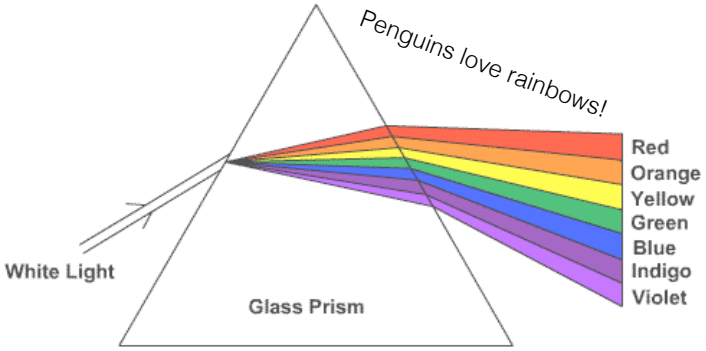
Light, Spectroscopy and the Spectrophotometer

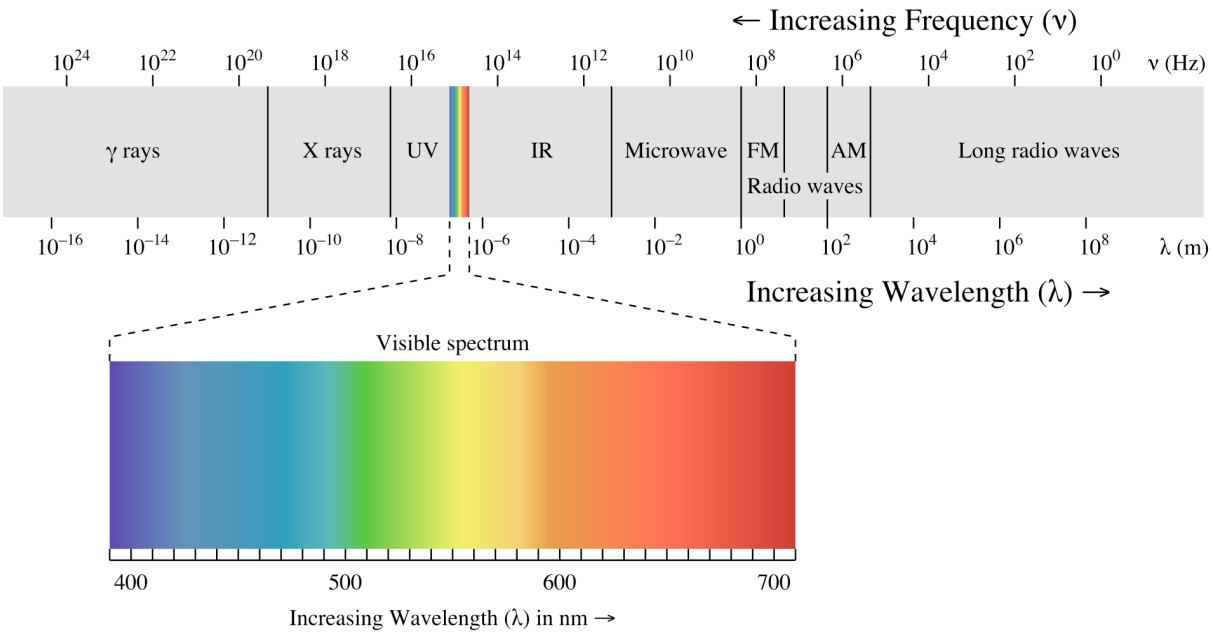
Wavelengths of Light

Spectroscopy is the study of the interaction between matter and radiated energy. In the past, spectroscopy was basically the study of how visible light dispersed according to its wavelength by a prism. Later, the concept was expanded to comprise any interaction with radiative energy as a function of its wavelength or frequency. Spectroscopic data is often represented by - you guessed it - a **spectrum**, which is a plot of the response of interest as a function of wavelength or frequency.



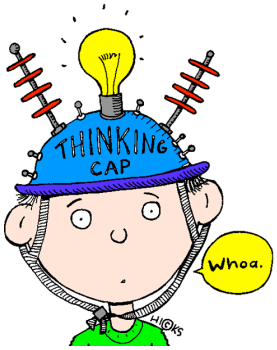
The quantitative measurement of the absorption or transmission properties of a material as a function of wavelength is called **spectrophotometry**, and the instrument we use is called a **spectrophotometer.**

So we're going to be looking at how light and matter interact. The nature of this interaction depends on two things: the wavelength of the light (which we will control) and the structure of the matter (which is what we are trying to study). Here's you new best friend, the electromagnetic spectrum. Isn't it pretty?



Ready, Set… Choose Your Wavelength!

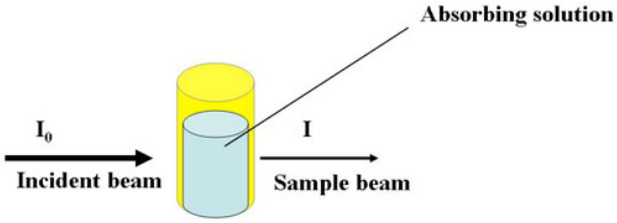
We want to use wavelengths which can excite electrons and move them to a higher energy state. These wavelengths are in the visible / UV range, but even within that range, we need to be more selective. It is important to note that molecules absorb only those wavelengths of light that contain just the *right amount* of energy to move the electrons to a higher energy state, resulting in peaks and valleys when absorbance is plotted against wavelength.



