Advanced lab techniques
Lecture 3

Spectrophotometry

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Purpose

Spectrophotometric is used to:

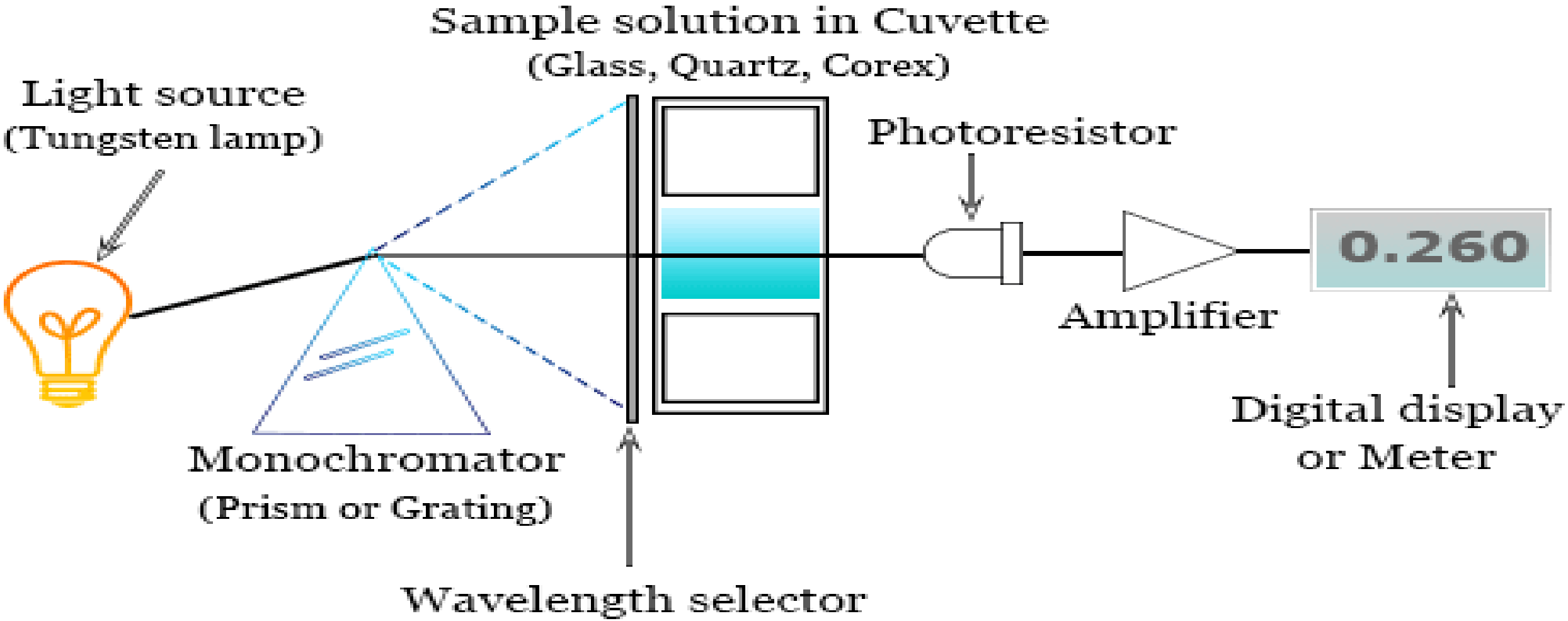
- Measure the concentration of the solution
- Identify organic compound by determining absorption maximum

