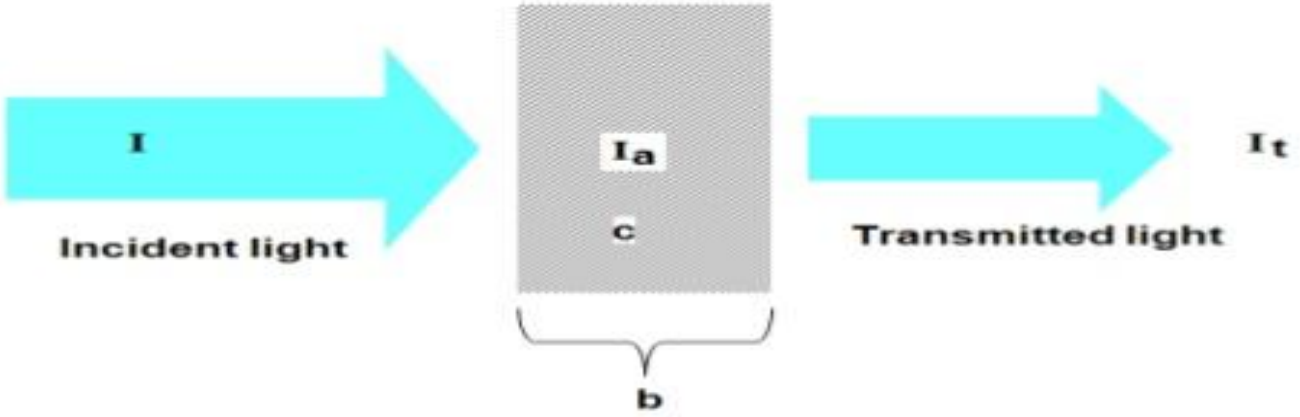
LAWS GOVERNING ABSORPTION OF RADIATION

- When a beam of light falls on a solution or homogeneous media, a portion of light is absorbed within the medium while the remaining is transmitted through the medium.
- Thus if I_0 is the intensity of radiation falling on the media, I_a is the amount of radiation absorbed and I_t as the amount of radiation transmitted then,

$$I_o = I_a + I_t$$



Where

I = Intensity of Incident light

I_a = Intensity of absorbed light

I_t = Intensity of transmitted light

c = molar concentration of sample

b = Length or thickness of the sample cell (cm)

Lambert's Law :

When a monochromatic light passes through an absorbing medium at right angles to the plane of surface of medium or solution, the rate of decrease in intensity with thickness of medium (b) is proportional to the intensity of incident light.

In other words the intensity of transmitted light decreases exponentially as the thickness of medium increases arithmetically.

$$-\frac{dI}{db} = KI \quad \ldots (1)$$

Where, I = Intensity of incident light of λ wavelength

b = Thickness of medium

K = Proportionality constant

Now integrating the equation, and putting $I = I_o$ when $b = 0$

$$I_n \frac{I_o}{I_t} = Kb \text{ or } I_t = I_o e^{-Kb} \quad \ldots (2)$$

Where, I_o = Intensity of incident light falling on thickness b

I_t = Intensity of transmitted light

K = Absorption coefficient for the given wavelength and medium

Converting from natural logarithms to base 10 logarithms

$$I_t = I_o \cdot 10^{-Kb} \quad \ldots (3)$$

Beer's Law:

Bernard and Beer independently stated that 'The intensity of incident light decreases exponentially as the concentration of absorbing medium increases arithmetically. This is similar to Lambert's law and thus,

$$I_t = I_o \cdot e^{-K'c} \quad \text{... (4)}$$

Where, K' = Proportionality constant

c = Concentration

By converting from natural logarithms to base 10

$$I_t = I_o \cdot 10^{-K'c} \quad \text{... (5)}$$

Now combining two laws, i.e. equations (3) and (5) we have

$$I_t = I_o . 10^{-\varepsilon cb} \quad \ldots (6)$$

Where, K and K' $= \varepsilon$

c = Concentration

b = Thickness of medium

Thus, $$\log \frac{I_o}{I_t} = \varepsilon c b \quad \ldots (7)$$

Where ε is the Molar extinction coefficient, a constant dependent upon the wavelength of incident radiation and the nature of absorbing material and the concentration is expressed in gram mole/litre

Since absorbance $A = \log I_o / I_t$

we can infer that

$A = \varepsilon bc$ (**Equation of beer – Lambert's law**)

Where:

A – Absorbance or optical density.

