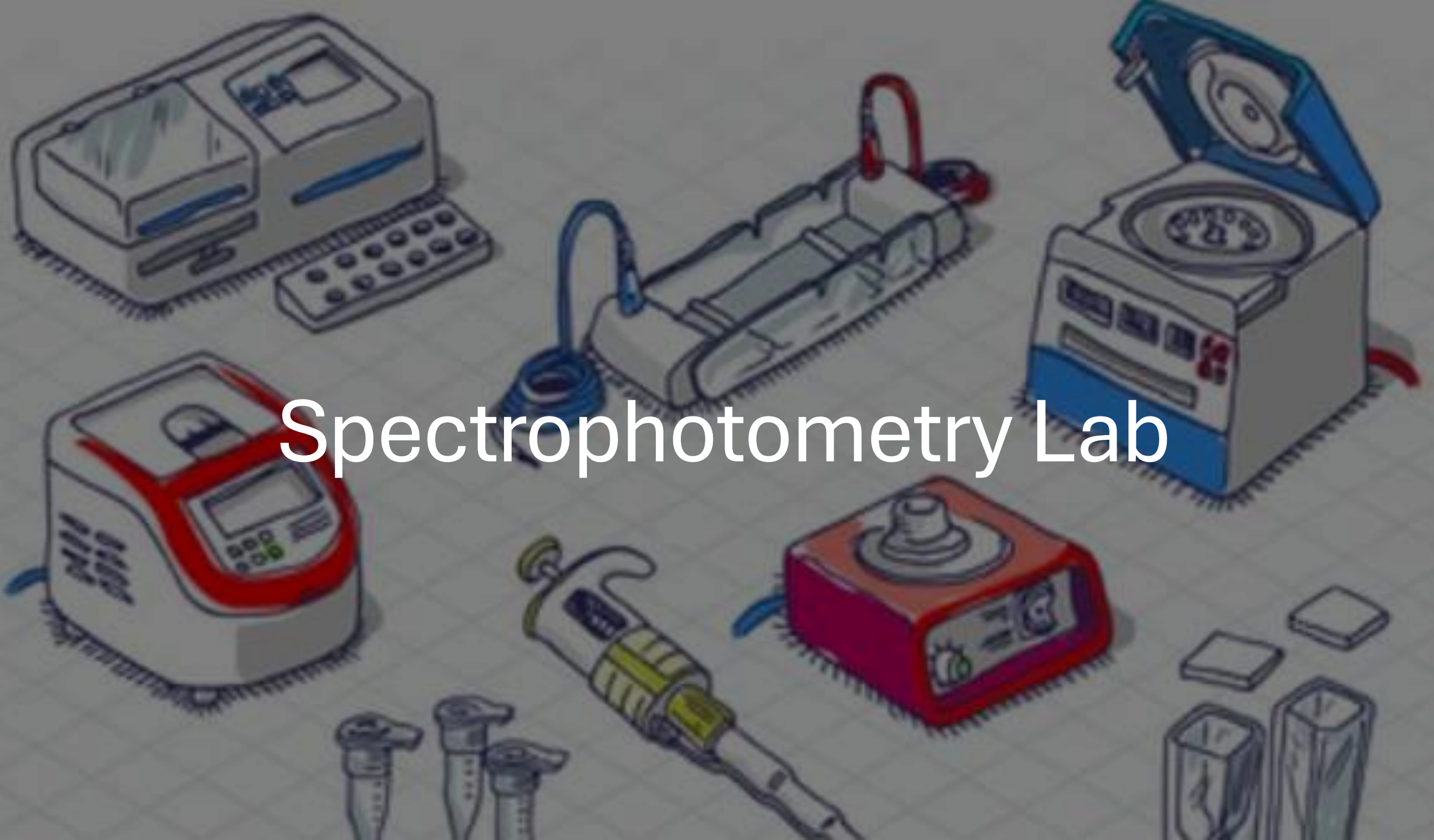


Spectrophotometry Lab



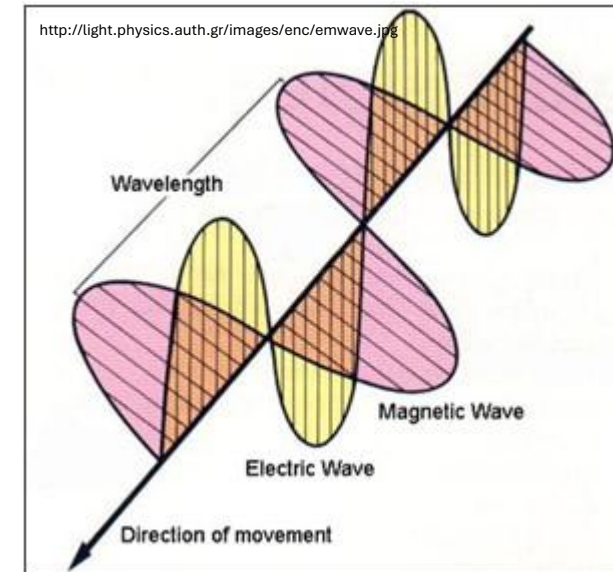
Background

- Spectrophotometry: A technique that uses **light to measure the concentration** of chemical substances. It is **based on the absorption** of radiation by a solution. It is one of the most commonly used methods for qualitative and quantitative analysis.
 - **Every** chemical **compound absorbs or reflects light** within a **specific wavelength range**. Spectrophotometry is the **measurement of the amount of light absorbed or reflected by a chemical substance**.
