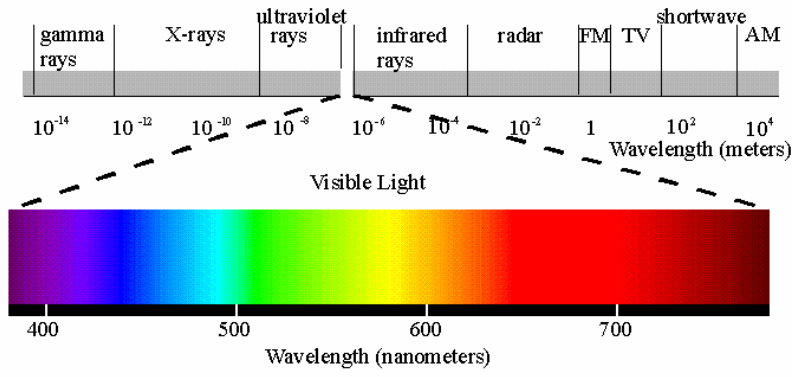
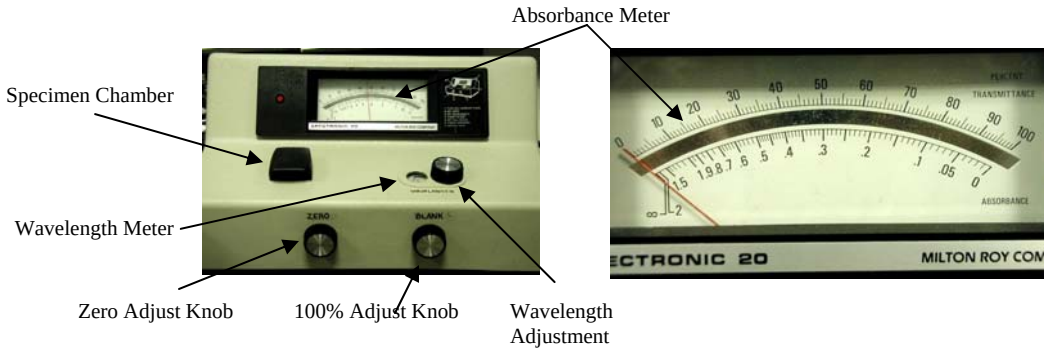
Topic 8: **Photosynthesis**

A. Introduction

Through photosynthesis, the abundant energy from the sun is collected and converted into chemical forms by photosynthetic organisms (i.e. plants) that can be utilized by plants as well as heterotrophic organisms. The chain of events encompassed by photosynthesis begins with the absorption of specific wavelengths of light by pigment molecules embedded in the thylakoid membrane of the chloroplasts. These pigments only absorb a small amount of the available radiation referred to as the **visible spectrum**. As seen below, the visible spectrum is only a small part of the **electromagnetic spectrum**, which is a representation of all the radiation coming from the sun.

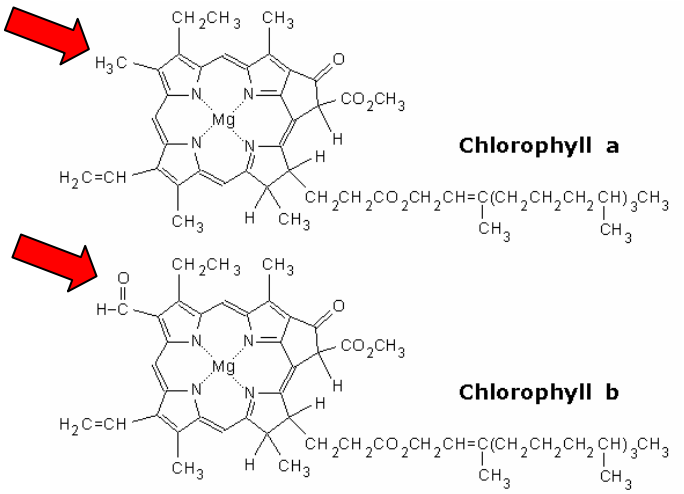


The pigments in photosynthesis absorb light with **wavelengths (λ)** in the visible light region of the electromagnetic spectrum. Some of these pigments include **chlorophyll a, chlorophyll b, β-carotene** (carotenoid), and **xanthophylls** (carotenoids). Each pigment has a preference regarding what wavelengths of visible light can be absorbed. When we purify pigments, we can investigate what wavelengths are preferentially absorbed. Alternatively, we can identify an unknown pigment molecule by measuring light absorption at different wavelengths and comparing the results to known pigments. The pattern of light absorption for a purified pigment is known as the **absorption spectrum** for that pigment. Absorption spectra can be determined using a machine known as a **spectrophotometer** which measures how much light a molecule absorbs. We will use the SPECTROPHOTOMETER 20 shown below:



