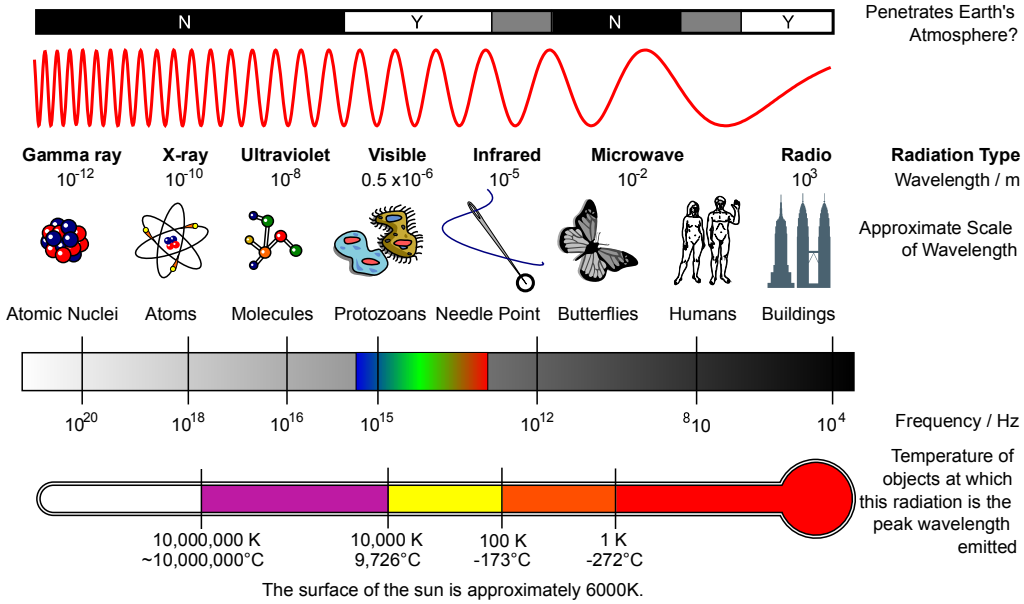
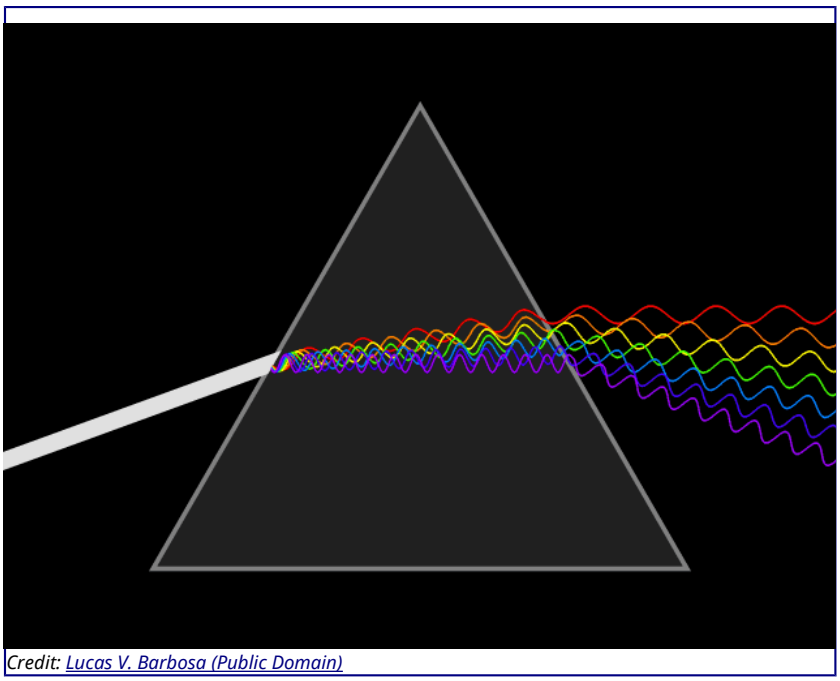
Properties of Light

Light is a type of energy that travels as a wave-particle. The **wavelength** of light is the distances between peaks in the waves as light travels. Wavelengths are measured in nanometers (nm) and different wavelengths of light represent differing colors. White light is a mixture of the visible light **spectrum**. Light of long wavelengths (infra-red) and very short wavelengths (ultra violet) are invisible to humans but can be observed by other organisms. As wavelength decreases, the energy of the light is increased.



Credit: By Inductiveload, NASA (CC-BY-SA-3.0)

Diffraction of light through a prism exposes the components wavelengths of light.