 - A spectrophotometer is an instrument that measures the number of photons (the intensity of light) absorbed after passing through the sample solution



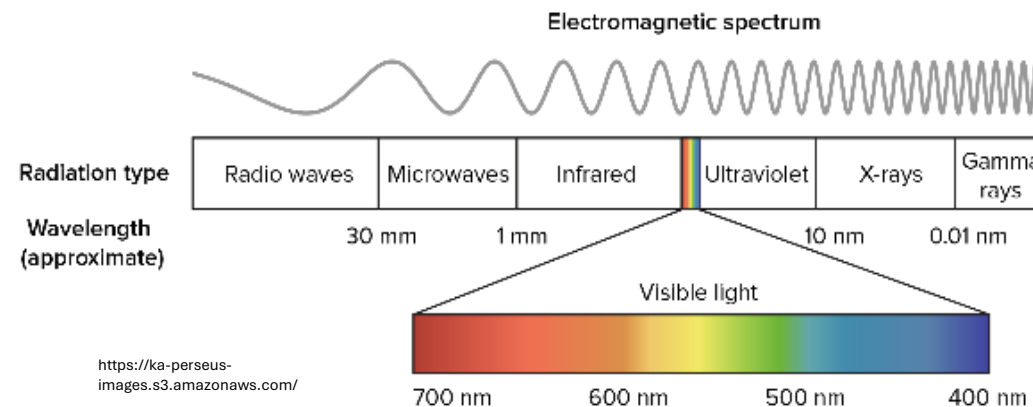
Background

- Light is a form of **electromagnetic** radiation, a type of energy that **travels in waves**.
- Light waves consist of **electric and magnetic fields** that are vertical to each other.
- The unit of measurement for electromagnetic radiation is the **wavelength** (λ), which represents the distance between the peaks of two consecutive waves and is measured **in nm**.