ε – Molar extinction coefficient

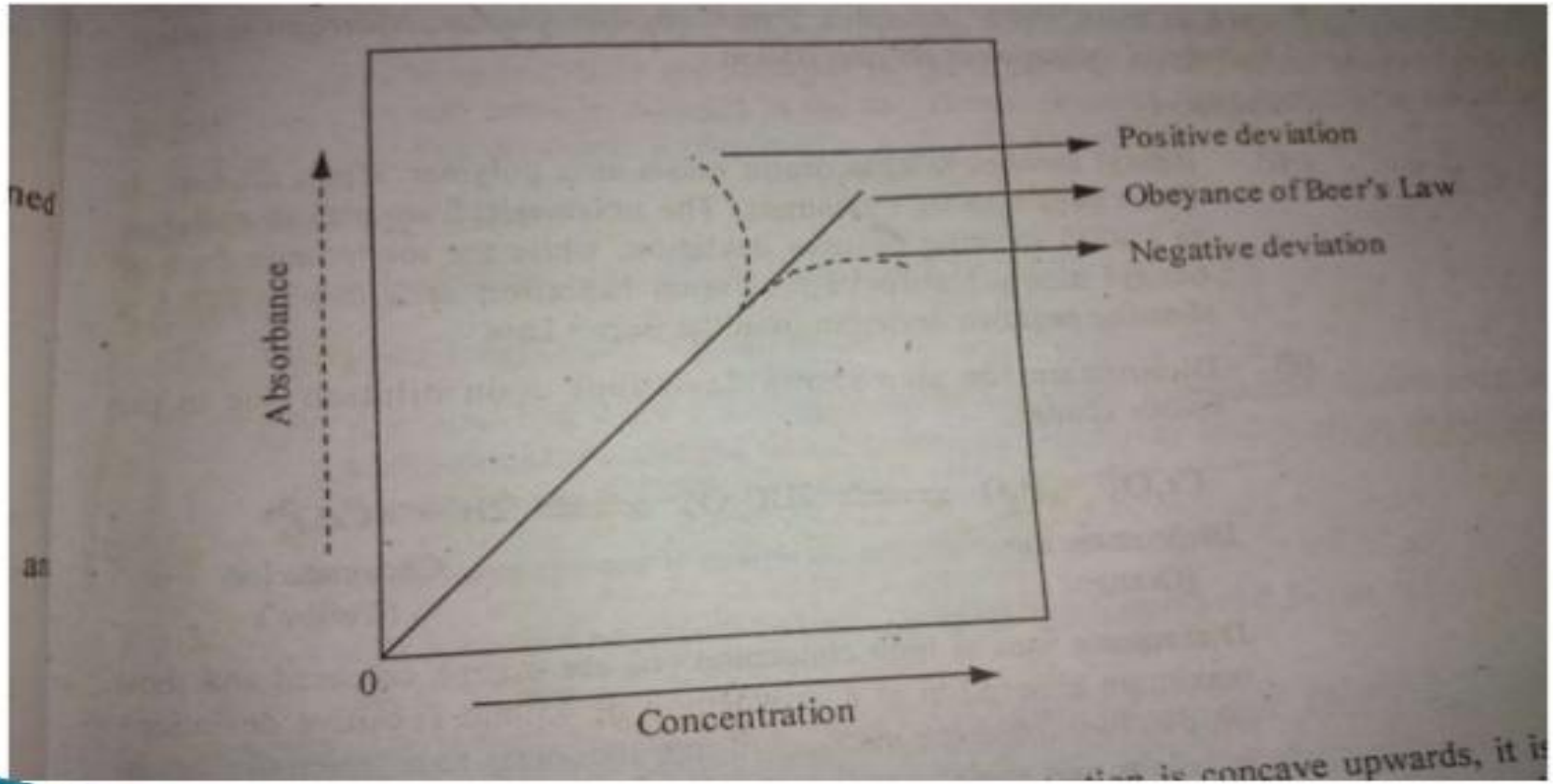
c – Concentration of the drug (mol/lit)

b – Path length (normally 10mm or 1cm)

Or $A = abc$

a –absorptivity, if concentration is expressed in grams/litre

DEVIATION FROM BEER'S LAW



REASONS FOR DEVATION FROM BEER LAMBERT'S LAW:

