The Spec0: A Single-Wavelength Homemade Spectrophotometer

Pérez, AA; Edelman, RD; Croom-Pérez, TJ; Tekely, CP; Golbeck, JH and Bryant, DA.

Grades 5-12

Measurable Outcomes

I. Students will learn about light and how it interacts with different materials.

II. Students will discover than an LED does not only emit light but it can also detect it and generate a measurable electric current.

III. Students will compose a simple circuit; intertwining engineering and science in one lesson.

IV. Students will discover that different wavelengths of light are absorbed differently by various materials, including living cells.

V. Students will be able to customize the Spec0 instrument to their specific needs. A potential for further experimentation besides growth determination.

Activity Summary

Students will elaborate a simple spectrophotometer utilizing inexpensive and accessible materials. The device will be used to indirectly quantify the growth of cyanobacteria and microalgae in the classroom setting. This device can be modified to utilize different light sources to discriminate unique properties and/or organisms via light.

Materials

- 2 Green 525 nm LEDs (forward voltage 3.5V, 4 Lumen, 8000mcd, T1-3/4 diameter, 45 degree viewing angle, 100 mA peak forward current)
- male-male USB cable
- 5V USB wall charger
- 2 Test lead set with alligator clips
- Plastic cuvettes
- Cuvette Holder
- Electrical Tape

- Wire stripper
- 150 ohm resistor
- Digital volt meter with leads
- Cyanobacteria or algae in liquid medium
- **Optional:** Solder

Content/Background

Light is part of the electromagnetic radiation spectrum (Fig. 1). In summary, light is energy transmitted through space in the form of waves whose distance between crests is denoted as the wavelength. Some forms of electromagnetic radiation have long wavelengths like radio waves (ranging in meters) and others have shorter wavelengths like gamma (γ) radiation (ranging in the picometers). It is important to note that wavelength is inversely proportional to the amount of energy present in radiation; in other words, the longer the wavelength, the less energy and vice versa.

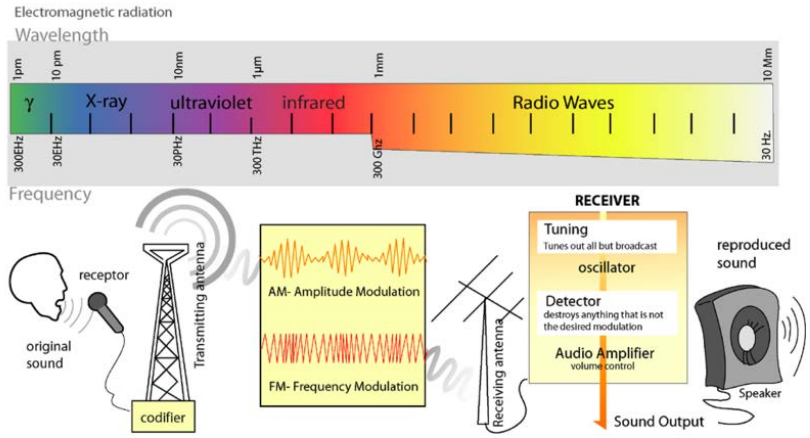

Fig. 1 The electromagnetic spectrum
(http://commons.wikimedia.org/wiki/File:Radio_transmition_diagram_en.png)

Because of the different properties conferred by different wavelengths, electromagnetic radiation can interact differently with objects. In fact, we are able to see different colors because of this; some wavelengths of visible light are not absorbed uniformly by all materials, but rather reflected. For example, plants appear green because their pigments absorb more readily the blue and red region of the spectrum, whereas the green region is not and thus is reflected to our eyes.

Scientists have devised numerous instruments to measure light. One of these, the spectrophotometer, is useful in determining the amount of light that is absorbed by a material. It works by passing a beam of light through a material and measuring the intensity of the light that reaches a detector on the opposite side. The solution will absorb some of the light, therefore the amount light that reaches the detector will be less than the original emitted light. This detector converts the light energy to electrical energy, which can be measured (Fig. 2).