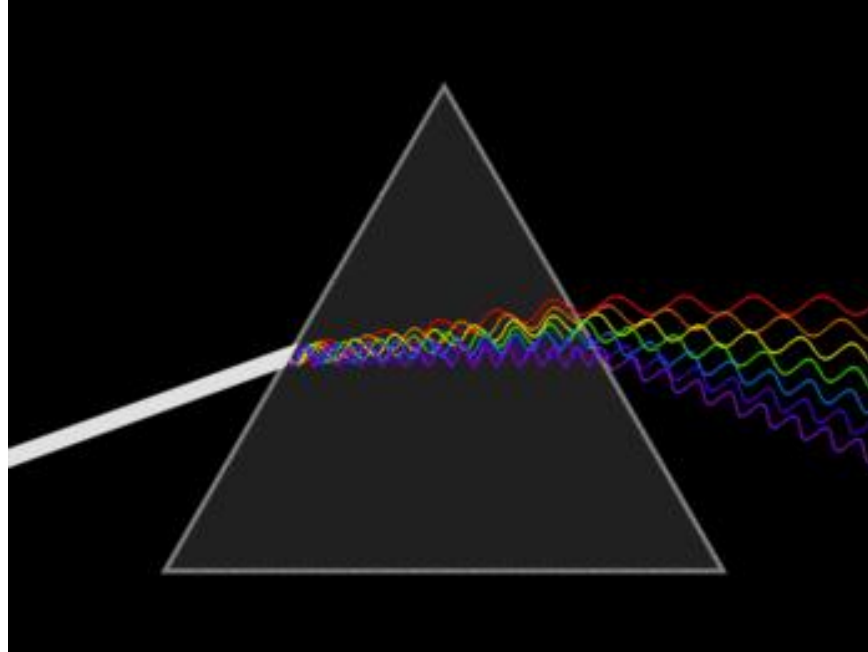
Background

- Each type of radiation consists of a characteristic range of different wavelengths.
- **Visible** light constitutes a small portion of electromagnetic radiation and is the only region of the electromagnetic spectrum that the human eye can see.
 - It consists of wavelengths between **400 and 700 nm**.
 - Red light has the longest wavelength and the least energy, while violet light has the shortest wavelength and the most energy.



