plants contain pigments that absorb and reflect various wavelengths of light. Chlorophyll A and B are the predominate pigments in green plants, but there are several accessory pigments, such as carotenes and xanthophylls, that absorb slightly different wavelengths of light than the chlorophylls (the autumn colors of maple trees are visual evidence of these accessory pigments). This gives plants a wider range of energy that can be absorbed by the plant. This absorption of light as a function of wavelength is known as the absorption spectrum. Plants have higher photosynthetic rates if they receive light in the wavelengths that correspond to where their photosynthetic pigments absorb the photons most strongly. Therefore, if we experimentally vary the wavelength of light reaching the plant we can vary the photosynthetic rate. Also, if we vary the intensity of light, we can also vary that rate.

In this experiment, you will examine how photosynthesis is affected by the amount of light available to the plant by varying the light intensity reaching spinach leaves (*Spinacia oleracea*). You will also examine how photosynthesis is affected by the wavelength of light available to the spinach leaves by passing light through different color filters. If the wavelength available to the plant affects photosynthesis, then there should be a difference in the photosynthetic rate and therefore a measurable difference in the amount of CO₂ consumed during photosynthesis. To determine this, you will measure the concentration of CO₂ present during a closed reaction. You will measure not only the amount of CO₂ consumed by photosynthesis, but also the amount of CO₂ produced by the normal cellular respiration of the leaves. You will also do an absorption spectrum of the photosynthetic pigments extracted from spinach and determine if there is a correlation between the rates of photosynthesis under the different colored filters, the wavelengths of light that those filters transmit, and the optimum wavelengths of light absorbed by the plant.

To test the amount of CO₂ consumed you will use a CO₂ sensor inserted into a flask containing spinach leaves (figure 2).



Figure 2. CO₂ sensor inserted in chamber containing spinach leaves

1. Procedure using CO₂ sensors

You may want to start section E first. While CO₂ data is being collected (section E) section A-D may be done.

A. Extracting the Photosynthetic Pigments from Spinach

1. Obtain a spinach leaf and place it in a 50 ml test tube.
1. Cover the leaf with 95% ethanol (EtOH) (approximately 20-30 ml)
2. Place the test tube in a boiling hot water bath on a hotplate and heat until the spinach-EtOH solution is just boiling. The photosynthetic pigments will extract out of the leaves into the EtOH. Remove from the hotplate.
3. Fold a piece of filter paper in quarters, and place it into a funnel. Moisten the filter paper with a few drops of EtOH
4. Put the end of the funnel into a test tube. Pour the pigment extract through the filter paper into the test tube
5. Take 0.5 ml of the filtered pigment extract and put it into a cuvette containing 3.0 ml of 95% EtOH
6. Pipette 3.0 ml of 95% EtOH into another cuvette for your blank

B. Determining the Absorption Spectrum of Spinach Photosynthetic Pigments

1. Set the spectrophotometer to 400 nm. Blank the spectrophotometer with 95% EtOH and then take the absorbance reading of your extract by reading the bottom absorbance scale. Note: you may do these readings at the same time as you do your % transmittance spectrum of the color filters (section C below), just be sure to **use the appropriate blank and read the correct scale.**
2. Set the spec to 420 nm. Re-blank with the 95% EtOH and take an absorbance reading of your spinach pigment extract.
3. Continue taking readings at 20 nm intervals up to 700 nm. Be sure to re-blank the spec at each wavelength before taking the reading of your extract.
4. When you are done with your readings dispose of the EtOH and the pigment extract in the “waste ethanol” container in the hood.

C. Determining the Percent Transmittance of Color Filters

1. Choose three different color filters to determine the % transmittance for. Cut a piece of each the filters the height of the cuvette and a width wide enough so that the piece will fit completely around the inside of the cuvette. Place each filter into a separate cuvette and fill those cuvettes with tap water.
1. Fill another cuvette with water for your blank.
2. Set the spec to 400 nm.
3. Blank the spec with water. Sequentially take the % transmittance for each color filter by reading the top % transmittance scale.

4. Set the spec to 420nm. Re-blank and read all cuvettes containing the color filters. Continue taking readings at 20 nm increments up to 700 nm. Be sure to re-blank the spec each time you change the wavelength.