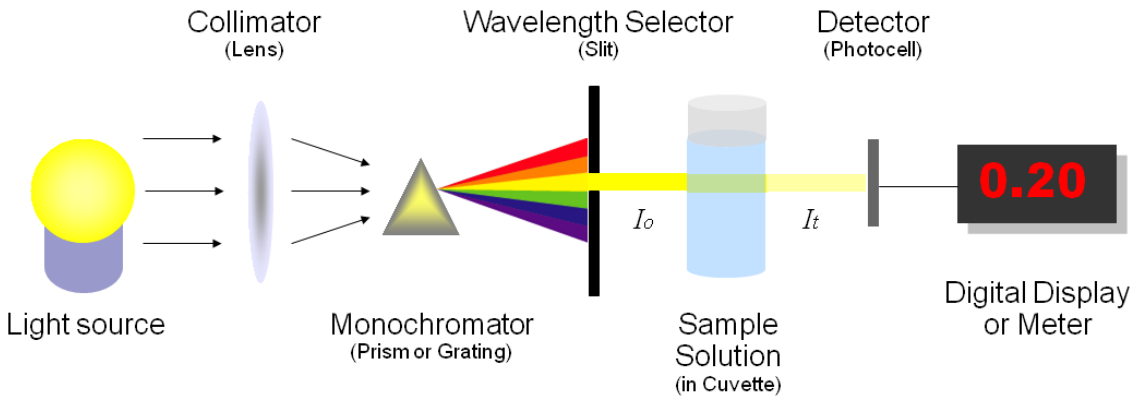

Fig. 2 How a spectrophotometer works

(http://chemwiki.ucdavis.edu/Core/Physical_Chemistry/Kinetics/Reaction_Rates/Experimental_Determination_of_Kinetcs/Spectrophotometry)

Photosynthetic organisms utilize myriad pigments to capture the energy of light for conversion into chemical energy useful for carbon fixation. These include an assortment of carotenoids, chlorophylls, and phycobiliproteins; all of these are capable of capturing varying ranges of light energy in different regions of the visible light spectrum. Scientists use their knowledge of the absorption of these pigments relative to the electromagnetic spectrum to classify and identify photosynthetic organisms such as microalgae (eukaryotes) and cyanobacteria (prokaryotes).

In this activity a spectrophotometer will be built to measure the growth of algae orcyanobacteria. This instrument will be built with accessible materials that you can find in a hardware store. Fig. 3 shows the schematic for the constructed single wavelength spectrophotometer, termed "Spec0" hereafter, utilizing the materials listed for this lesson plan. The theory behind this instrument lies in the use of a pair of LEDs as both a light source and a light detector when assaying how much light traverses a sample being tested. LEDs use a small semiconductor crystal made of negative (n-type) and positive (p-type) materials to generate light using current. An LED can be used as a detector because light received from a similar wavelength LED can induce "movement" of electrons in the semiconductor, yielding an electrical current that can be measured using a voltmeter device.

Growth of an algae/cyanobacterial culture can be measured using the Spec0 instrument. In theory, the more density in the culture, the less light will be available to reach the LED detector resulting in a decrease of electric current readout.

Schematic Figures for Construction of Spec0 Single-Wavelength Spectrophotometer

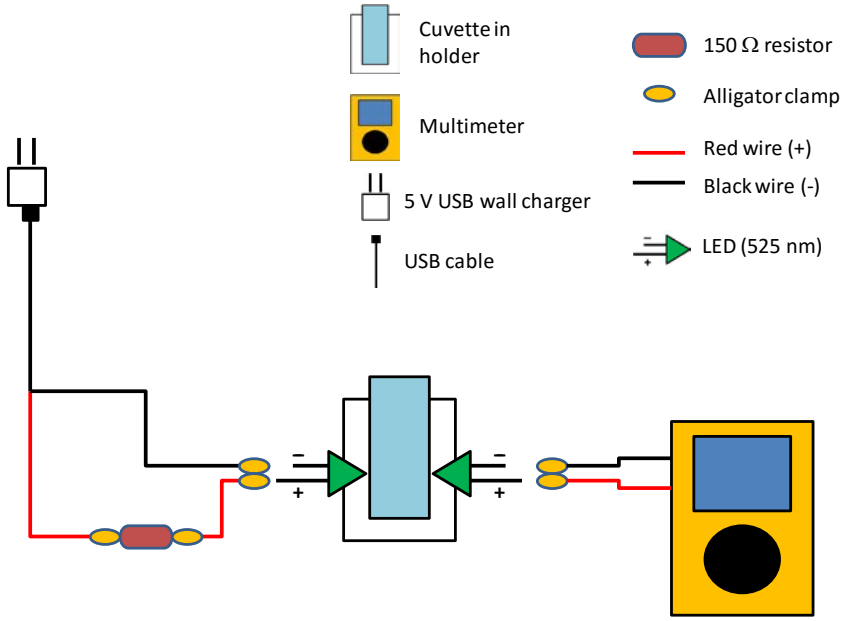

Fig. 3 Schematic for building the Spec0

The development of the cuvette holder depends on the dimensions of the cuvettes used. Its purpose is to simply serve as a scaffold (Fig. 4A) to hold the cuvette so that the pathlength between the LED providing the light source and the LED acting as the detector through the sample is reproducible the same. The cuvette holder can be made of any material that can provide sturdiness to the scaffold. It is preferable to paint or use material that is black to prevent reflection of light. It is important to drill a traverse hole (Fig. 4B) that will snuggly accommodate the diameter of the LEDs utilized and allow the LEDs to face directly to each other (Fig. 4C).