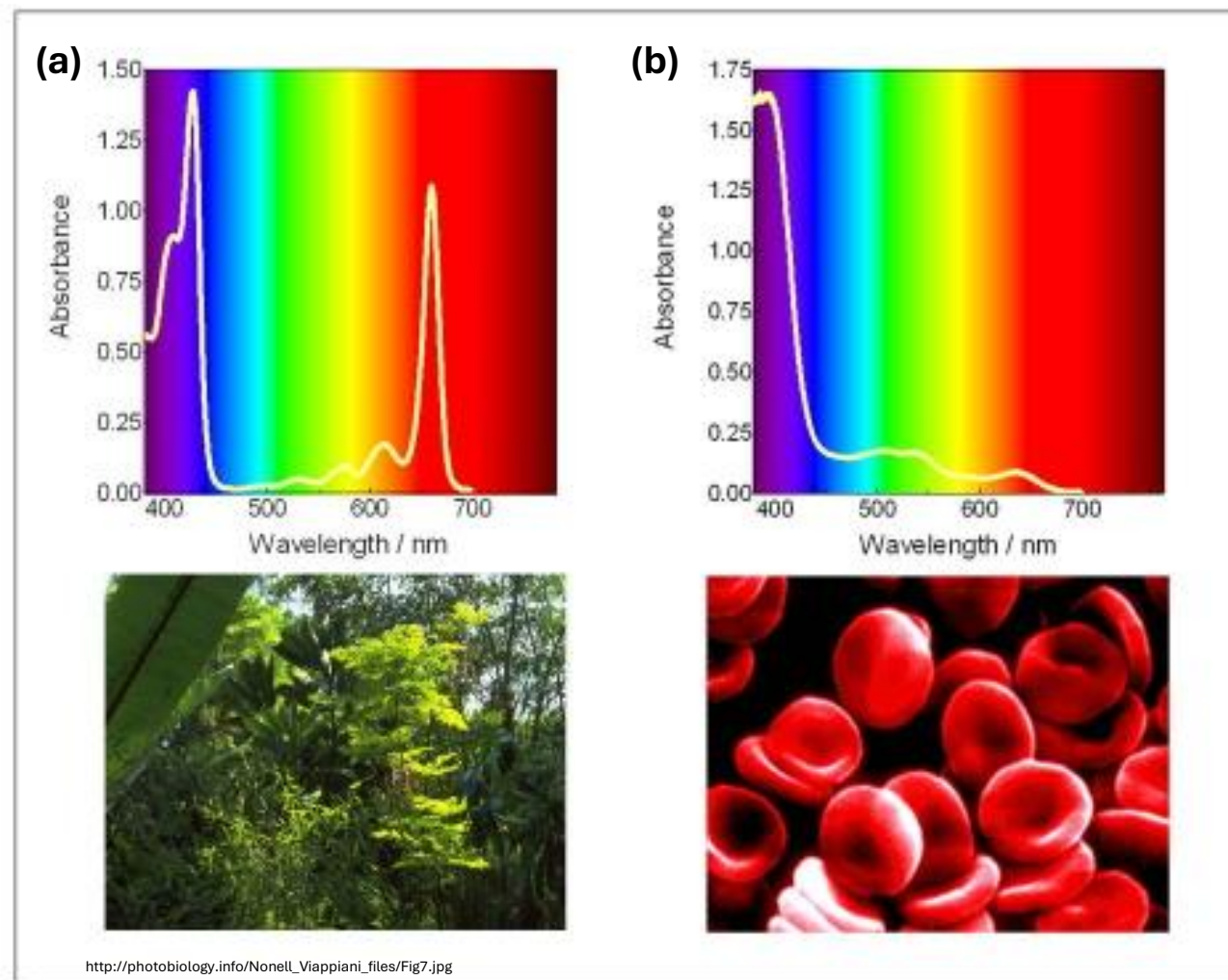
Background

- Visible light from the sun appears white. Its composition of different wavelengths (colors) is **revealed when it passes through a prism**: because the different wavelengths of light are bent at different angles (refraction), they spread out and form what we see as a rainbow.
 - This property allows us to distinguish and **isolate specific λ**



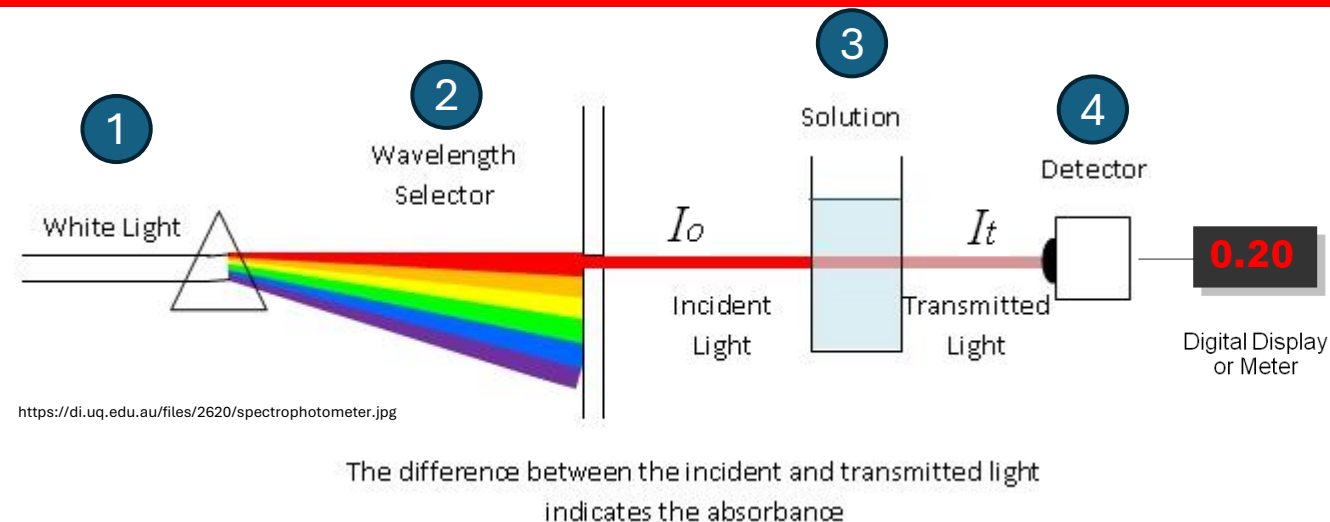
Background

- Pigments = molecules that **absorb** only **specific wavelengths** of visible light and **reflect the rest**
 - Range of wavelengths absorbed = absorption spectrum.
 - Wavelengths that are not absorbed, along with the reflected light = what we perceive as color. (e.g., plants appear green to us because they contain many chlorophyll a and b molecules, which reflect green light).