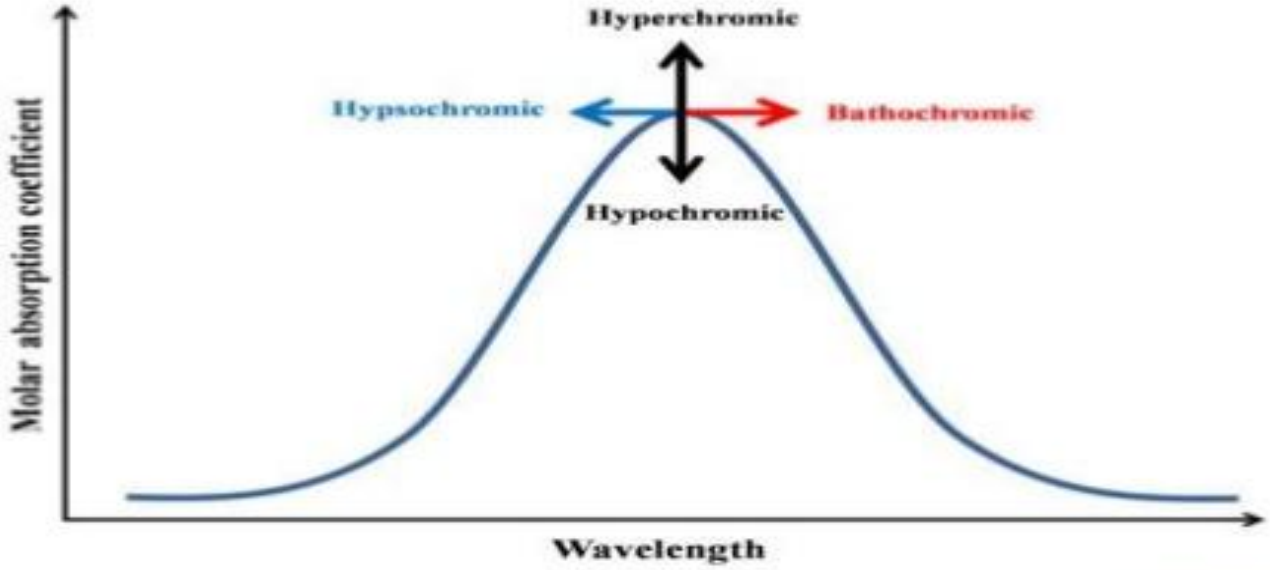
❖ **True deviation :** True deviations are related to the concentration of the absorbing substance. Beers law holds good only for dilute solutions.

❖ **Chemical deviation :** Chemical deviations arise if the absorbing species undergo chemical changes such as association, complex formation, dissociation, hydrogen bonding, hydrolysis, ionization or polymerization

❖ Instrumental Deviation :