Spectrophotometry

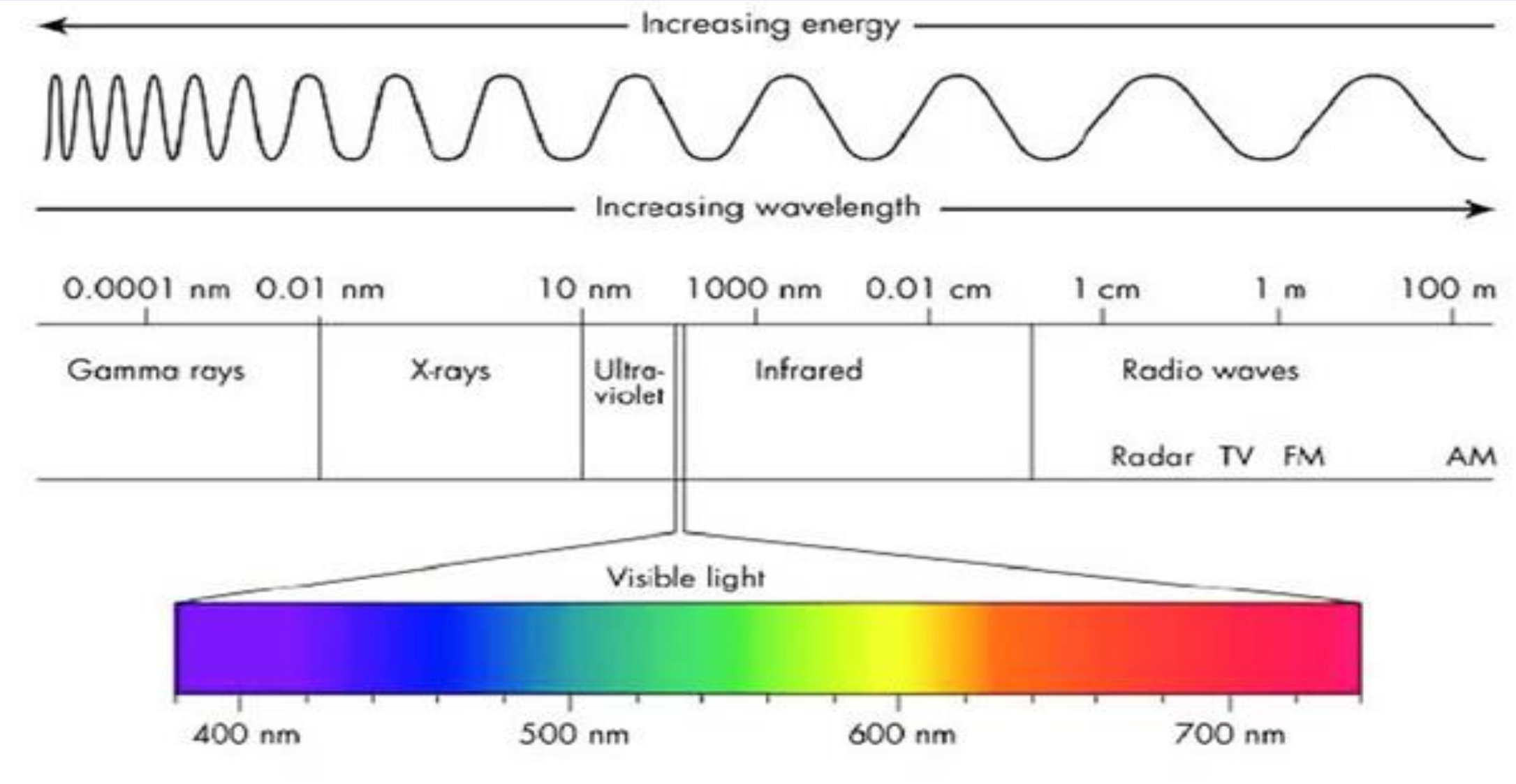
- **Photometry** measures light intensity without consideration of wavelength
- **Spectrophotometry** measures light intensity of a specific narrow range of wavelength
- Spectrophotometer techniques are mostly used to measured the concentration of solutes in solution by measuring the amount of the light that is absorbed by the solution in cuvette placed in the spectrophotometer

- Spectrophotometry is mainly concerned with the ultraviolet(200-400nm)and visible (400-800nm) regions.