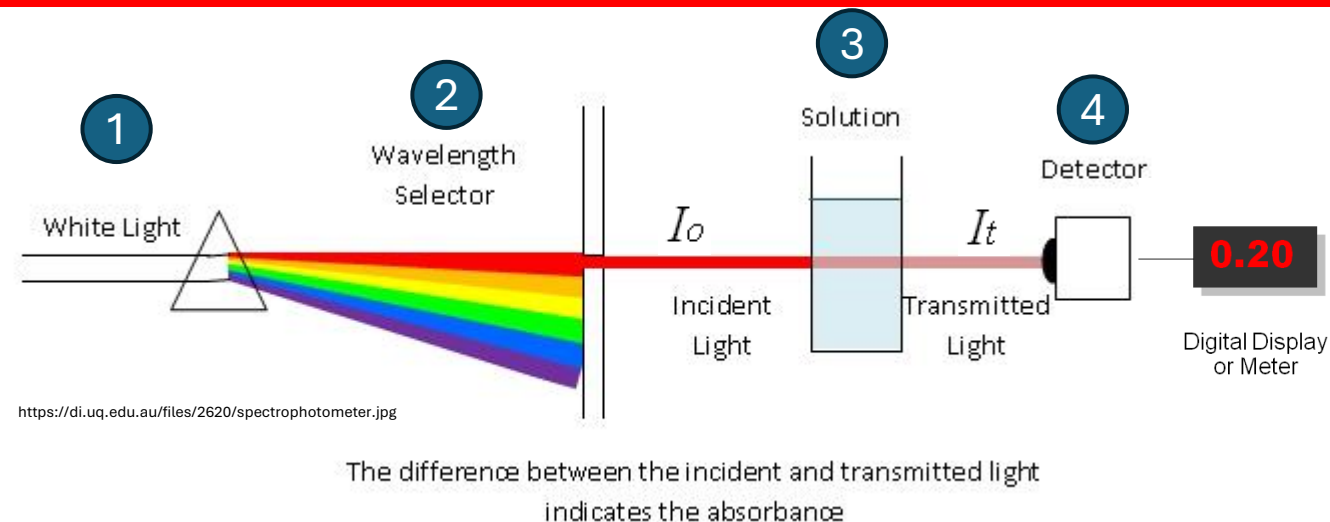
Absorption spectrum of (a) chlorophyll and (b) Fe(II) protoporphyrin IX, the prosthetic group of heme. The sharp absorption of chlorophyll in the red part of the spectrum makes grass green, and its lack in heme makes blood red.

Principle of Operation of a Chemical Laboratory Spectrophotometer



- The first component of the spectrophotometer consists of the **radiation source**. In visible-spectrum spectrophotometers, the source is a standard incandescent lamp (white light).
- The second section is the one that **separates the desired wavelength from the others** produced by the source. The separation system is a standard prism or, more commonly, a diffraction grating that isolates the desired monochromatic radiation.

Principle of Operation of a Chemical Laboratory Spectrophotometer

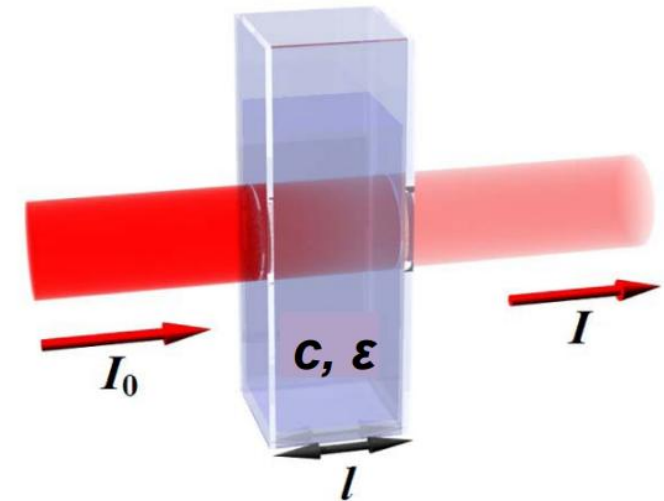


- The third component is where the **sample** to be measured is **placed**. The radiation passes through a cuvette—that is, a transparent, colorless container holding the sample to be analyzed.
- The last component is the **radiation meter**. The amount of light that passes through (is not absorbed by) the solution is measured by a photodetector (light detector) located on the opposite side of the cell.