B. Autumn Colors

Autumn colors are largely due to the differential rate of breakdown of the various photosynthetic pigments that were all present within the leaves throughout the growing season. The green that is dominant throughout the growing season is from generally two types of chlorophylls (**chlorophyll "a"** and the other is often **chlorophyll "b"**). These are usually the first to be broken down in Autumn. In addition to the chlorophylls, the thylakoid membrane system (which you'll learn about in several weeks) of the chloroplasts of photosynthetic cells contain a variety of antennae or accessory pigments. These include various **carotenoids**, which may be yellow, red or purple. The most important carotenoids are **B-carotene** and various **xanthophylls**. These accessory pigments absorb light in wavelengths of light other than those of the chlorophylls therefore, increase the range of light that can be utilized. These accessory pigments may also help to absorb excess light that could damage the photosynthetic machinery. When the chlorophylls break down in the Fall, the carotenoids are revealed.



To test for the presence of this variety of pigments in leaves that are apparently all green, we will do a simple extraction and chromatographic separation of pigments from healthy green leaves.

C. PIGMENT ANALYSIS:

Your **INSTRUCTOR** will prepare a pigment mixture for you to analyze by extracting the pigments from 10g of spinach leaves. In a Waring Blender, 10g of spinach and 100ml of **ACETONE** are combined and macerated for 3-5 minutes. This will be followed by vacuum filtration to separate the cellular material from the soluble extract. Only the compounds soluble in organic solutions (acetone) will be present. The pigment extraction will be collected in a 200-

400ml beaker and this will be **YOUR SOURCE** for the pigment separation and analysis experiments.

1. Separate individual lipid-soluble pigments via paper chromatography:

a. Obtain a small amount of the extract in a beaker or vile.

b. Using a paint brush, place the extract on a pencil line 2.5 cm from the bottom of the sheet of chromatography paper. Add extract to form a dark, thin line. Allow to dry briefly between applications. Label the top in pencil, with your name.

c. Place 100 ml 9:1 v/v petroleum-ether/acetone solvent into a chromatography tank. The height of this liquid in the tank should be 1.5-2.5 cm.

d. Hang the folded top edge of the chromatography paper over a string spanning the top of the tank. Make sure the bottom of the chromatography paper is submerged in the chromatography solvent mixture by 1-1.5 cm, but below the line of leaf extract that was applied.

e. After sufficient time to separate the pigments, but before the solvent front reaches the top of the chromatography paper, remove and dry the chromatograms.

f. The pigments will have separated according to their differing polarities. Can you see the chlorophylls and carotenoids?

Are ALL potential pigments present for this analysis?

What is meant by separation due to different polarities?

What colors can you detect?

What order are these color bands in starting from the top?

Below, List the color bands identified, their position and PREDICT what pigment they represent:

2. Analysis and identification of pigments isolated from Spinach leaves:

Objective: To identify the pigment molecules separated by paper chromatography by determining the absorption spectrum of each and comparing it to the absorption spectrum of known pigments.

1. Turn on Spectrophotometer so it warms up (~15 minutes). Refer to **Appendix A** for the operating procedure of the Spectronic 20.

2. Isolate an individual pigment by cutting out a single colored band and then cutting the chromatography paper into strips approximately 1 inch long. Be careful not to combine different colored pigments on your cut paper. Then place the pieces into a test tube (use separate test tubes for each pigment being purified).

3. Add 5 ml of petroleum ether/acetone (9:1 v/v) and elute the pigments into solution. USE THE MINIMUM SOLUTION NECESSARY! Cork the test tube tightly and mix by inverting the test tube. Elute the pigments for 5 minutes or until all color is removed from the paper.

4. Do this for all of your pigment bands. Keep each band separate so that if you have a minimum of four bands, you should have four test tubes in the next step.

5. Add the petroleum ether/acetone pigment extract to a glass test tube for use in the spectrophotometer. *Do not write on the glass test tube but do not mix it up with your other samples.*

6. Adjust the spectrophotometer wavelength to **400nm** and insert a test tube containing 10ml of petroleum ether/acetone with **NO PIGMENT**. This tube is your **BLANK**. Set the % Transmittance to 100%. Remove the BLANK.