Spectrophotometer

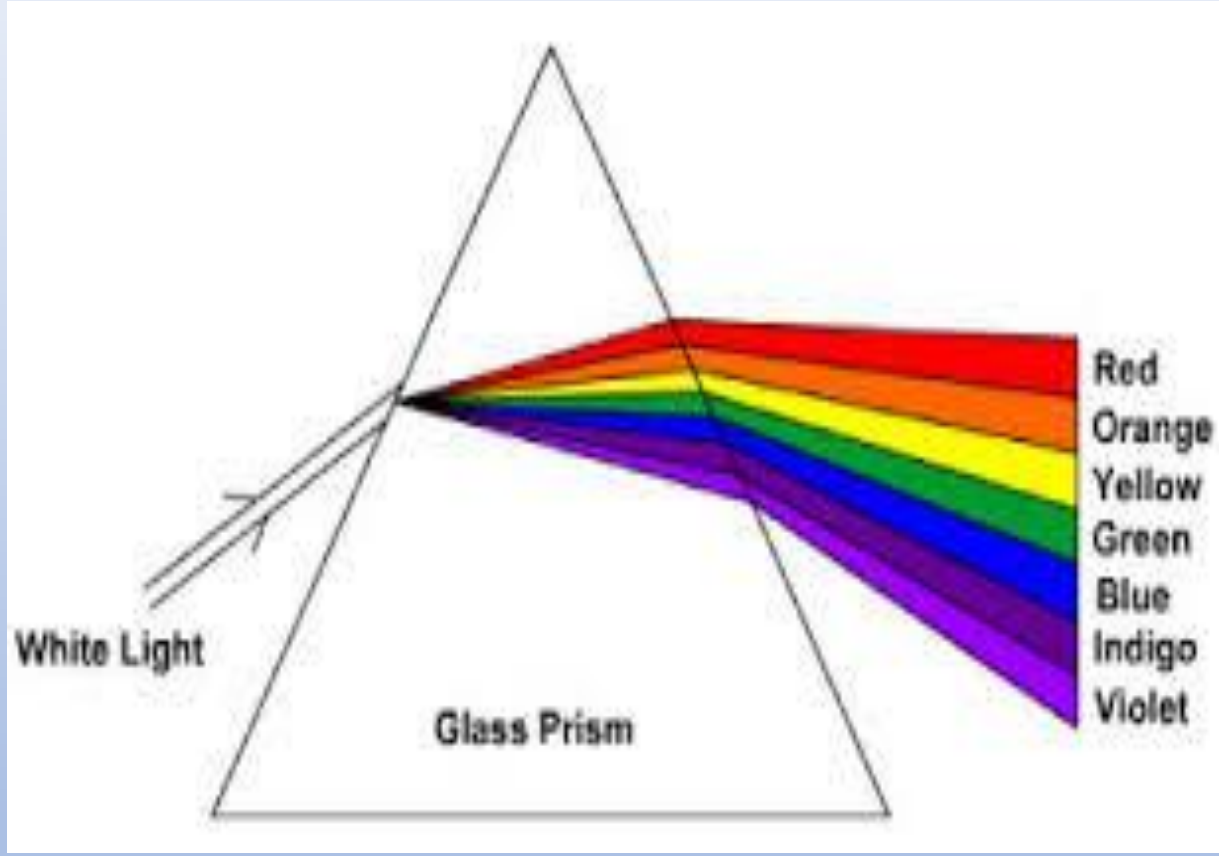


Regions of the electromagnetic spectrum



Light source and monochromators

- Lamps must give polychromatic light
- For visible regions: tungsten or tungsten –halogen lamp
- For UV & visible range: xenon lamp
- Can also use laser
- The monochromator helps select a specific wavelength (filters, prisms, fiber optics)



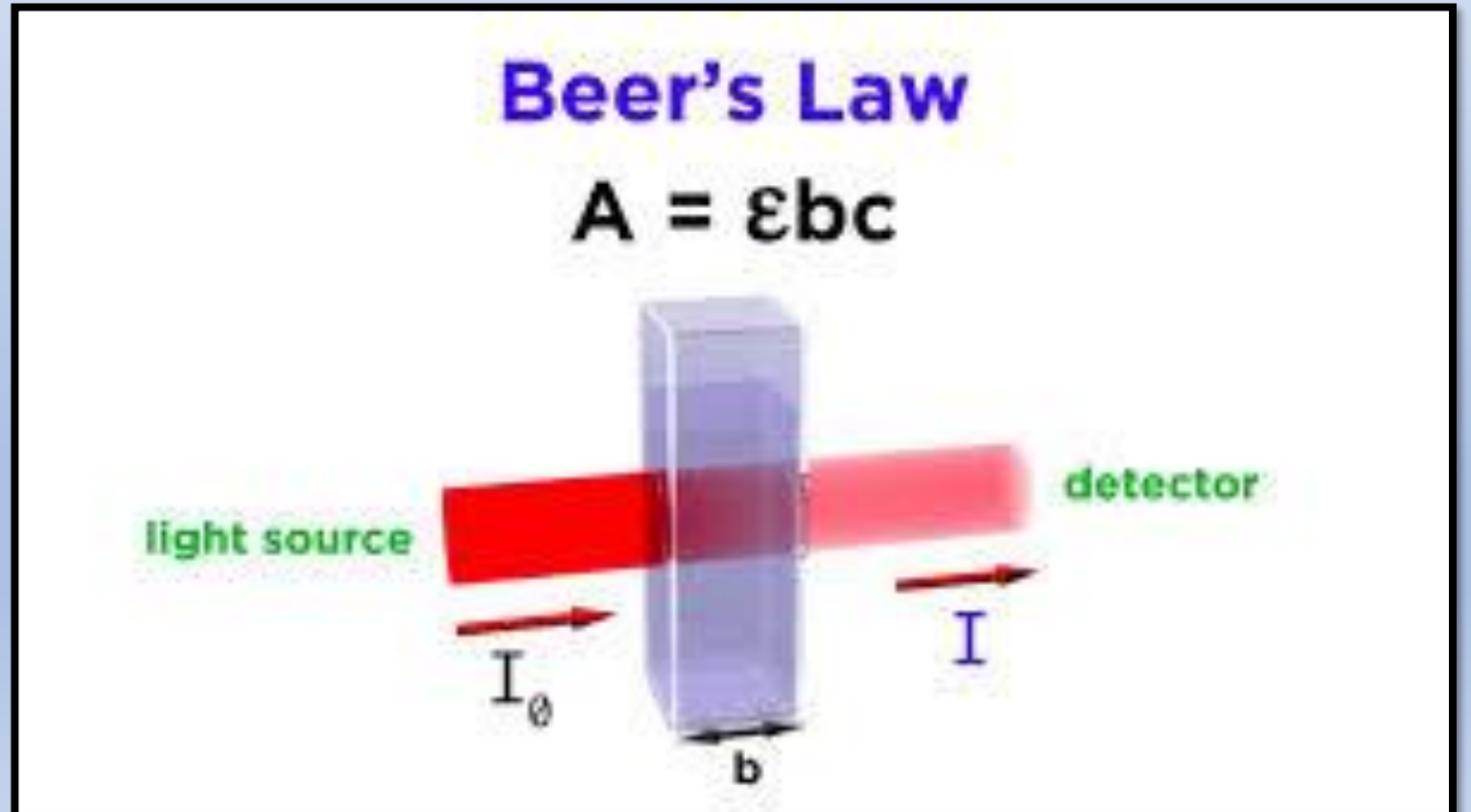
Lambert-Beer's Law

State that the concentration of a substance is directly proportional to the amount of light absorbed by that substance or inversely proportional to the logarithm of the transmitted light.

