Technology Networks

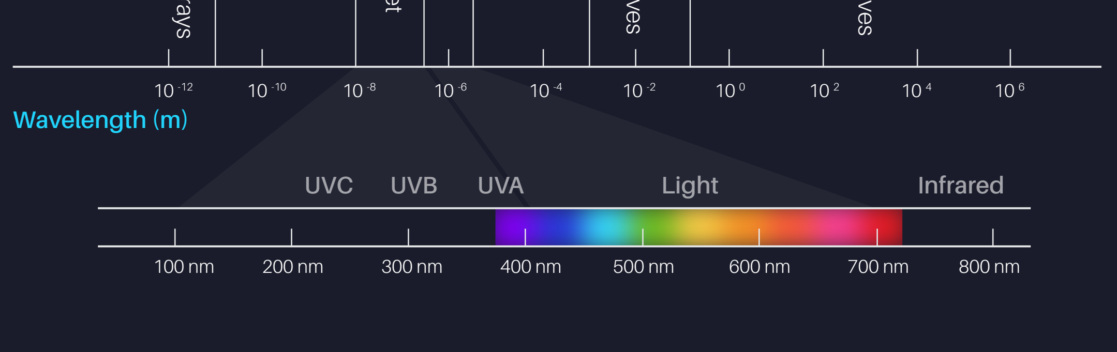
Ultraviolet-Visible Spectroscopy

Ultraviolet-visible spectroscopy (UV-Vis spectroscopy) is a cornerstone technique in many biological and chemical labs. Its ability to derive useful information from the fundamental physical characteristics of samples has earnt it a place in disciplines from nucleic acid quality control to drug identification.

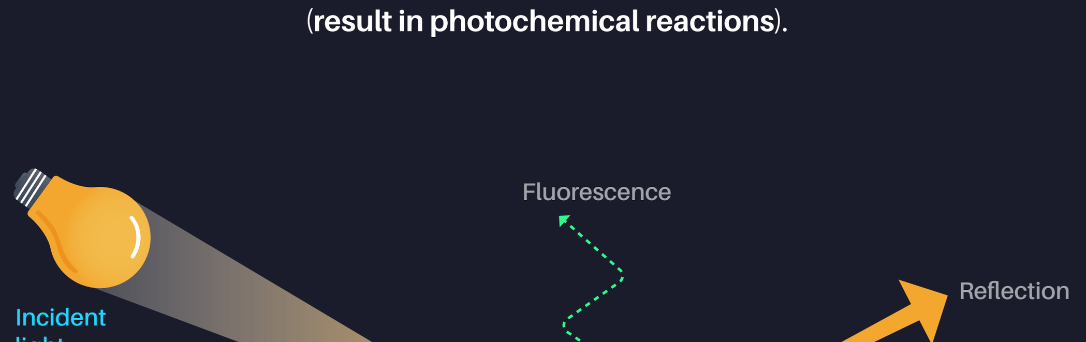
In this infographic, we will take a look at how UV-Vis spectroscopy works, what it can tell us, its strengths and limitations and the uses to which it is being applied.

How does UV-Vis spectroscopy work?

First commercialized in 1941, UV-Vis spectroscopy takes advantage of radiation within the UV and visible ranges of the electromagnetic spectrum, extending from wavelengths of around 100 to 800 nm.