D. Measuring light intensity

To measure the light intensity reaching the plant material you will use a **quantum meter** to measure the number of photons emitted between 400-700 nm from the light source (in this case a fluorescent light). The photosynthetic photon flux (PPF), is measured in $\mu\text{mol m}^{-2} \text{s}^{-1}$ (micromoles of photons per square meter per second).

1. Plug in the fluorescent light.
2. Turn the dial of the quantum meter to the “electric lamp” position.
3. Hold the sensor at the level of the leaves. The sensor top surface should be horizontal and directly under the light source. Be careful not to shade the sensor with your hand or any other object.
4. Read the PPF off the display (units are $\mu\text{mol photons m}^{-2} \text{s}^{-1}$)
5. Turn off the meter.



Figure 3. Quantum meter

E. Using Vernier Logger Pro 3 and a CO₂ Probe for Obtaining Photosynthesis /Respiration Rates

Respiration Rate in the Dark

1. Be sure the CO₂ Gas Sensor is connected to the Vernier Lab Pro interface and that Lab Pro is connected to the computer. If the Lab Pro power cord is not plugged in do so now. The Lab Pro will beep when the power is connected.
2. Prepare the computer for data collection. Open **Logger Pro 3** by clicking on the Logger Pro icon in the computer desktop dock (figure 4).



Figure 4. Logger Pro Icon

3. Go to **File** in the menu and click on **Open**.
4. Click on **Biology with Computers**, and then double click on **Experiment 31B, Photosynthesis-Respiration CO₂**. The Logger Pro window for measuring CO₂ production will open. For photosynthesis/respiration in spinach, the vertical (y) axis has carbon dioxide concentration scaled from 0 to 5 ppt (parts per thousand), the horizontal (x) axis has time scaled from 0 to 10 minutes and the data rate is set to 20 samples/minute.
5. Obtain two spinach leaves from the front table. If the leaves are damp, blot them dry between two pieces of paper towel.
6. Carefully place the leaves into the respiration chamber, using forceps if necessary. Try to lay the leaves as flat as possible in the chamber.
7. Wrap the entire respiration chamber in aluminum foil so that no light reaches the leaves.
8. Place the CO₂ Gas Sensor into the bottle as shown in Figure 2. Place the sensor/chamber on its side under the fluorescent light on black construction paper. Even though this is the “dark” control, the light should be turned on (why?). Wait 3-5 minutes for the chamber to acclimate before proceeding.
9. To begin measuring the CO₂ concentration in the chamber, click the green button in the Logger Pro menu bar (fig. 5). Data will be collected for 10 minutes.



collect/stop
button in
LoggerPro
menu bar

Figure 5. LoggerPro menu bar

10. When data collection has finished you will determine the rate of photosynthesis/respiration of the spinach leaves:
 - a. Identify the portion of the curve where the CO₂ concentrations starts a steady increase (or decrease). Highlight this portion of the curve by moving the mouse pointer to the point where the data values begin to increase (or decrease). While holding down the mouse button, drag the pointer to the point where the data ceases to rise (decline) and release the mouse button. If your chamber had totally equilibrated before the start of the run you will not need to do this step, since the entire curve would be linear.

- b. Click on the Linear fit (regression) button to perform a linear regression. A floating box will appear with the formula for a best fit line.
 - c. Record the slope of the line, m , as the rate of photosynthesis/respiration.
 - d. Label the line by choosing **Text Annotation** from the **Insert** menu. Type the experimental condition (i.e. dark) in the text box. Drag the box to a position near its respective curve.
11. Remove the aluminum foil from around the respiration chamber. **Do NOT remove the CO₂ sensor from the respiration chamber.**

Photosynthesis/Respiration Rate under Filtered Light

1. Wrap the respiration chamber with one of the cellophanes used in step C (% transmittance spectra) above. Make sure the bottom and neck of the respiration chamber are covered. **Do NOT remove the CO₂ sensor from the respiration chamber.**
2. To determine the light intensity coming through the filter, place the quantum meter sensor under a piece of the same color of cellophane you have used to cover the respiration chamber. Move the respiration chamber as close to the lamp as reasonable to obtain a reading (directions in step D above). Do not let the lamp touch the respiration chamber. Wait 5-10 minutes prior to beginning data collection.
3. After the five to ten-minute acclimation period is up, begin data collection by repeating steps 9-10 above. When you click on the green Collect button, you will be given a set of options, be sure to choose **Store Latest Run**. This will allow for all trials to be on the same figure.
4. Once all data for the filtered light condition has been collected, remove the cellophane from around the respiration chamber. **Do NOT remove the CO₂ sensor from the respiration chamber.**