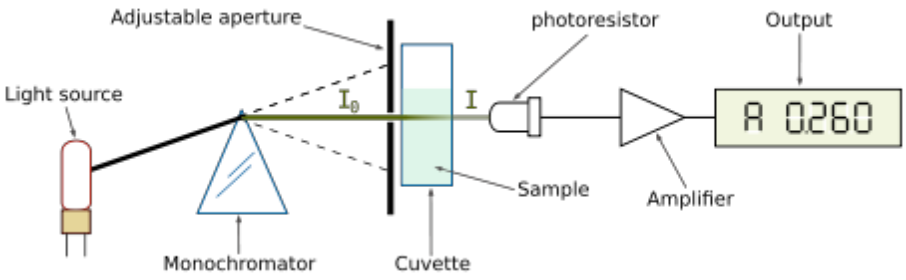
Students often ask me what happens if you use longer or shorter wavelengths (i.e., outside the UV / visible range). Well, you can - but you'll need different instrumentation and you would measure different properties of matter. Absorption of longer, low energy wavelengths (infrared) stretches and bends bonds in molecules; consequently IR spectroscopy is used to identify chemical structures. Shorter wavelengths (X-rays and gamma rays) are sufficiently energetic to strip electrons from molecules completely. Wow.

Light vs. Matter - Quantified

You probably already know that when you pass light through a substance, some of the incident energy is absorbed and some is transmitted.



The spectrophotometer measures transmitted light through a sample with respect to the incident light very precisely. Check this out:

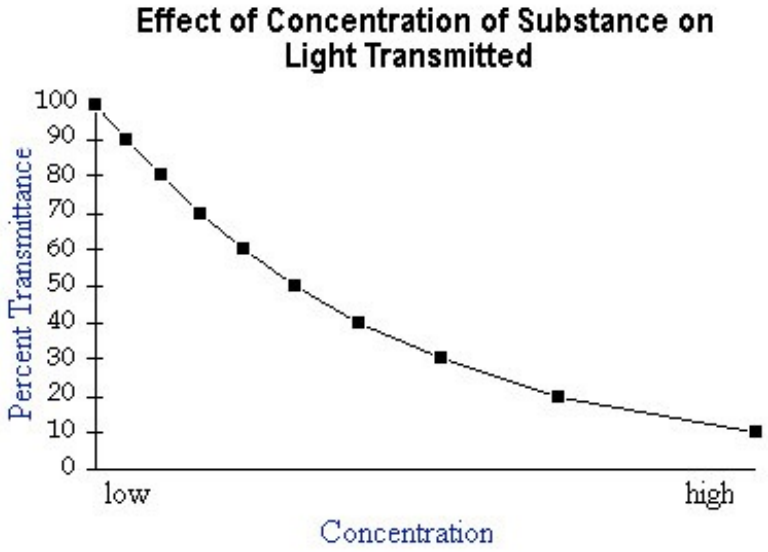


OK. As you can see, the spectrophotometer measures how much light is ***transmitted*** through our sample (compared to how much light entered the sample). Transmittance is simply the percentage of incident light reaching the photocell:

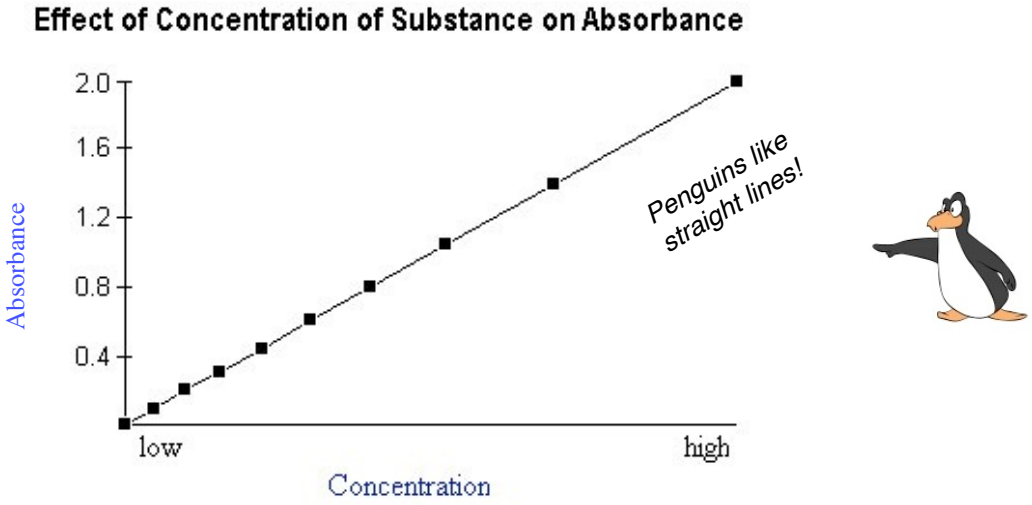
$$\%T = 100 \times (I / I_o)$$

where I is the light reaching the photocell and I_o *is the light striking the cuvette.*

Wait a second… here's a graph of **transmittance** as a function of concentration:



As you can see, the relationship is exponential, which is awkward to work with. The good news is that since transmittance decreases *exponentially* with concentration, *log 1/T increases linearly with increasing concentration*! That's great because straight lines are a lot more convenient than curved ones! Here's a graph of **absorbance** as a function of concentration:



The best news of all is that YOU don't have to do the math - the spectrophotometer does it for you! All YOU have to do is tell the spectrophotometer that you want to know absorbance (**ABS**) not %T. But just so you know… A = - log %T

The relationship between absorbance and concentration is known as the Beer-Lambert law: $A = \varepsilon l C$, where ε is the extinction coefficient (a property of the light-absorbing substance), l is the light path in cm and C is the concentration of the light-absorbing substance. Most cuvettes have a light path of 1 cm. Just FYI.