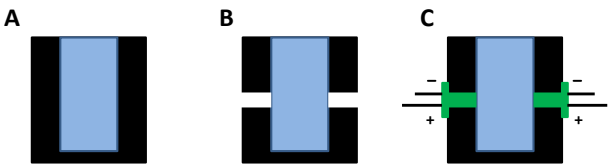

Fig. 4 Drilling of the cuvette holder for proper positioning of the LED components

Another important step in the construction of the Spec0 is the proper stripping of the male-male USB cable. Cutting the male-male USB cable in the middle (Fig. 5A) will provide materials for the production of two Spec0 devices (Fig. 5B). Carefully strip the USB wire in order expose the copper wiring from the + (black) and – (red) wires (Fig. 5C). Remember to connect the red wire to the 150 Ω resistor utilizing alligator clamps. Failure to include the resistor component will damage the LED component.

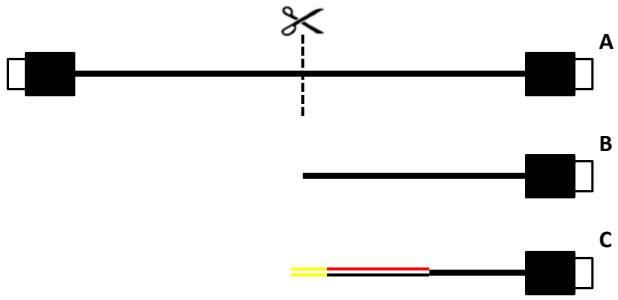

Fig. 5 Stripping the male-male USB cable

As with all electrical components, proper care must be taken to avoid electric shock. Utilize electrical tape to cover all exposed conductive surfaces (e.g. copper wiring) before utilizing the device for the first time.

Suggested Activities:

Data Analysis Interpretation:

Utilizing the Spec0 device we will follow the growth of your algae/cyanobacteria. **Note:** The Spec0 device **will not** provide you with a quantification of the cell mass, but an indirect measurement of dense your culture is.

Measure the current output provided by the volt meter to plot a graph for growth. You will observe that the current output will decrease as the cell density increases. **Discuss** with your class what would be the best way to interpret this data graphically in order to showcase growth.

Standardization:

Develop a standard curve by measuring the optical density (OD) of your cells utilizing a conventional spectrophotometer, and the current output of the same sample. Plot with your students the OD vs. current output over a period of time. This data will allow you to correlate the current output (x-axis)

with OD (y-axis) of your samples and allow you to have a more accurate interpretation of the biomass present in your culture. **Note:** research online the correlation between OD of your organisms and the amount of cells per unit of volume.

Studying different pigments:

Cyanobacteria and algae contain myriad pigments to capture a wide range of the electromagnetic spectrum. Assign your students (in groups) to investigate the different type of pigments that are present in their photosynthetic organism. Assign a pigment to each group of students to research in detail (e.g. structure, absorptive properties, function inside the cells etc.). Using the researched information, investigate what single wavelength LEDs would be most appropriate to screen for this particular pigment *in vivo.*

Important! Different LEDs will require different resistors to operate. Refer to the specifications provided with your LEDs. You can also utilize the online tool in the references segment to calculate the type of resistor you would need to operate your modified Spec0.

As a class effort, you can simultaneously screen different pigments of your organism. This is particularly useful to make inferences of how pigment production behaves when growth parameters are changed (e.g. growth in dark, growth at different temperatures, use of different growth medium recipes etc.)

Note: The provided instructions suggest the use of a green LED to correlate biomass more efficiently as it is not readily absorbed by pigments naturally found in these cells. Fig. 6 shows the absorption spectrum of *Chlorella vulgaris*, a microscopic algae, to better illustrate the choice of the green LED.

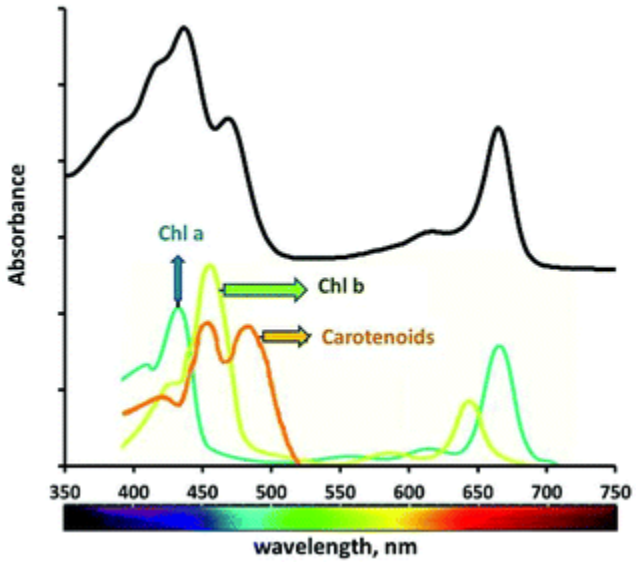

Fig. 6. The absorbance spectrum of *Chlorella vulgaris.*
Eroglu E, Eggers PK, Winslade M, Smith SM, Raston CL. 2013. Enhanced accumulation of microalgal pigments using metal nanoparticle solutions as light filtering devices. Green Chem **15:**3155-3159.

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A good online tool to calculate the type of resistor you would need for your LED
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