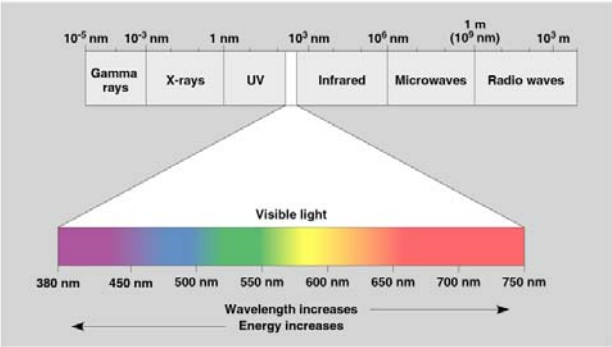
Absorption Spectra of Photosynthetic Pigments

Light is a form of electromagnetic radiation. Visible light is a combination of many wavelengths in the range of 380 - 750 nm that we see as different colors. Each wavelength is associated with a specific photon, or particle of energy. In general, shorter wavelengths have more energy than longer ones.



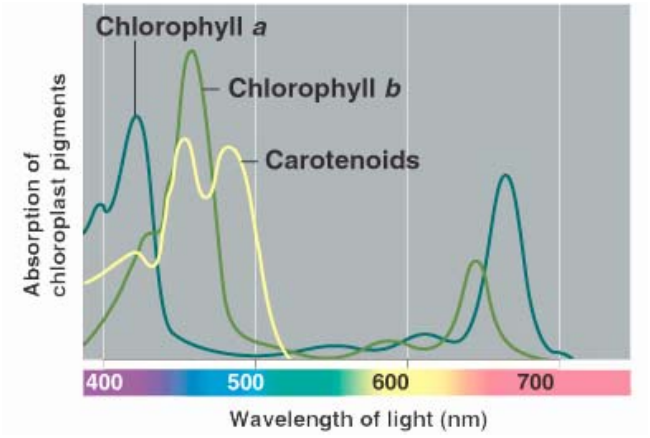
Electromagnetic and Light Spectrum

The pigments of the leaf have the property of absorbing and transmitting certain light waves, while reflecting others. The "color" we see is the wavelength(s) of light reflected by the pigment molecules. To provide energy for the process of photosynthesis (the transformation of light to chemical energy), light must be absorbed by the pigments. The light absorbing photosynthetic pigments do not absorb all wavelengths of light equally. The **absorption spectrum** of the leaf pigments will help you determine which light waves are absorbed by the leaf pigments.

A spectrophotometer is an instrument that can measure the absorption of light by a substance at specific light waves. An explanation of how a spectrophotometer works and instructions for its use are in the Spectrophotometer handout. You will determine the absorption spectra for 4 leaf pigments: carotene, chlorophyll a, chlorophyll b and xanthophyll.

Paper chromatography is used to separate the different light-absorbing pigments from a leaf extract. Once separated, the pigment strips are cut apart and re-suspended alcohol to establish an absorption spectrum.

This will be a class exercise. All of the chromatograms will be pooled to concentrate the pigment extract. We will be using as many spectrophotometers as we have available. It's always best to have more than one set of data when doing science. Volunteers will be required to participate in all stages of the exercise: preparing extracts, operating the spectrophotometers and recording the data on the board for the other students. Do not hesitate to volunteer for at least one activity.



Materials Needed

Spectrophotometers (Turned on at Beginning of Lab)
Spectrophotometer cuvettes
4 250-ml Beakers
500 ml Alcohol
4 Small labels
4 Wood stirrers
1 China marker (grease pencil)
Sharp scissors for each student

Procedure

While several students are completing steps 1 and 2 for all of the chromatograms, other students can be doing the following:

- The students who plan to collect the spectrophotometer data should review the procedure for operating the spectrophotometer from the *Using the Spectrophotometer* handout from the Biology 101 Laboratory Exercises website and be getting the spectrophotometer ready for operation. There should be three students for each spectrophotometer. (See below.)
- One student group can reproduce the Absorbance chart on the board, making sufficient columns for each pigment for the number of spectrophotometers available and for an average value.
- One student group can label the 4 beakers for each of the pigments ("A" for chlorophyll a, "B" for chlorophyll b, "C" for carotene and "X" for xanthophyll) and put a small amount of alcohol in each beaker, along with a wooden stirrer. This group will also be in charge of preparation steps 3 - 5 below.

Preparation of the pigment extracts

1. Cut each of the four pigment bands (carotenes, xanthophylls, chlorophyll a and chlorophyll b) from your two chromatograms. Be sure to keep track of which strip is which pigment. Yes, you must cut along the "zigzag" lines. Each strip must have just one pigment.
2. Crumple up the pigment strips and place each strip in its appropriate beaker located at the side table. The beakers will be labeled "A" for chlorophyll a, "B" for chlorophyll b, "C" for carotene and "X" for xanthophyll.
3. When all of the chromatogram strips have been placed in the beakers, stir the paper-alcohol mixtures with the wooden stirrers provided. The pigments will be transferred from the paper to the alcohol. If necessary, add more alcohol.
4. Pour extract into 4 cuvettes for each spectrophotometer available. Be sure that you know which cuvette has which pigment. Avoid getting paper fragments in the extract. Pour alcohol into a fifth cuvette. This will be the blank.
5. Take one set of the four pigment extracts and a "blank" containing alcohol to each of the spectrophotometers available.