Photosynthesis/Respiration Rate under White Light

1. Using the quantum meter, adjust the height of the fluorescent light so that the intensity of light reaching the leaves is as close as possible to that measured for the cellophane trial above.
2. Place the respiration chamber under the light. **Do NOT remove the CO₂ sensor from the respiration chamber.** Wait 5-10 minutes prior to beginning data collection, then repeat steps 9-10 above.
3. Remove the plant leaves from the respiration chamber, using forceps if necessary. Clean and dry the respiration chamber .

Troubleshooting

1. Use no more than 2 spinach leaves
1. Once the level of CO₂ reaches 5 ppt you will have reached the maximum level of the probe. If this occurs, take the probe out of the respiration chamber for a minute or so to let out excess CO₂, then restart the experiment.

2. Photosynthesis experiment using Floating disks

You may use disks of spinach leaves to look at the rate of photosynthesis under various conditions. Since oxygen is a product of photosynthesis, measuring the rate of its production would enable us to measure the rate of photosynthesis. Leaf tissue has gas-filled intercellular spaces so disks cut from a leaf will float. If we remove the air from those intercellular spaces and replace it with liquid the disks will sink. As photosynthesis occurs the oxygen that is produced will diffuse into those intercellular spaces and once enough gas has accumulated in those spaces the disks will once again float. By observing the number of those leaf disks that start to float over time we will be indirectly measuring oxygen production as an indication of photosynthetic activity.

In this lab you will test to see how the rate of photosynthesis is affected by the amount of light available to the plant by varying the light intensity reaching the spinach disks. You will also see if some colors (wavelengths) of light are better for photosynthesis than others. If so, then there should be a difference in the photosynthetic rate and amount of oxygen produced and thus a difference in the rate the disks float to the surface. You will also do an absorption spectrum of the photosynthetic pigments extracted from spinach and note if there is a correlation between the rates of photosynthesis under the different colored filters, the wavelengths of light that those filters transmit, and the optimum wavelengths of light absorbed by the plant.

Procedure:

A. Light intensity and color variations

1. Decide at what distances from the light source you want to run your light intensity experiment. Do a minimum of three distances. Place books or whatever else is handy under the lights to get the distances desired. Measure from the light to where the plant disks will be, then refer to Appendix to determine the PAR value (photosynthetically active radiation - $\mu\text{mol photons/m}^2/\text{sec}$) for each distance.
2. For your experiment with the color filters, pick one of the distances used for the light intensity experiment. The distance closest to the light might be best. Use that distance for all color filters used. Use a minimum of three colors.
3. On a cutting board cut out disks from spinach leaves using a cork borer. Try not to get any of the central leaf vein (figure 6). To prevent drying, place the disks into a beaker containing 20 ml of 0.2% NaHCO_3 (sodium bicarbonate). Think about how many disks should be used. Too few disks will not give an

accurate representation, too many will crowd others from the light. Be sure to keep the number of disks used consistent from one treatment to the next.

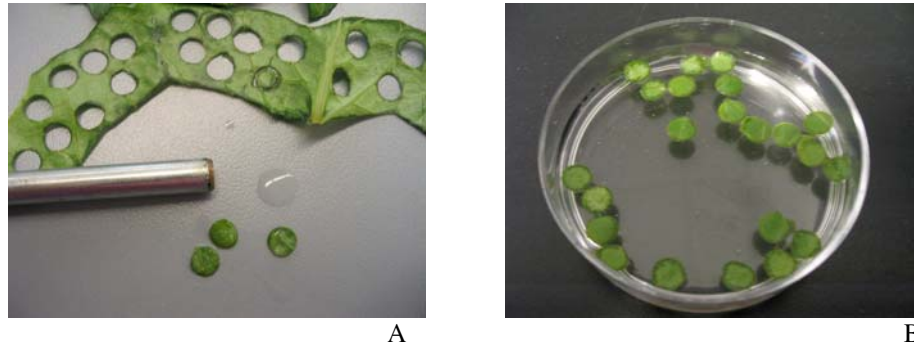


Figure 6. Cork borer used to cut disks from spinach (A). Disks placed into dish with 0.2% sodium bicarbonate (B).