Calculating the concentration of a substance, Lambert-Beer's Law

- Experimental measurements with spectrophotometers are usually expressed in terms of **absorption** (A), which is defined as: $A = \log(I_0 / I)$
 - I_0 = the **intensity of the radiation** incident **on the solution**
 - I = the **intensity of the radiation** emerging **from the solution**

Absorption has no units (it is dimensionless)



Calculating the concentration of a substance, Lambert-Beer's Law

- Beer-Lambert Law: The **absorption** A (for a monochromatic beam of radiation) of a solution of an absorbing substance is **proportional to its concentration**.
 - A more concentrated solution absorbs more light
- The mathematical expression for the law is: **$A = \epsilon \cdot C \cdot l$**
 - ϵ = **molecular absorption coefficient** (characteristic of each substance at a given wavelength)
 - c = the **concentration** of the substance in the solution, in **M**.
 - l = the **path length** of the radiation through the solution (i.e., the internal length of the cuvette in which the solution is contained)



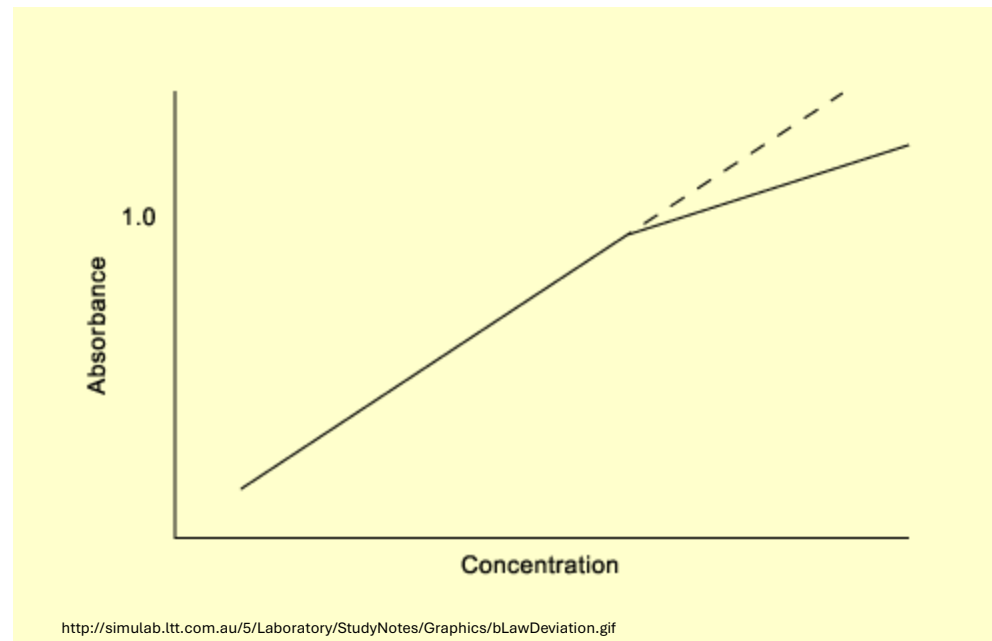
Aqueous solution of rhodamine (a fluorescent dye) is illuminated with a green laser pointer. Note how far the green light travels before it is fully absorbed (the length of the yellow-green line).



The same green laser illuminating an aqueous rhodamine solution of lower concentration. Note the laser light is visible to a greater depth than observed in the photo above, because the less concentrated solution absorbs the light less efficiently (i.e., the more dilute solution has lower c).