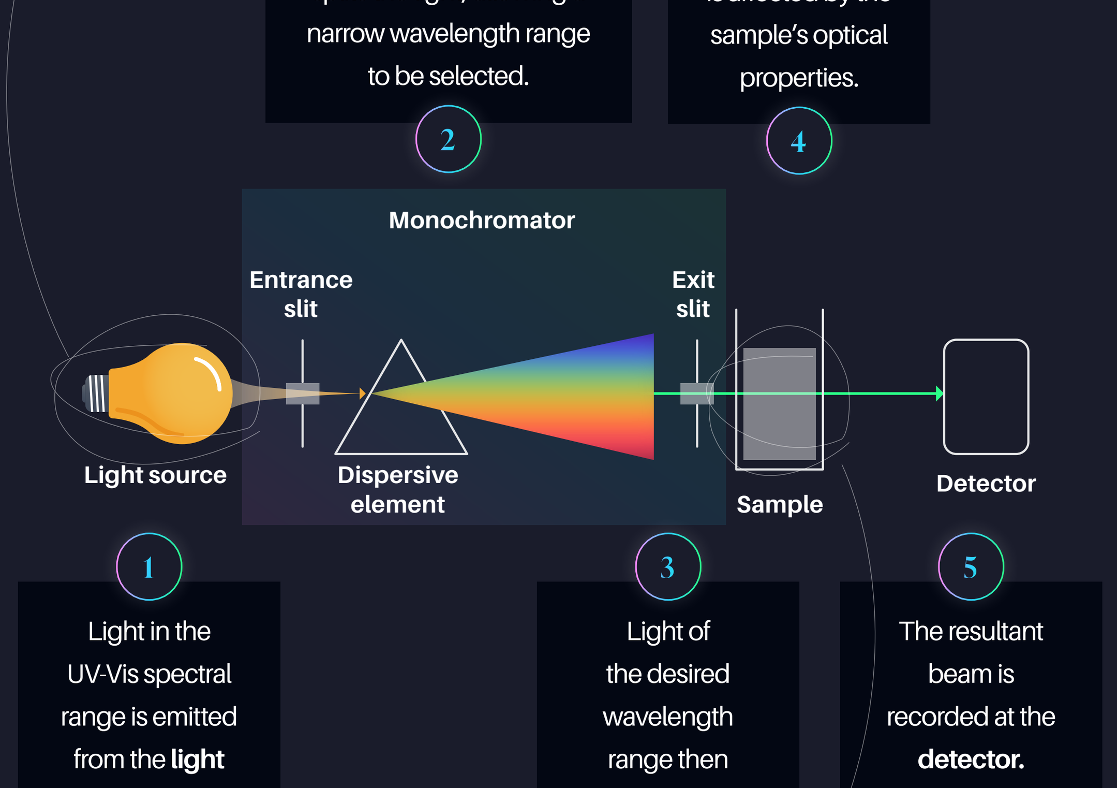


When the light waves meet matter, they may be **reflected, absorbed, transmitted**, **scattered**, absorbed and re-emitted (**fluoresced**) or absorbed and break bonds (**result in photochemical reactions**).



Typically, in UV-Vis spectroscopy, **absorbance** is measured.

An ideal light source would give a stable output of constant intensity over the whole UV and visible wavelength range. In the past, two bulbs were required — a deuterium arc lamp for the UV region and a tungsten-halogen lamp for the visible region— but now the whole range can be covered using a xenon flash lamp.

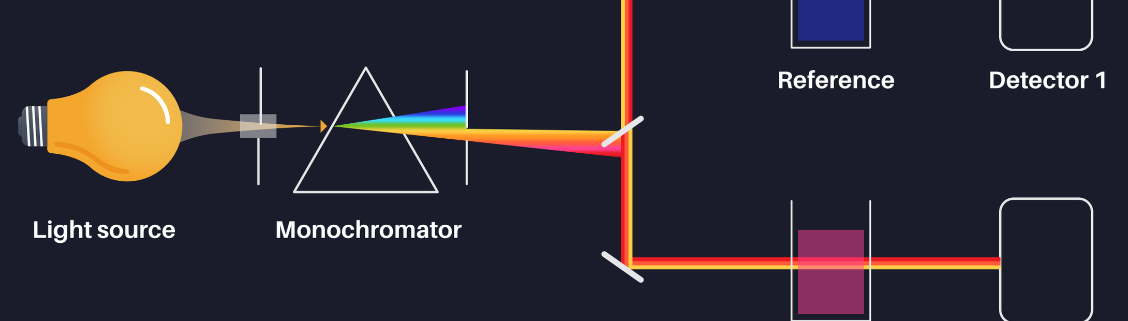


It is important to choose the right instrument and cuvette type for your sample. For example, most plastic cuvettes are not suitable for UV absorption studies as they absorb UV light.

Sample cuvettes with a pathlength of 1 cm are most common, although others with shorter path lengths are available for small sample volumes. For really small sample volumes, such as when testing DNA samples, instruments are available that don't require a cuvette and use only a microliter of sample. Specialist instruments also enable air and gas analysis.

In **single beam spectrophotometer** instruments like the setup shown above, a "blank" reference sample is first used to calibrate the instrument. In others, the light exiting the monochromator is split and simultaneously passed through reference and test samples, the signals from which can then be compared and results computed.

These are called **double beam spectrophotometers** and offer greater accuracy as the reference and sample are measured at the same time, compensating for any light or instrument fluctuations that may occur over time.