4. Fill small petri dishes with approximately 20 ml of 0.2% NaHCO_3 solution (one dish for each condition being tested). Don't forget to set up controls.
What should your controls be?
5. Pull a vacuum on the disks – choose from one of two methods:
 - a. Take your spinach disks to the vacuum pump. Place the disks in the side-arm flask containing 0.2% sodium bicarbonate. Stopper the flask and turn the vacuum pump on. Pull a vacuum for about 30-45 seconds. You will be drawing the air out of the intercellular spaces of the leaf disks and replacing it with sodium bicarbonate. Turn the pump off. Gently release the vacuum by slowly pulling the stopper out of the flask. The disks should sink to the bottom.
 - b. Pull the plunger out of a 30ml syringe. Hold a finger over the end of the syringe and pour some of the 0.2% sodium bicarbonate solution containing disks into the barrel of the syringe. Put the plunger back in and push out all air. Place a finger over the end of syringe and pull down on plunger to create a vacuum. Hold for 10-15 seconds; release finger. Disks should sink; if not repeat pulling vacuum (figure 7).



Figure 7. Syringe with spinach disks

6. Transfer the disks in the liquid to a beaker, keeping them as much out of the light as possible.
7. Transfer the appropriate number of disks carefully with forceps to the small petri dishes.
8. Place the petri dishes on a piece of black construction paper under the light at the distances you have decided. For those dishes that will have a color filter over them, place those filters on now as well. Be sure to have your controls set up (figure 8).

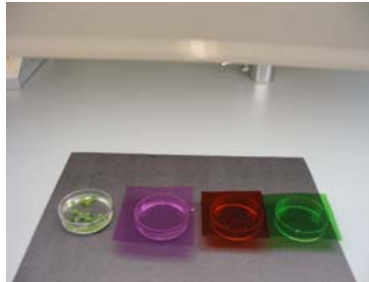


Figure 8. Set-up for testing different wavelengths of light

9. The data you collect will be of the number of disks floating. You will need to decide when you will collect your data. Are you going to collect your data at certain intervals (how long will those intervals be and how many), or will you wait until all disks have floated and compare times between conditions, or will it be some combination of the two. You also need to decide what you consider to be floating disk – one that has totally gone to the surface or one that has come up off the bottom and is sort of sideways. Just be consistent.
10. Turn on the lights; start timing. Record your data.

B. Absorption spectrum of Pigment Extract and % Transmittance Spectra of Colored Filters

1. Obtaining the pigment extract (use one of following methods)

a. Using EtOH

1. Obtain a spinach leaf and place it in a 50ml test tube.
2. Cover the leaf with 95% EtOH
3. Place the test tube in a boiling water bath on hotplate and heat to boiling (pigments will extract out into the EtOH). Remove from heat.
4. Fold a piece of filter paper in quarters, place into a funnel and moisten it with a few drops of 95% EtOH
5. Put the end of the funnel into a test tube. Pour the pigment extract through the filter paper into the test tube
6. Take 0.5 ml of the filtered pigment extract and put it into a cuvette containing 5ml of 95% EtOH

7. Pipette 5 ml of 95% EtOH into another cuvette for your blank
- b. Using acetone
1. Obtain a spinach leaf and place it in a mortar. Add approximately 5 ml of acetone. When using acetone it is important to use glass pipets, plastic pipets will dissolve in the acetone. Gently grind the leaf with a pestle to release the photosynthetic pigments
 2. Fold a piece of filter paper in quarters, place into a funnel and moisten it with a few drops of acetone.
 3. Put the end of the funnel into a test tube. Pour the pigment extract through the filter paper into the test tube.
 4. Take 0.1 ml of the filtered pigment extract and put it into a cuvette containing 5ml of acetone.
 5. Pipette 5 ml of acetone into another cuvette for your blank.