7. Insert a tube of unknown pigment sample. Record the % Transmittance in the provided table. Convert the % Transmittance to Optical Density using Appendix B. Readings below 0 should be recorded as 0 % Transmittance. Remove the sample from band #1 and repeat for all of your pigment samples before moving onto the next wavelength.

8. Include a sample of crude pigment extract to analyze in addition to your multiple purified pigments.

****IMPORTANT: DO ALL OF YOUR SAMPLES BEFORE MOVING****
TO NEXT WAVELENGTH!

9. Remove your sample, increase the wavelength by **20nm** and re-insert the BLANK. Set the Transmittance to 100% and record the optical density or % Transmittance for each of your sample pigments.

10. Repeat step 8 until you have finished with your measurements at **700nm**. Record all values in the provided table.

Do the pigments absorb all wavelengths equally or do they exhibit preferred absorption?

Do the absorption patterns from the different bands differ from one another?
Do any of the bands being analyzed seem to have similar patterns?

Table 1. Pigment % Transmittance and Optical Density in Relation to Wavelength.

Sample:

λ	% T	O.D.

Sample:

λ	% T	O.D.

Sample:

λ	% T	O.D.

Sample:

λ	% T	O.D.

Sample:

λ	% T	O.D.

Sample:

λ	% T	O.D.

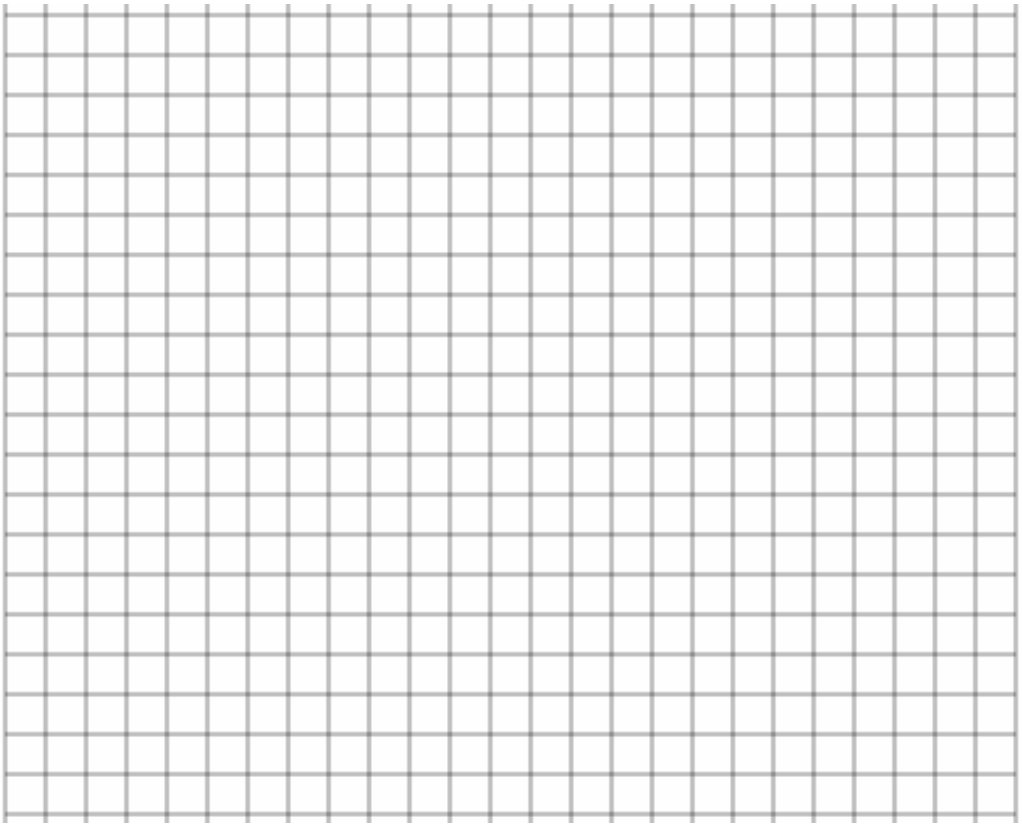
11. **Plot** the optical density against the wavelength for EACH CANDIADATE PIGMENT and the crude extract using Excel (alternatively use provided graph paper). Use different colors or line patterns to clearly distinguish one line from the next. Using Figure 7.5 on page 119 of your textbook, IDENTIFY the pigments. Clearly label each pigment band as the number it was on the paper AND as the type of pigment it represents. (If for assignment, plot each sample on a separate graph...use the same scales for all graphs.)

Note the patterns of wavelengths absorbed and not absorbed.