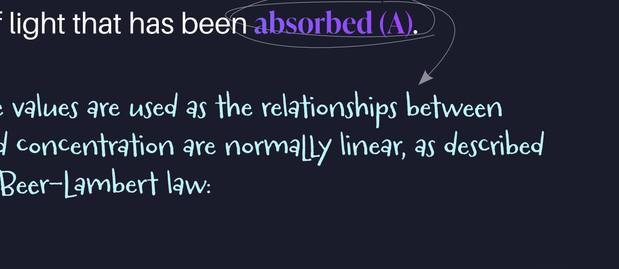
Transmittance is normally expressed as a percentage or a fraction of 1

The light that is **transmitted (T)** through the sample and **arrives at the detector (I)** can then be compared to the **light that was incident on the sample (I_0)**, the difference of which is the amount of light that has been **absorbed (A)**.

For most applications, absorbance values are used as the relationships between absorbance and both path length and concentration are normally linear, as described by the Beer-Lambert law:

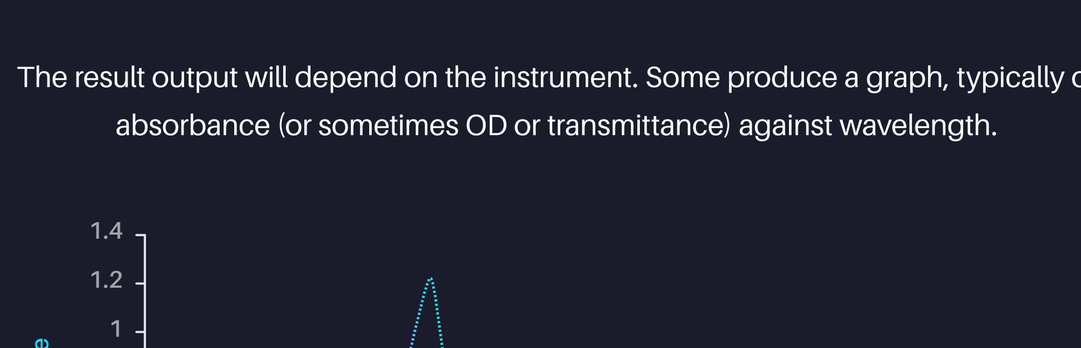
$$A = \log_{10} (1/T) = \log_{10} (I_0/I)$$

These values can be compared to the reference measurements to calculate the sample's absorptive properties and thus determine information such as the purity of a DNA sample or concentration of a drug compound.



Absorbance is sometimes referred to interchangeably as the optical density (OD). However, OD also includes light lost due to scattering as well as absorbance and hence is normally associated with samples containing suspensions such as cell and bacterial cultures. While UV-Vis spectroscopy is routinely used to measure the turbidity of bacterial cultures, most instruments are not optimized for light scattering measurements and may overestimate the amount of absorbance. This can also lead to variations were the same sample to be measured on different instruments and can be problematic for comparison of absolute values.

The result output will depend on the instrument. Some produce a graph, typically of absorbance (or sometimes OD or transmittance) against wavelength.

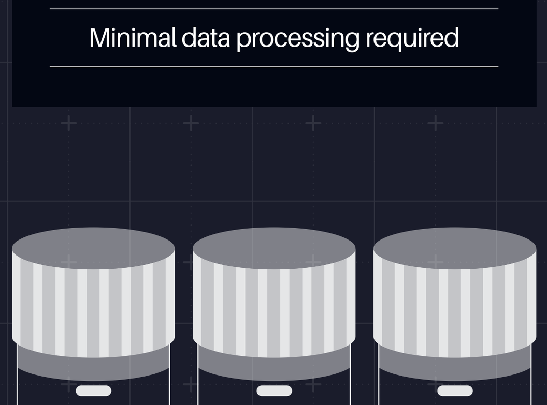


Others may just provide a value, such as the OD measured at a given wavelength, common when measuring bacterial culture growth.

What are the strengths and limitations of UV-Vis spectroscopy?

Strengths

- Measurements can be made quickly
- Accurate
- Provides qualitative and quantitative analysis
- Non-destructive
- Easy-to-use
- Inexpensive
- Minimal data processing required



Limitations

- Samples must absorb in the UV-Vis spectral region
- In single beam instruments, variations in light intensity or system optical performance between the blank and test samples being read can lead to inaccuracies
- Samples prone to photobleaching may be damaged during measurement
- Samples containing suspended solids or bubbles can cause scattering, resulting in inaccurate and potentially non-reproducible results
- Stray light in the instrument may cause measurement inaccuracies
- Complex samples containing multiple absorbing species can pose an analytical challenge, especially if the spectra overlap
- Absorptive properties can be affected by temperature, pH, contaminants and impurities
- Most instruments designed for homogenous liquid samples

What is UV-Vis spectroscopy used for?