2. The absorption spectrum of the pigment extract

- a. Set the spectrophotometer to 400 nm. Blank the spectrophotometer and then take the absorbance reading of your extract. Note: you may do these readings at the same time as you do your % transmittance spectrum of the color filters, just be sure to use the appropriate blank and read the correct scale.
- b. Set the spectrophotometer to 420 nm. Re-blank and take an absorbance reading of your extract.
- c. Continue taking readings at 20 nm intervals up to 700 nm, being sure to re-blank at each wavelength before taking the reading of your extract

3. % Transmittance of color filters

- A. Cut a piece of one of the color filters used in your experiment such that the piece will fit completely around the inside of a cuvette. Fill the cuvette with tap water. Do this for each color filter you used.
- B. Fill a cuvette with water for your blank. Set the spec to 400 nm.
- C. Blank the spectrophotometer with the water blank then sequentially take the % transmittance reading for each color filter.
- D. Set the spec to 420nm, re-blank with water, and read all cuvettes containing the color filters. Continue taking readings at 20 nm increments to 700 nm, being sure to re-blank at each change of wavelength.

Questions

1. Graph the absorption spectrum of the pigments extracted from the spinach leaves – note the peaks of maximum absorption in figure legend.
2. Graph the % transmittance of the three colored cellophanes – note areas of maximum transmittance for each color in figure legend.
3. Graph the preliminary data of the effect of light on the photosynthetic rate of spinach. Include no light, filtered light, and white (full) light conditions.
4. Data analysis –
 - a. Compare graphs #1 & #2. Under which one of the three cellophanes used would you expect the spinach to have the greatest photosynthetic rate? Which the least? Why?.
 - b. Do your data from graph #3 make sense in the context of the data from graph #1 & #2? Explain.

Tips and Extension Experiments

1. Absorption spectrum

- a. the peaks for the absorption spectrum of spinach pigments are the same whether extracted by soaking or grinding in acetone, isopropyl alcohol, 95% ethanol, or in boiling ethanol (figure 9). You may need to filter no matter what method you use.
- b. compare absorption spectra of pigments isolated from different plants, especially one with varied colors in leaves
- c. collect leaves from a particular tree in the summer and freeze in plastic bags until needed. Collect leaves from the same tree in the fall when colors are changing. Extract pigments and compare spectra of leaves from summer vs. fall
- d. cut out the bands of each pigment from chromatogram (will need to combine similar bands from several chromatograms to get enough pigment), soak in a small amount of the solvent (can just use isopropanol or ethanol), then do absorption spectrum of each individual pigment.

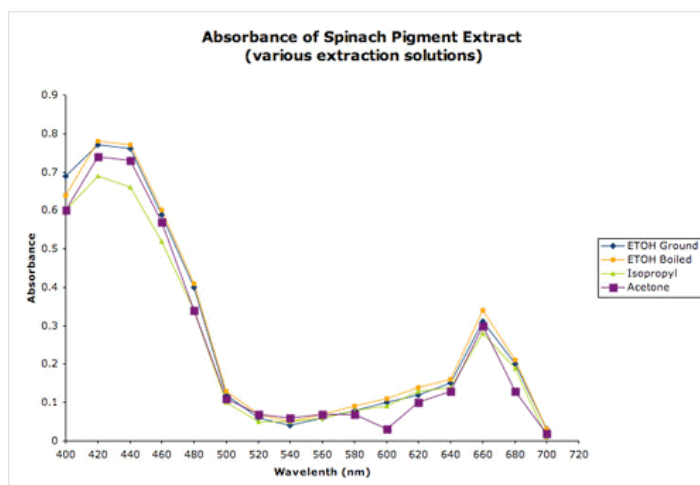


Figure 9. Absorption spectra of spinach pigments extracted in various solvents

2. Floating Disks

- a. prepare a 0.2% sodium bicarbonate solution (CO₂ source) and then add 1 drop of a dilute liquid soap (breaks surface tension)
- b. cut uniform disks from leaves with hole punch, cork borer or sturdy straw, avoid major veins (use spinach, ivy, plants that have smooth, hairless surface)
- c. put disks in small dishes OR may leave disks in syringes, place in conditions testing
- d. Use screw-in fluorescent bulbs instead of incandescent bulbs or shop lights so don't need heat sink

Supply sources

Roscolux colored filters/gels were for photosynthesis experiments. Ask for color sampler when ordering. The sampler gives the transmittance spectra of all gels.