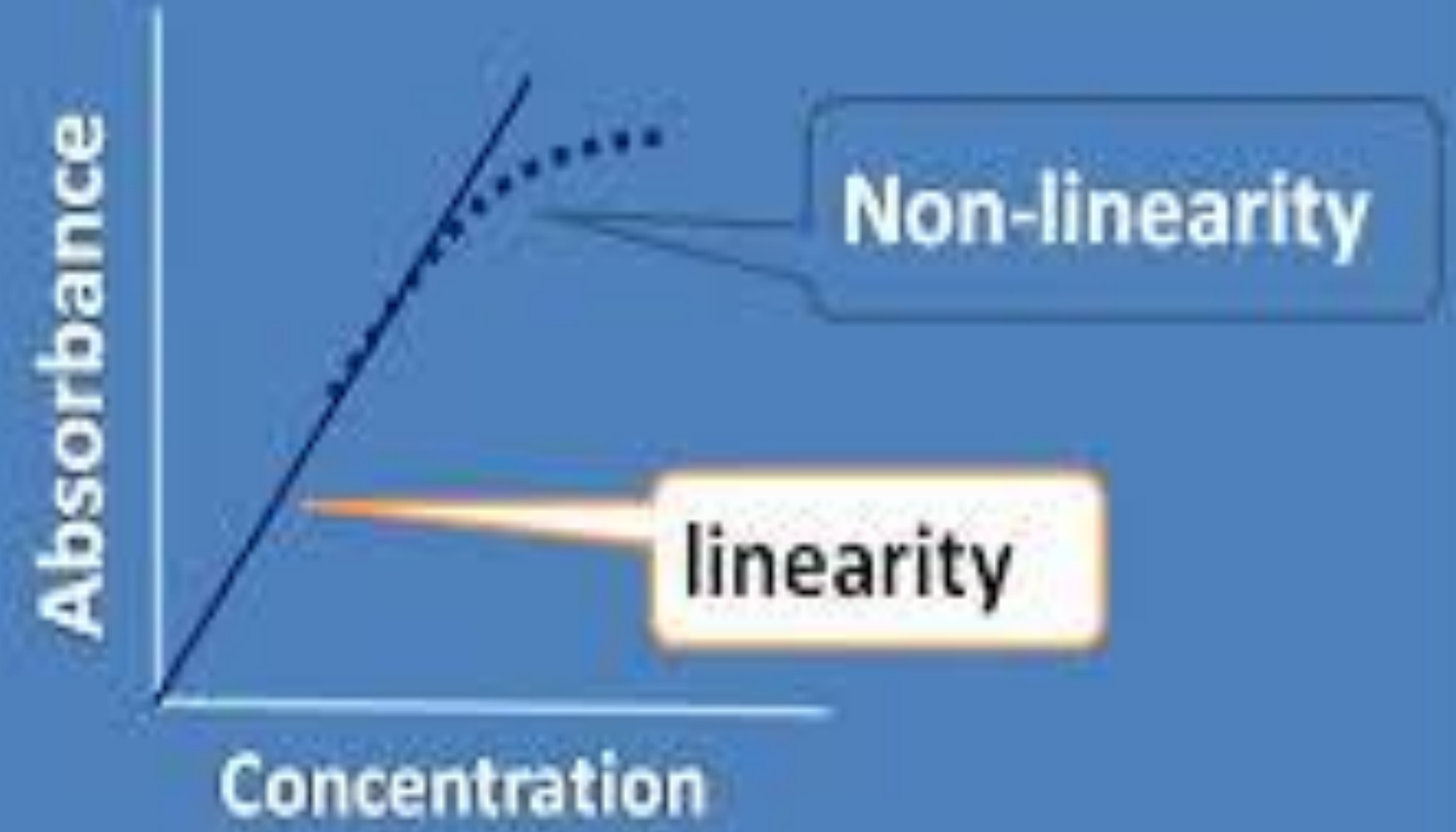
$A = -\log I / I_0$

$A = -\log T$

$A = 2 - \log \%T$

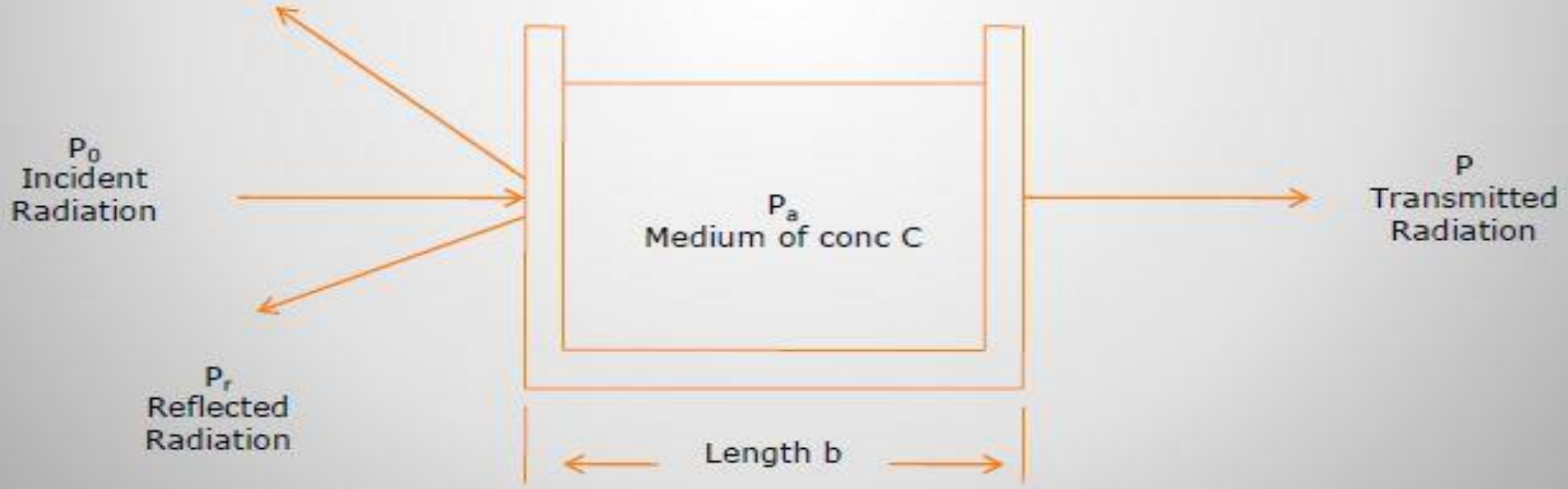


Deviations from Beer-Lambert Law?



Fundamental Laws of photometry

- When light is incident upon a homogeneous medium, a part of the radiant power of the incident light is reflected, a part is absorbed and the remainder is transmitted.