What does this tell you about the pigments?

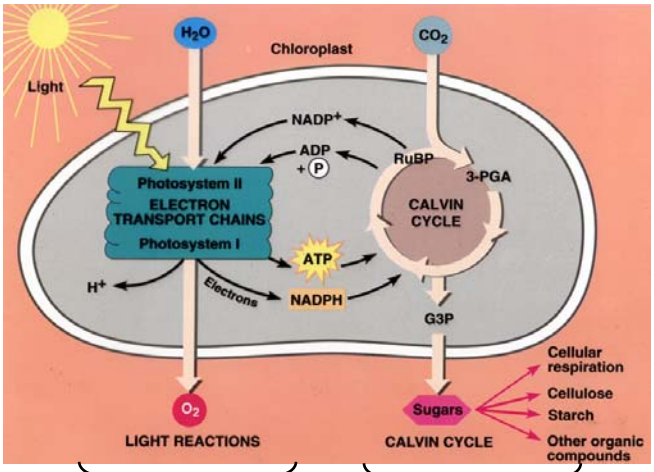
What pigment have you identified?

Relate the pigment identified to the color of the leaf and explain why it was or was not originally seen in the leaf.



D. Photosynthesis produces measurable products

Photosynthesis can be divided into two phases referred to as the **light reactions** (also know as the thylakoid reactions) and the **dark reactions** (more appropriately called the light-independent reactions). Both stages produce biological molecules that can be detected and measured and represent photosynthesis. The dark reactions produce the **carbohydrate** molecules that are the final energy storing forms of the energy originally harvested from visible light. The light reactions produce the energy needed for the dark reactions (i.e. ATP and NADPH) but also produce **Oxygen** as a waste product. We can measure the oxygen produced and use the rate of oxygen production to represent the rate of photosynthesis. The figure to the right demonstrates the overall process:



The reaction of photosynthesis can be written:

$$6CO_2 + 12H_2O \rightarrow C_6H_{12}O_6 + 6O_2 + 6H_2O$$

This reaction supplies the energy requirements for cellular processes such as cellular respiration via the production of carbohydrate ($C_6H_{12}O_6$). The production of O_2 as a waste product has unequivocally changed the atmosphere surrounding Earth and provides the oxygen needed for respiration to occur in aerobic organisms. However, from a growth point of view, the carbohydrates and energy (ATP, NADPH) produced by photosynthesis are more important because they provide the components needed for growth to occur.

In the next section we will take advantage of the waste product production and you will use carbon dioxide probes to measure CO_2 fixation in **LEAVES**. Two variables will be investigated: Light **QUALITY** (i.e. red, green, white wavelengths of light) and Light **QUANTITY** (i.e. varying distances between the light and leaves). These experiments provide invaluable insight into the mechanisms of photosynthesis.

2. The effect of different light wavelengths on the rate of photosynthesis:

Objective: To measure the rate of photosynthesis through the rate of carbon dioxide consumption and to determine the effect of light quality (wavelength) on photosynthesis.

This section will be accomplished using a Vernier CO_2 gas probe connected to a computer and the collected data will be recorded temporarily using the Logger-Pro data analysis program. You will be responsible for setting up the experimental conditions, initiating the data collection, recording the data generated from the Vernier CO_2 gas probe, and graphing the data.

1. Open the file for the photosynthesis experiments as follows:

    Logger Pro
        File
            Open
                Vernier Software

Logger Pro (2.1)
Experiments
Biology with Computers
Exp 31BS Photosyn-Resp (CO2)
Exp 31B CO2 Gas.MBL

This will open the correct window. You can confirm this by checking the readout in the lower right-hand corner that indicates the "Carbon Dioxide-Live in ppt". If it does not say "ppt" then ask for assistance from the instructor.

2. Now set-up the parameters for data collection as follows:
   Setup
   Data Collection
   Sampling
   Experiment Length = Set to 120 minutes
   Sampling Speed = Set to 60 samples/minute

When you begin to collect the probe will automatically plot the data in real time in the graph window. Also the readings will be displayed in the table on the upper right portion of the screen.

3. Place pieces of tape on the table at 10cm, 26cm and 70cm from the light source.

4. Cut leaves off of the available plants half way along the petiole. Weigh 5g of leaf tissue and insert it into the clear plastic leaf chamber.

5. Place the CO_2 gas probe firmly into the top of the chamber and place the chamber/probe into the cardboard cylinder so that the front side of the plastic box touches the tape.