SLD Lighting

318 W 47th St

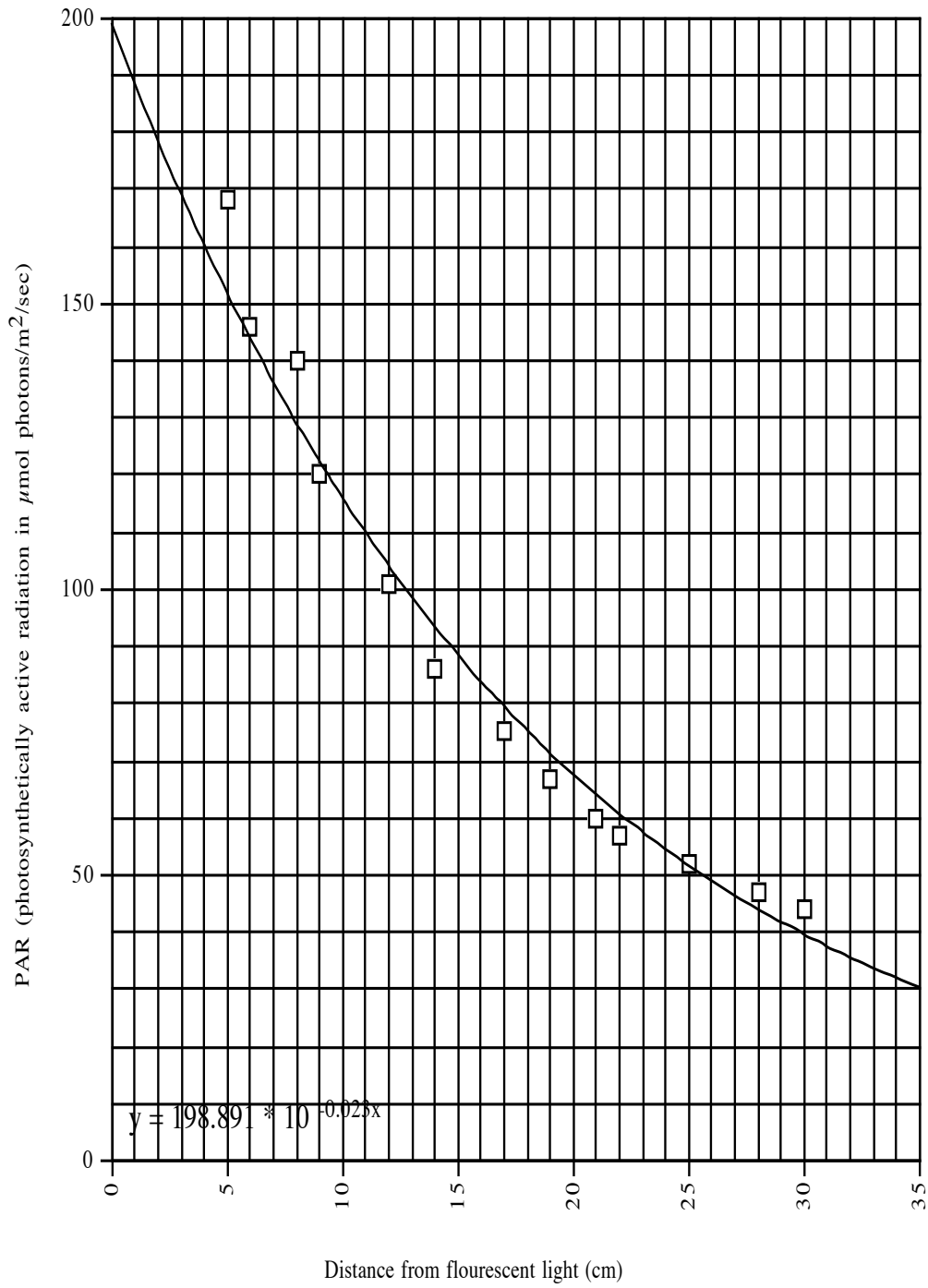
NY, NY 10036

1-800-245-6630

Fax – 1-212-956-6537

www.sldlighting.com





Determining PAR value. Measure distance from fluorescent light then use the graph above to get PAR value.