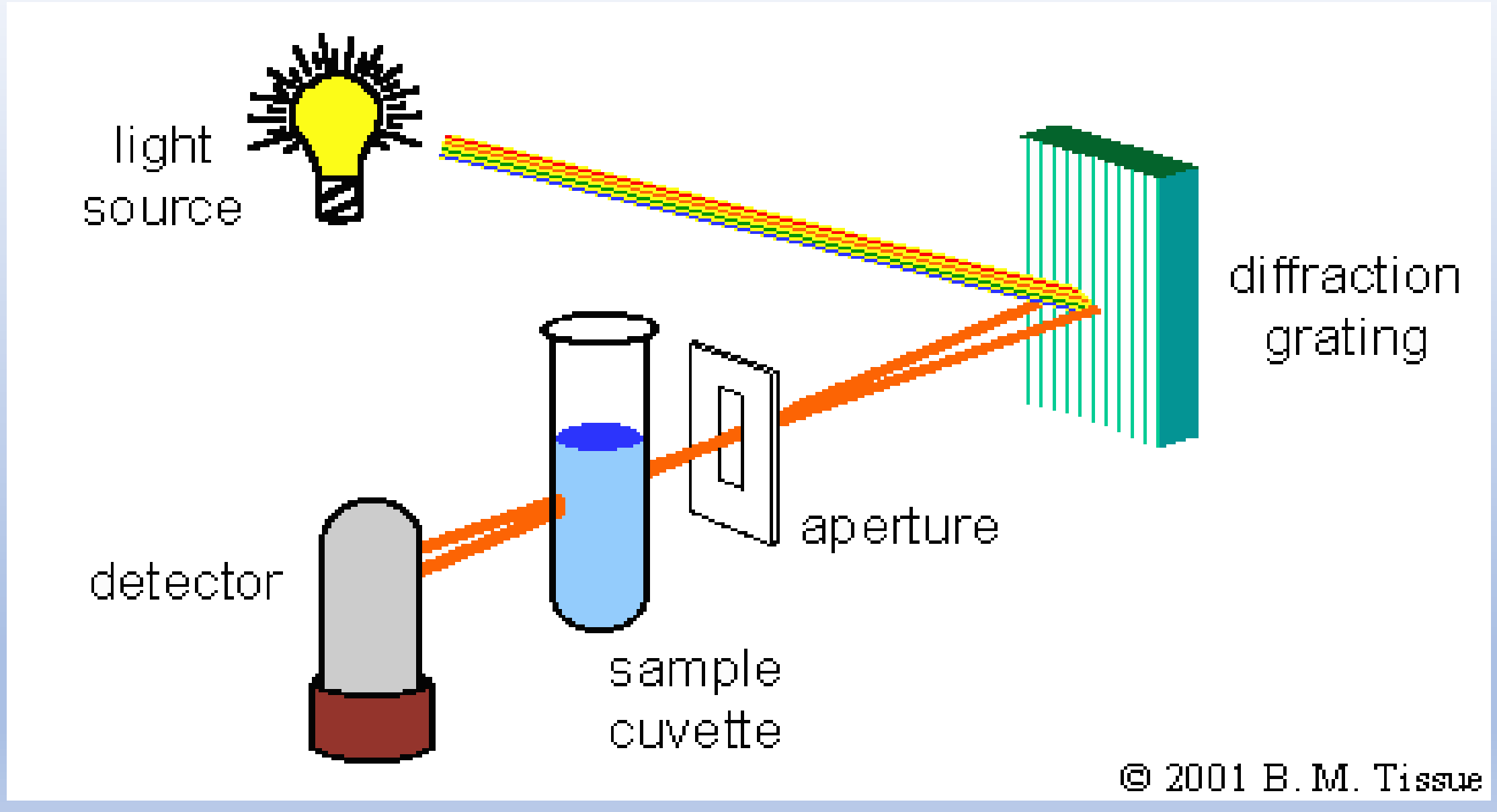


Single beam spectrophotometer

Single Beam spectrophotometers are often sufficient form making quantitative **absorption** measurements in the UV –Visible spectral region. The concentration of an analyte in solution can be determined by measuring the absorbance at a single wavelength and applying the **Beer-Lambert Law**.