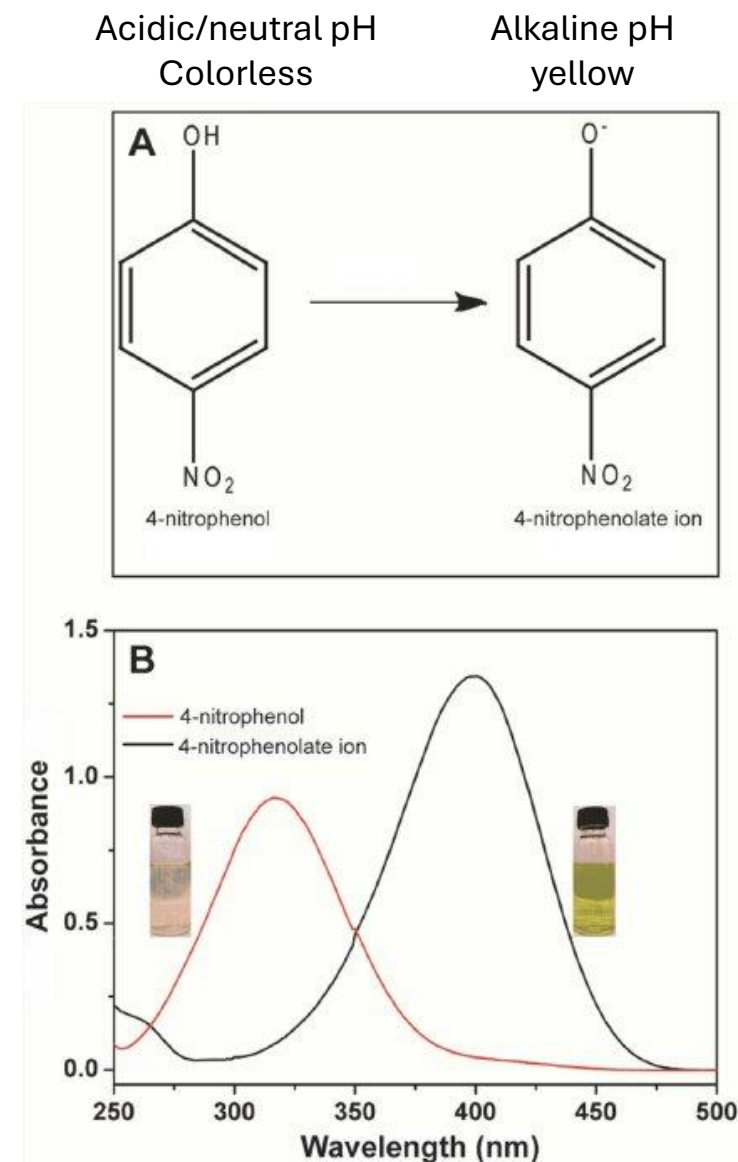
Calculating the concentration of a substance, Lambert-Beer's Law

- Beer's law loses its linearity at the high- and low-concentration ends of the relationship. Consequently, the **best results** (minimum total error) are achieved when the absorbance ranges **between 0.2 and 0.8**. For absorbances below 0.1 and above 1.0, significant errors are likely to occur.



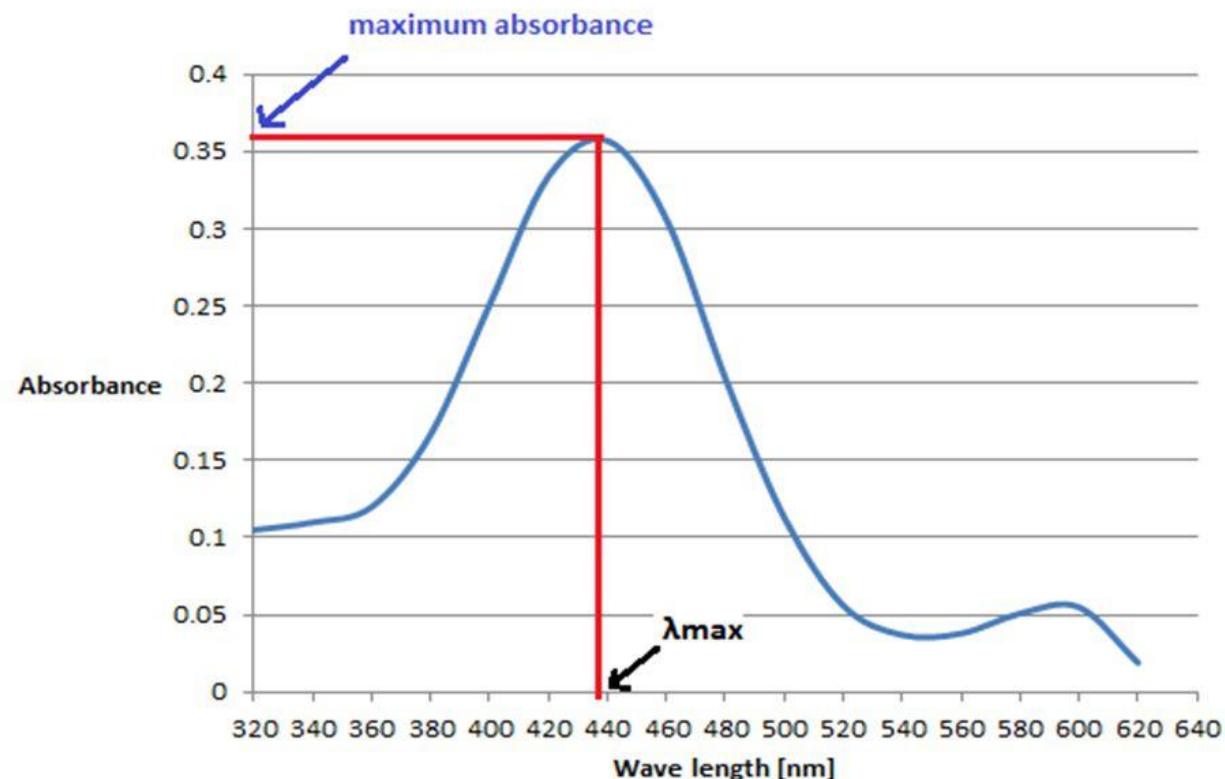
Lab: Determination of the Spectrum and Concentration of a p-Nitrophenol Solution