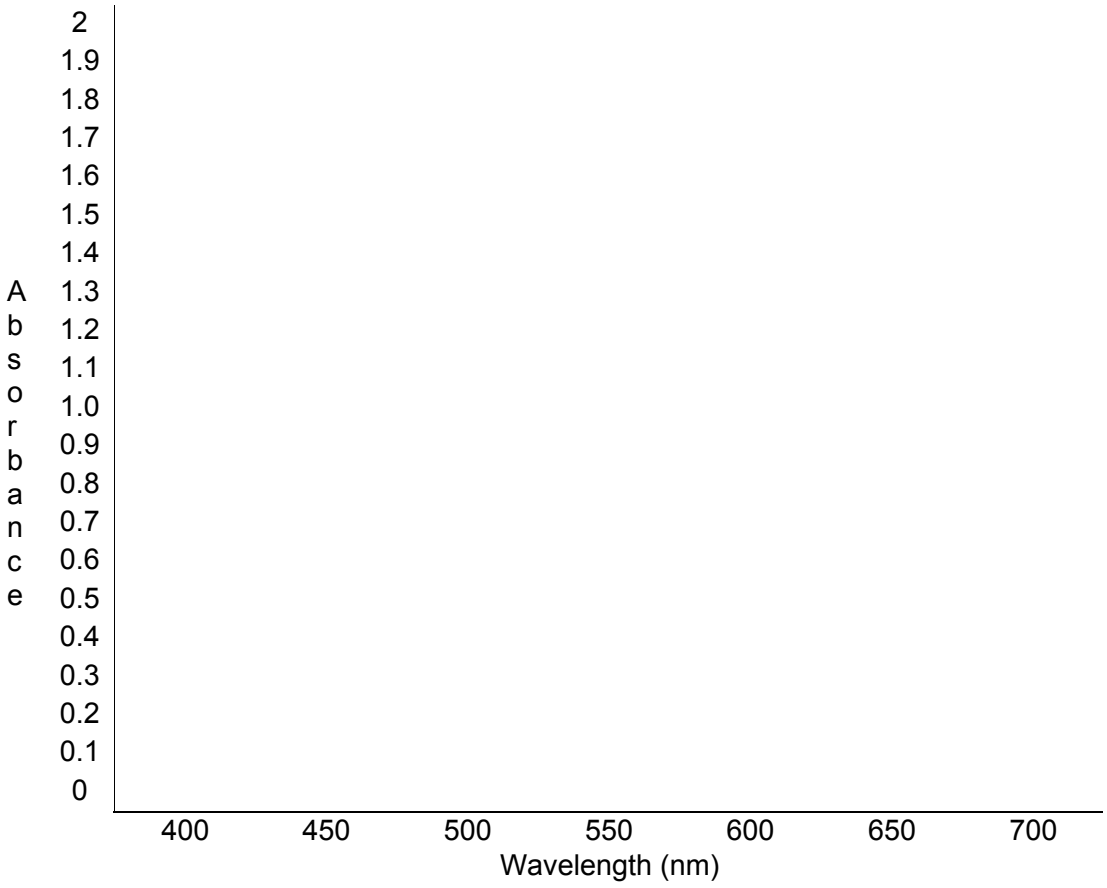
Collecting the absorption data
You will measure light absorption at 50 nm intervals from 400 nm → 700 nm for the absorption spectra of the leaf pigments, but you will start at 400 nm.

1. Set the wavelength to 400nm and make any other instrument adjustments that are mentioned in the spectrophotometer manual.
2. Standardize the absorbance to "0" using the blank cuvette containing alcohol, following the instructions available in the spectrophotometer handout. Each time you change the wavelength selection, you must repeat this step. Remove the alcohol blank cuvette. The spectrophotometers should have spaces for four cuvettes.
3. Place each of your pigment samples in one of the chambers. Keep track of which chambers the pigments are in.
4. Close the cover and read (and record) the **absorbance** on the meter for your first pigment. Repeat for each of the pigments by shifting the cuvette chambers into the correct position for reading the absorbance.
5. Record the absorbance numbers for 400 nm for each of the four pigments on the chart on board for the class data.
6. Change the wavelength to 450 nm and repeat steps 1 – 5 for 450 nm wavelength.
7. Change the wavelength to 500 nm and repeat steps 1 – 5 for 500 nm wavelength.
8. Change the wavelength to 550 nm and repeat steps 1 – 5 for 550 nm wavelength.
9. Change the wavelength to 600 nm and repeat steps 1 – 5 for 600 nm wavelength.
10. Change the wavelength to 650 nm and repeat steps 1 – 5 for 650 nm wavelength.
11. Change the wavelength to 700 nm and repeat steps 1 – 5 for 700 nm wavelength.

Absorbance of Leaf Pigments

Wavelength (nm)	Absorbance			
	Chlorophyll a	Chlorophyll b	Carotene	Xanthophyll
400				
450				
500				
550				
600				
650				
700				

Graph the class average data for each pigment.
Absorbance should be on the y axis and wavelength on the x axis.



Discussion Questions

1. What wavelength is absorbed best by chlorophyll a? Are there additional wavelengths absorbed well by chlorophyll a? What wavelength(s) is(are) absorbed poorly by chlorophyll a?

2. Is the absorption spectrum for chlorophyll b different from that of chlorophyll a?

3. Would you expect a plant to photosynthesize if exposed to **only** the green wavelengths of light? Why or why not?

4. What are the advantages of having accessory pigments?