Instrumentation

- Single-beam spectrophotometers can utilize a fixed wavelength light source or a continuous source.
- The simplest instruments use a single-wavelength light source, such as alight-emitting diode(LED),a sample container , and a photodiode detector.
- Instruments with a continuous source have a dispersing element and aperture or slit to select a single wavelength before the light passes through the sample cell.
- In either type of single-beam instrument ,the instrument is calibrated with a ***reference cell*** containing only solvent to determine the Po value necessary for an absorbance measurement.


light source
diffraction grating
aperture
detector
sample cuvette
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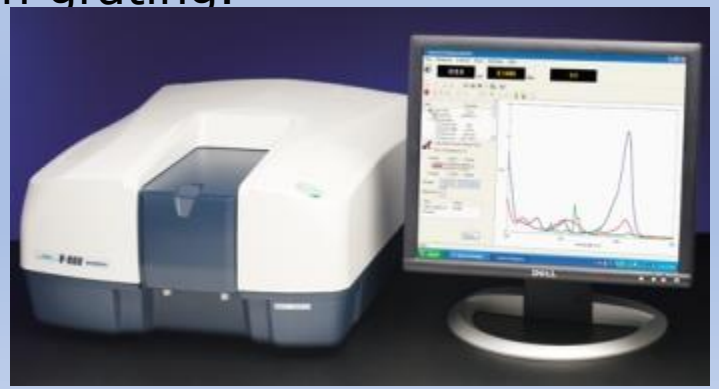
Double beam spectrophotometer

- ***The light source***

- You need a light source which gives the entire visible spectrum plus the near ultra-violet so that you are covering the range from about 200 nm to about 800 nm.

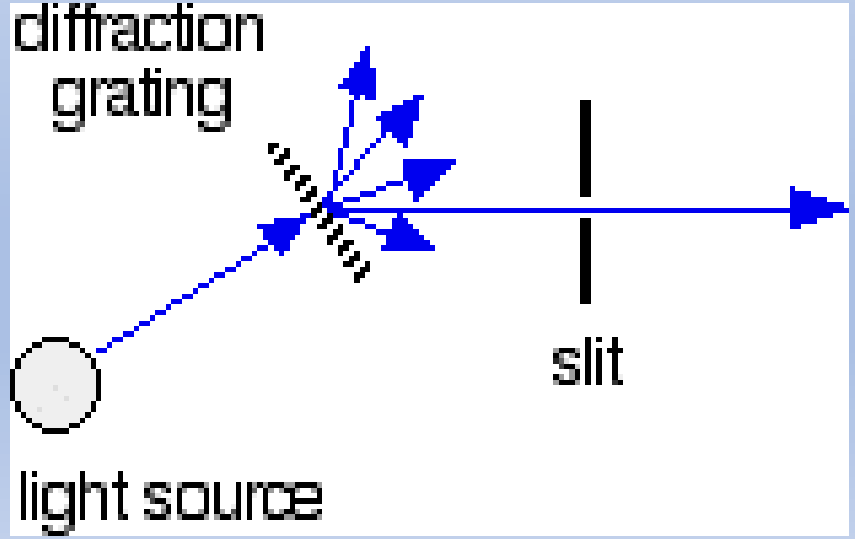
- You can’t get this range of wavelengths from a single lamp, and so a combination of two is used-a deuterium lamp for the UV part of the spectrum, and a tungsten/halogen lamp for the visible part.

- The combined output of these two bulbs is focused on to a diffraction grating.