Credit: Lucas V. Barbosa (Public Domain)

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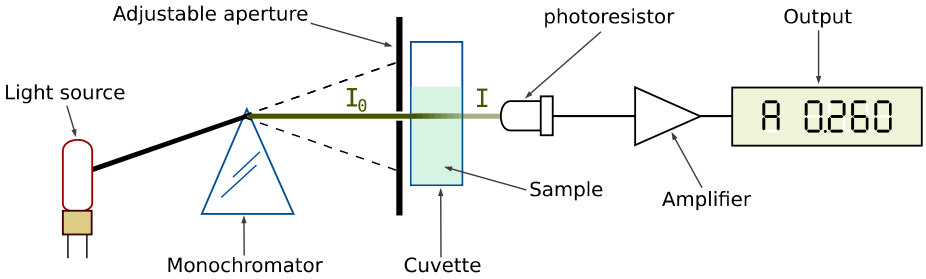
Spectrophotometry

Spectrophotometers (***spectro***-image/color ; ***photo***-light ; ***meter***-measure) are used for chemical analysis of solutions based on properties of absorption or transmission.



Schematic of a spectrophotometer. The monochromator is a prism that splits the light. A single wave-length of light is focused through the aperture to pass through the solution in the cuvette.
Credit: GYassineMrabetTalk [GFDL or CC BY-SA]

Transmittance refers to the amount of light that passes through the solution.

$$T = \frac{I}{I_0}$$

Transmittance of a light source through a cuvette. The intensity of light, $\mathbf{I_0}$, decreases as it passes through the solution. The light detected by the sensor, $\mathbf{I}$, reflects the transmittance of the solution.If light is being absorbed by chemicals in the solution, this results in a lower transmission. **Absorbance** is therefor inversely related to transmittance as expressed by the equation:

$$A = -\log_{10} T$$

Follow the virtual demonstration at: http://www.virtual-labs.leeds.ac.uk/pres/spectrophotometry/ (CC-BY-NC-SA) for a more in-depth explanation of spectrophotometry.

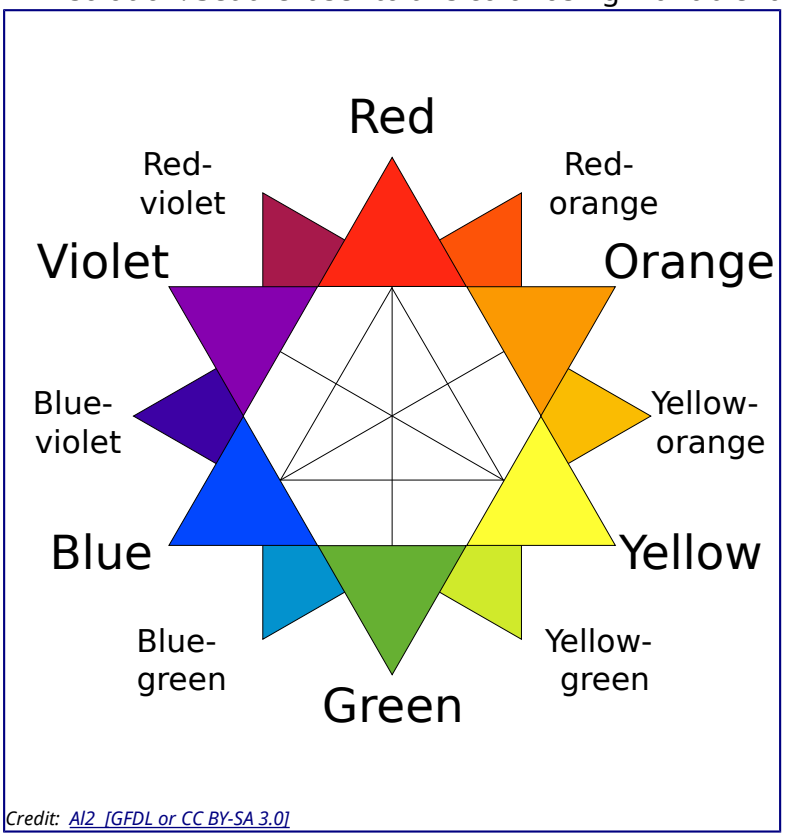
Beer's Law

Beer's Law is a relationship between the concentration or amount of a dissolved substance in a solution that is reducing the amount of transmitted light due to the absorption of the radiant energy. **Lambert's Law** states that the reduction of transmittance was related to the length of the path of light. As the light path increases through a substance, there is a reduction in transmittance. Collectively, these ideas are referred to as **Beer-Lambert Law**, but most observers will control the path length and simply refer to it as Beer's Law.