6. Cover the cylinder opening and top with aluminum foil to establish a "dark" treatment.

7. Start collecting data by clicking on "COLLECT". Keep the leaves in the dark for 20 minutes.

8. Following the dark treatment, switch filter/treatments and turn on the light. Leave the foil in place on top of the apparatus. Proceed through the following sequence of light filters, exposing the plant material to each filter + light for 15 minutes apiece.

   a) White light (no filter)→@ 70cm
   b) Green light filter → @ 26cm
   c) Red light filter → @ 10cm
   d) Green light filter → @ 26cm
   e) Blue light filter → @ 10cm

9. If the CO_2 level plateaus during a treatment, turn the light off, cover the cylinder hole with aluminum foil, and allow the leaf tissue to rest in the dark for 20 minutes.

Treatment	Slope (m)
White	
Blue	
Red	
Green	

What happened to CO_2 levels during the initial dark treatment? Why?

What happened to CO_2 levels during the white light treatment? Why?

What wavelengths of light stimulate photosynthesis the most?

What wavelengths of light have the least effect on photosynthesis?

How do the red and blue rates of photosynthesis compare to each other?

How can you explain the difference seen between them?

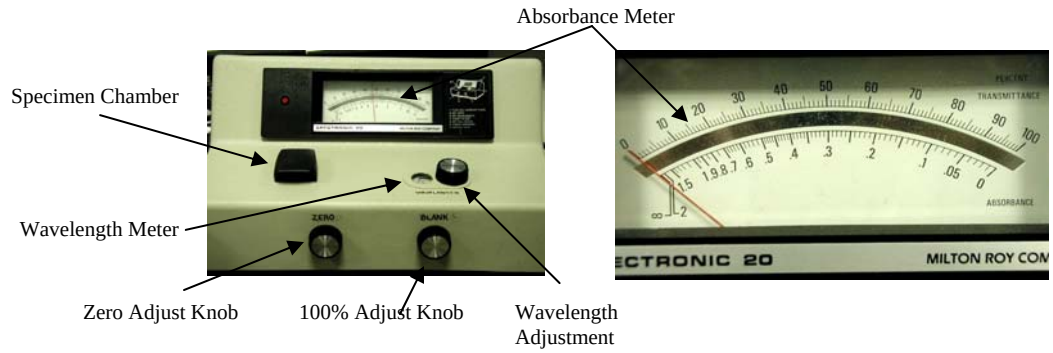
3. Effect of Light Intensity on Photosynthetic Rate: (See Instructor - optional).

1. Use the previously described setup and program for this experiment.
2. Keep bean leaf tissue in the dark for 10 minutes.
3. Expose the bean leaf tissue to light at 80, 40, 20, and 10 cm from the light source.
4. Limit each light treatment to 10 minutes.
5. Calculate the rates of CO2 assimilation for each treatment.
6. Record your data below:

Light Treatment	Rate
80 cm	
40 cm	
20 cm	
10 cm	
Dark	

Explain what your results indicate regarding the relationship between light intensity and rate of photosynthesis. Propose a hypothesis explaining the results.

Appendix A: The SPECTRONIC 20



The Spectronic 20 spectrophotometer is a very important and useful scientific tool allowing you to measure the absorption patterns of individual pigments and combinations of pigments in solution. The Spectronic 20 requires care and consideration during its use.

Basic Operation:

1. Turn on and allow it to warm up for 10-15 minutes.

2. With specimen chamber closed and empty, adjust to desired wavelength.

3. Set needle to 0% transmittance with the zero adjust knob.

4. Place a reagent blank in the chamber and adjust the needle to 100% transmittance with the 100% adjust knob.

5. Place sample(s) to be read into chamber and record absorbance.

6. To read at a different wavelength, repeat steps 2-5.

7. To obtain valid readings when the wavelengths noted are printed in red, one must convert the machine. This is easily done by disconnecting the current, opening the hatch beneath the machine, inserting the red filter supplied in the holder and changing the bulb from #CEA-59X to #CEA-30 (red). This is necessary in obtaining data on chlorophylls.

8. When graphing data, one must have log absorption. This can be read directly with some difficulty, but is usually obtained by converting percent transmittance using the attached table.

9. Chlorophyll will fluoresce or transmit red light when its resonance system is energized by ultra-violet light. This red is the color absorbed by chlorophyll from white light. It reflects (doesn't use) green light. The absorption spectrum demonstrates this.

Appendix B: LOG TABLE FOR SPECTRONIC 20

see handout from Instructor

Appendix C: OUTLINE FOR WRITTEN REPORTS

see handout from Instructor