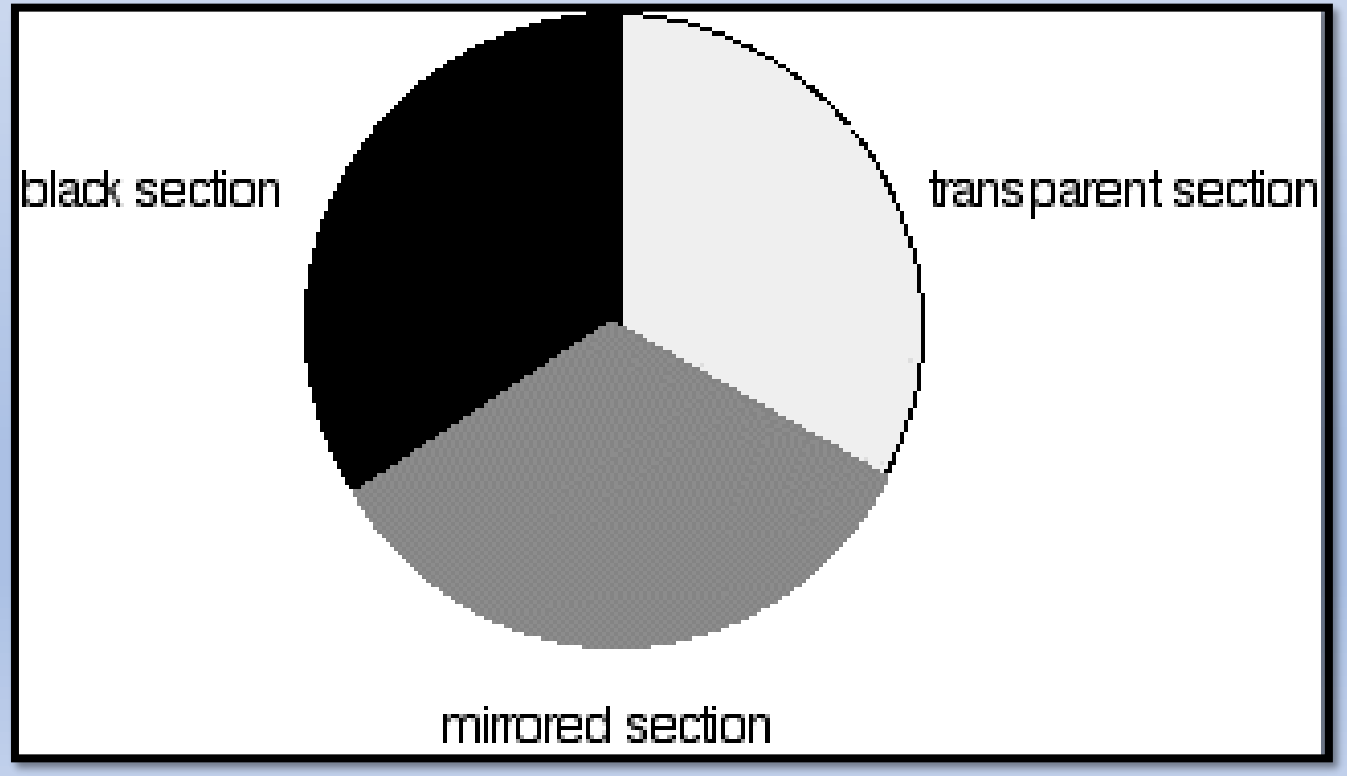
The diffraction grating and the slit

- You are probably familiar with the way that a prism splits light in to its component colors.
- A diffraction grating does the same job, but more efficiently.
- The blue arrows show the way the various wavelengths of the light are sent off in different directions.
- The slit only allows light of a very narrow range of wavelengths through into the rest of the spectrometer.
- By gradually rotating the diffraction grating, you can allow light from the whole spectrum (a tiny part of the range at a time) through into the rest of the instrument.



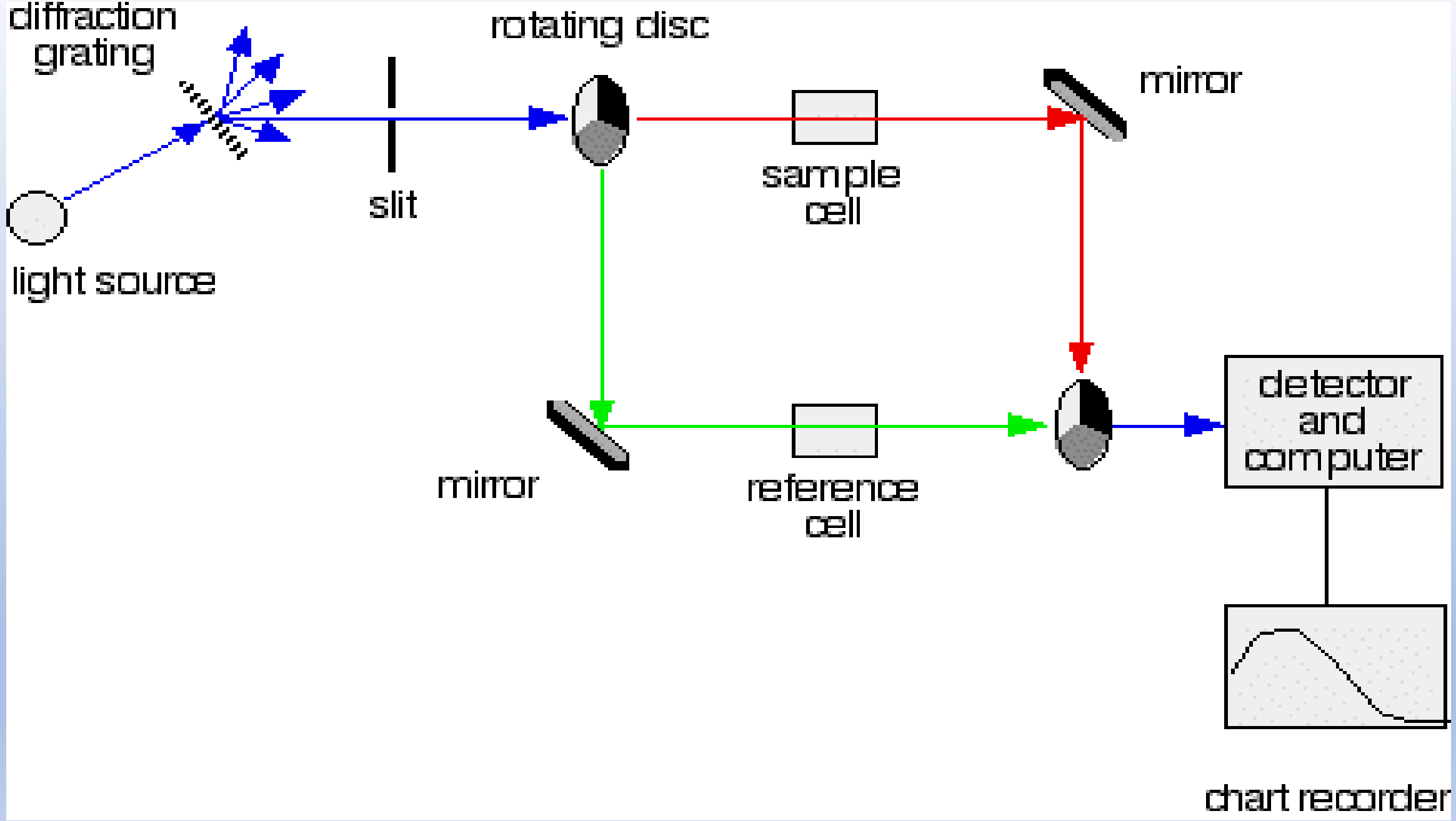
The rotating disks

- Each disk is made up of a number of different segments.
- Those in the machine we are describing have three different sections-other designs may have a different number.