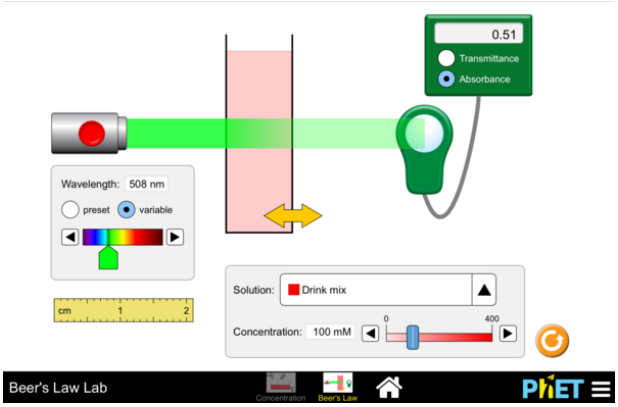
Exploring Beer's Law (virtual)

Use the link below to launch a simulation where you can alter the properties involved in spectrophotometry and examine the Beer-Lambert Law. Increase and decrease the concentration slider in the simulation:

1. What happens to the contents in the cuvette?
2. How does this change the Transmittance and Absorbance readings?
3. Click "variable" and use the slider. What happens to the readings when the Wavelength of the laser is a similar color as the solution in the cuvette?
4. Consult the color star below and find the color wavelength that is opposite of the color of the solution. Set the laser to this color using "variable" and the slider.



Credit: Al2 [GFDL or CC BY-SA 3.0]



Follow link to launch simulation

1. What is the effect on Transmittance and Absorbance with this color?
2. Using the previous observations (using variable wavelength slider), how would you use the relationship on Transmittance/Absorbance to best measure the concentration of a solution?

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Experimental Background

Bovine Serum Albumin (BSA) is a protein that circulates in the blood of cows. Purified BSA can be used with Biuret solution in serial dilutions to generate a **Standard Curve**. The standard curve will illustrate the relationship between concentration (the **dependent variable**) and absorbance at 540 nm (the **independent variable**). We can then use this curve to estimate the concentration of unkown samples.

1. On a graph, do you remember which axis is the dependent and which is the independent variable?
2. In the table below, can you identify which samples are the **negative controls** and which are the **positive controls**?
3. What is the prediction of the absorbance or color intensity of the different tubes?

Dilute BSA Standards

1. Label 9 tubes 1-9
2. Combine the components of the table below to generate appropriate concentration of solutions

	BSA Standard Dilution						Blank	Unknowns	
	1 1.0 mg/ml	**2** 0.8 mg/ml	**3** 0.6 mg/ml	**4** 0.4 mg/ml	**5** 0.2 mg/ml	**6** 0.1 mg/ml	**7** 0 mg/ml	**8** ? mg/ml	**9** ? mg/ml
ml BSA	1	0.8	0.6	0.4	0.2	0.1	0	-	-
ml H_2O	0	0.2	0.4	0.6	0.8	0.9	1	-	-
ml unknown	-	-	-	-	-	-	-	1	1
ml Biuret	4	4	4	4	4	4	4	4	4

3. Place tube 1 (**1mg/ml**) into a cuvette for measuring absorbance (**A**) in the SpectroVis Plus. This will find the peak absorbance value.
4. The instructor will begin to set-up the units for distribution
5. Enter the LabQuest 2 application and press on the green Start button to generate a full spectrum
   - tap on the file cabinet icon to store this data
6. On the Meter Screen, tap on **Mode**
   1. Change the mode to "Events with Entry"
   2. Enter the Name: Concentration
   3. Enter Units: mg/ml
   4. Select OK
   5. If message appears about saving run, choose Discard
7. Sequentially read each sample at the stored wavelength (between $\mathbf{A_{540nm}}$-$\mathbf{A_{600nm}}$) and record values in table below

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1. Plot each BSA dilution in plot.ly as a scatterplot
2. Generate best-fit line for these standards with the equation of the line
3. Use the equation of the line to estimate the concentration of the unknown sample.

Tube #	BSA (mg/ml)	A_{540}
1	1.0	
2	0.8	
3	0.6	
4	0.4	
5	0.2	
6	0.1	
7	0.0	
Do not plot the values below. Use the plot to estimate the concentrations.		
8	?	
9	?	

LabQuest2 and SpectroVis Tutorial

https://youtu.be/8ZVYkMW03Rs

Scatterplot Tutorial

You can watch this tutorial at 1.25X and pause when needed.

https://youtu.be/jeRMxXvbI7g

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