- In acidic/neutral pH, p-Nitrophenol is colorless → we cannot detect it.
- By changing the pH → p-Nitrophenol becomes yellow → we can detect it



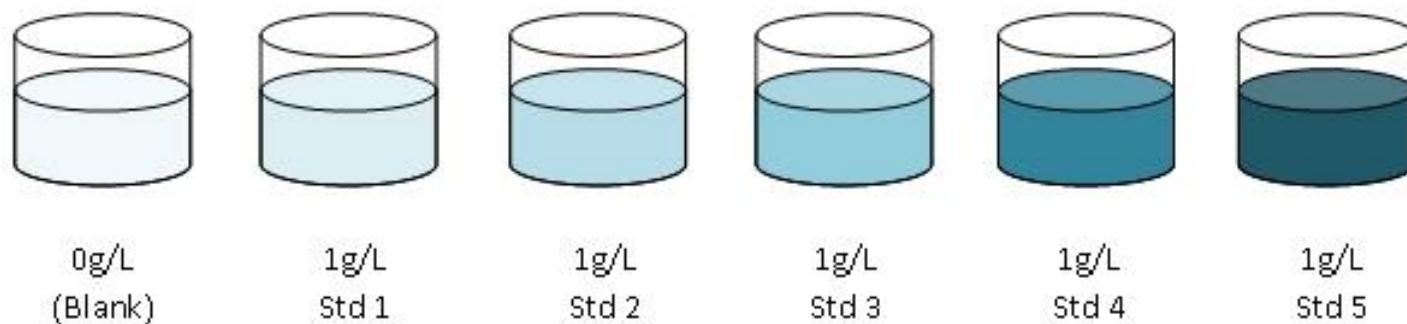
Lab: Determination of the Spectrum and Concentration of a p-Nitrophenol Solution

- The absorbance of a solution of known concentration of p-nitrophenol is measured across a range of wavelengths to determine λ_{max} .
- Knowing the **λ_{max}** of a substance is important for two reasons:
 - to achieve **maximum sensitivity**, since a given concentration will yield maximum absorption at that wavelength
 - to **minimize variations** in absorption due to small changes in wavelength during the measurements.



Lab: Determination of the Spectrum and Concentration of a p-Nitrophenol Solution

- After determining λ_{max} , this value is **selected** on the corresponding scale of the spectrophotometer.
- **Standard solutions** of the substance **at various concentrations** are prepared, and their optical densities are determined for the specific wavelength λ_{max} .



Lab: Determination of the Spectrum and Concentration of a p-Nitrophenol Solution

- A **curve** showing the change in **optical density** as a function of the **concentration** of the standard solutions (**standard curve**) is plotted. The curve is linear if the solution of the substance follows the Lambert-Beer equation.
- The **optical density** of the **unknown** solution of the substance is measured, and its unknown **concentration is calculated** using the standard curve.