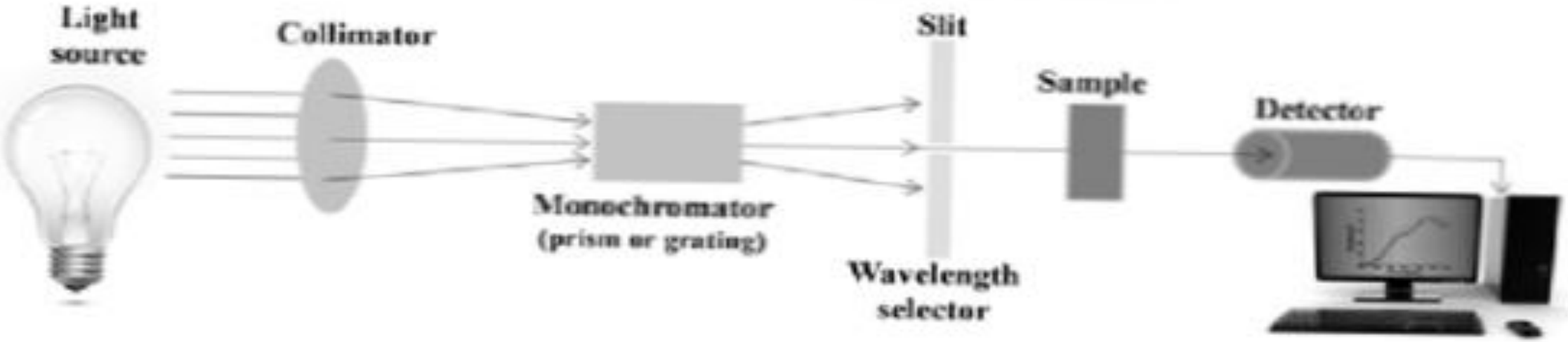


The sample and reference cells

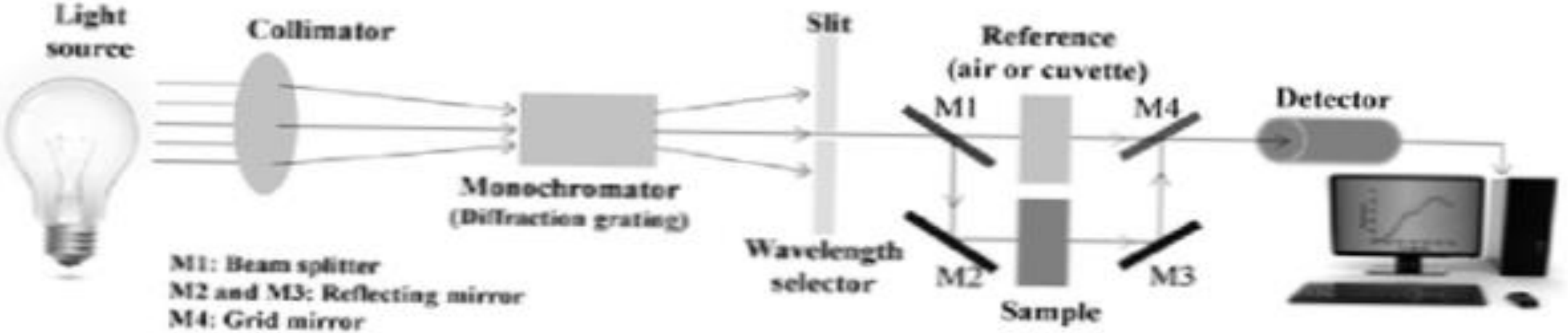
- These are small rectangular glass or quartz containers.
- They are often designed so that the light beam travels a distance of 1 cm through the contents
- The sample cell contains a solution of the substance you are testing usually very dilute.
- The solvent is chosen so that it doesn’t absorb any significant amount of light in the wavelength range we are interested in(200-800nm)
- The reference cell just contains the pure solvent.


diffraction grating
rotating disc
mirror
slit
sample cell
light source
detector and computer
mirror
reference cell
chart recorder

Single Beam Spectrophotometer



Double Beam Spectrophotometer



Applications

- ✓ The applications of UV/Vis Spectrometer are quite vast.
- ✓ Mainly it is used for qualitative and quantitative determinations such as enzyme assays, molecular weight determination.
- ✓ Ts routinely used in analytical chemistry for the quantitative determination of different analytes, such as metal ions, highly conjugated organic compounds, and biological macromolecules.
- ✓ Spectroscopic analysis is commonly carried out in solutions but solids and gases may also be studied.