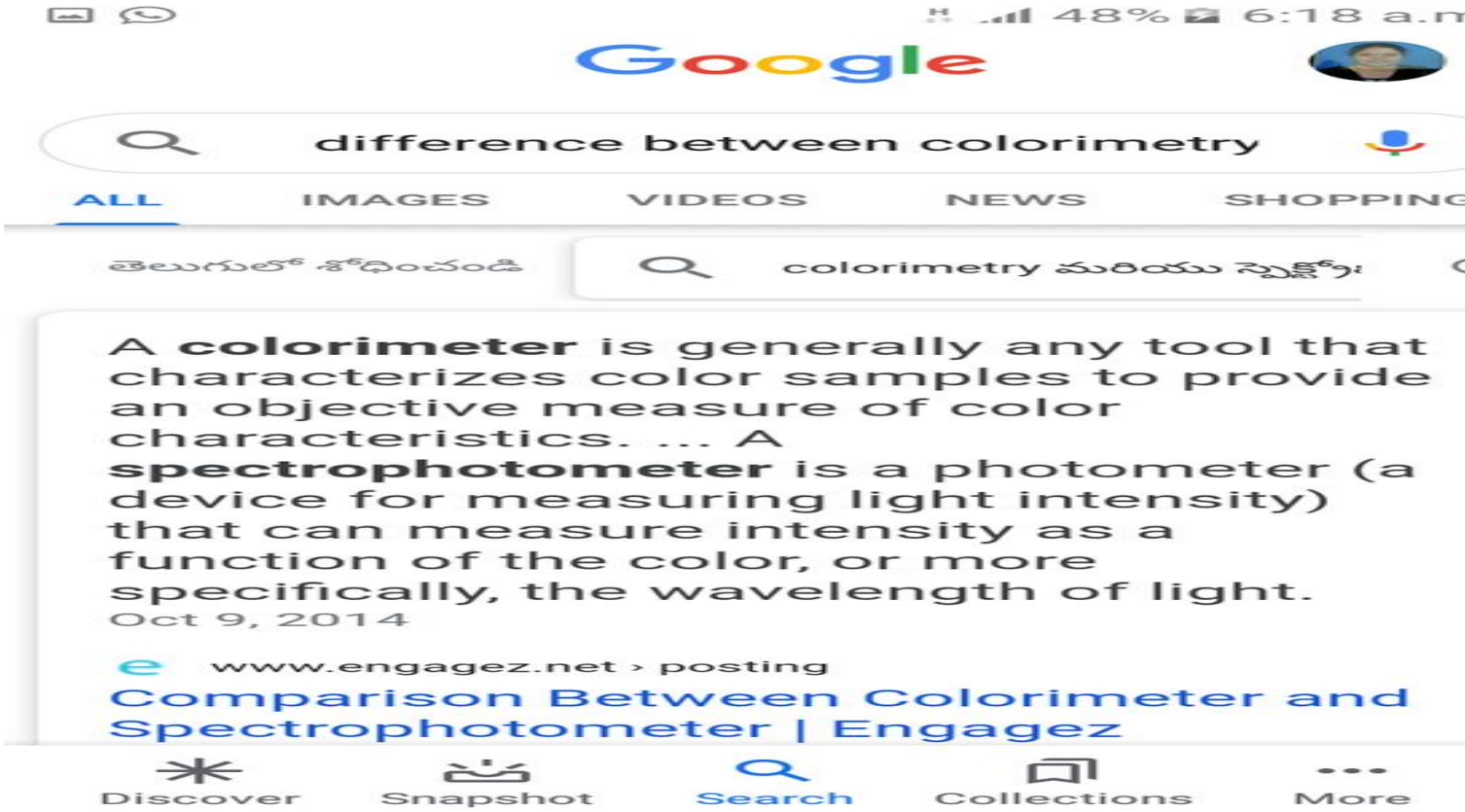
- Only monochromatic light gives beers law use of polychromatic light gives negative deviation.
- Any fluctuations in intensity of light, change in the sensitivity of detector, improper slit width can lead to deviation from beer lamberts law.

ABSORPTION AND INTENSITY SHIFTS:



LIMITATIONS

- Deviations in absorptivity coefficients at ***high concentrations (>0.01M)*** due to electrostatic interactions between molecules in close proximity.
- **Scattering of light due to particulates** in the sample.
- **Fluoresecence or phosphorescence** of the sample.
- Changes in refractive index at high analyte concentration.
- Shifts in chemical equilibria as a function of concentration.
- Non-monochromatic radiation, deviations can be minimized by using a relatively flat part of the absorption spectrum such as the maximum of an absorption band.
- Stray light.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colorimetry
A **colorimeter** is generally any tool that characterizes color samples to provide an objective measure of color characteristics. ... A **spectrophotometer** is a photometer (a device for measuring light intensity) that can measure intensity as a function of the color, or more specifically, the wavelength of light.
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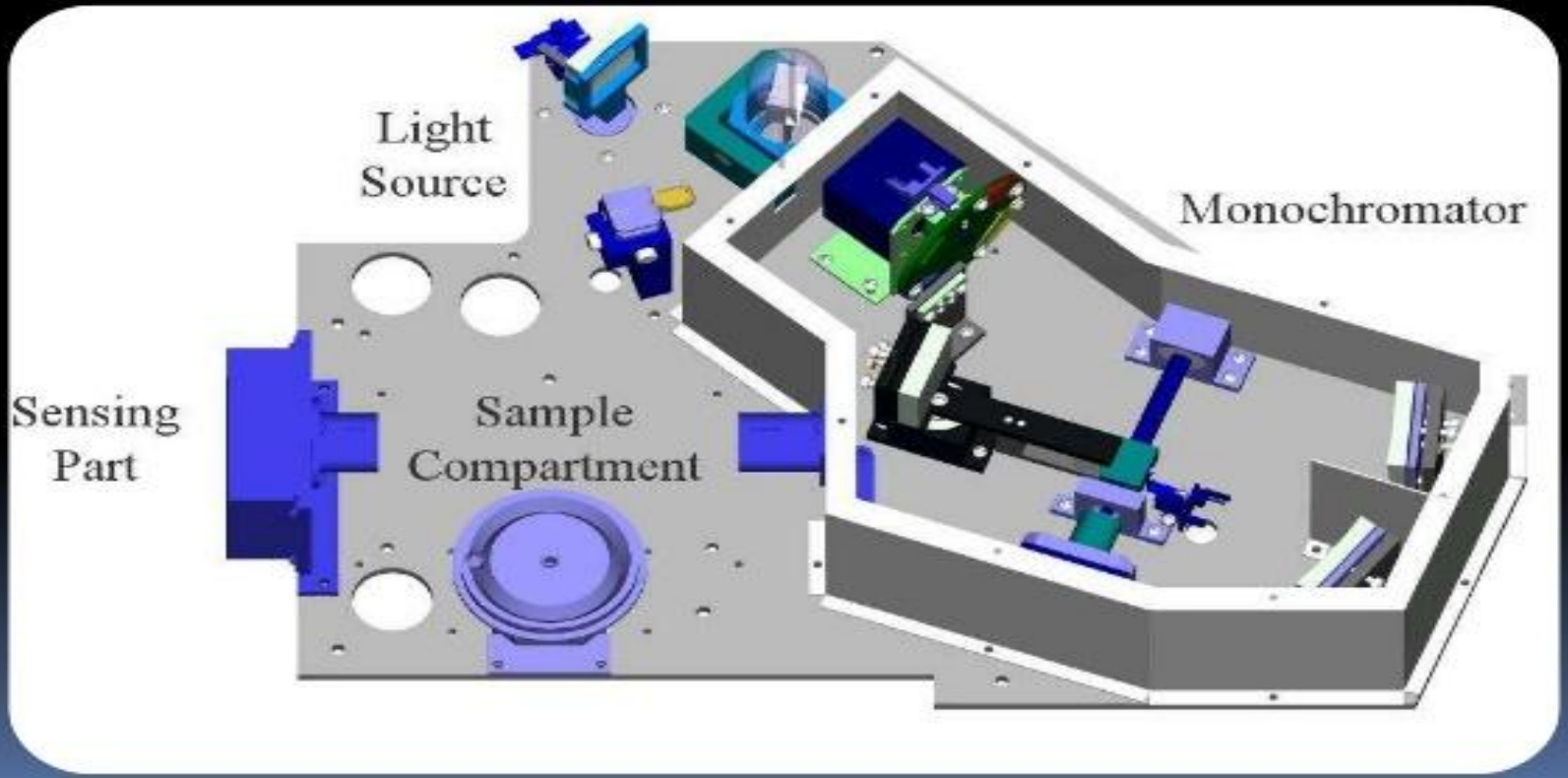
Spectrophotometers?

There are several similarities between colorimeters and spectrophotometers, but the two are still vastly different. The biggest difference is in capability and usage. Spectrophotometers are incredibly powerful and can offer more in-depth measurements than a colorimeter, such as spectral data. This is why they are primarily used for precise measurements in research and development or laboratory use. Colorimeters, in comparison, are simpler and are common in production and manufacturing, such as for quality control.

Some other differences include:

- **Versatility:** Spectrophotometers have many adjustable options and can work well for different types of samples and measurements.
- **Cost:** As mentioned, spectrophotometers are often more expensive than colorimeters due to their powerful technology.
- **Accuracy:** Colorimeters aren't as accurate or precise as spectrophotometers.

Components of Spectrophotometer



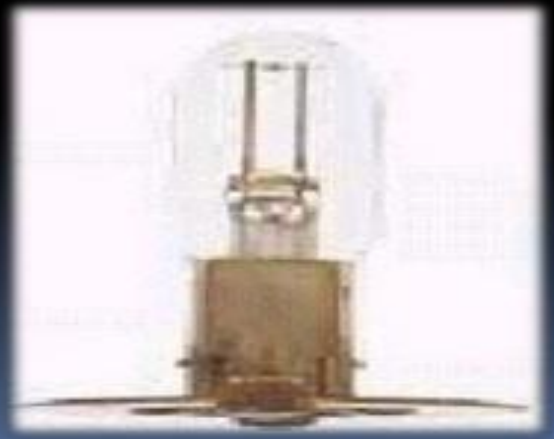
Light Source

- to provide a sufficient of light which is suitable for marking a measurement.
- Tungsten Lamp
- Hydrogen Lamp
- Xenon Lam

I) Tungsten Lamp

- It is the most common light source used in spectrophotometer wavelength
- range of about 330 to 900 nm
- It has long life

about 1200h.



II)Hydrogen / Deuterium Lamps

For the ultraviolet region, hydrogen or deuterium lamps are frequently used.

their range is approximately 200 to 450 nm.

Deuterium lamps are generally more stable and has long life about 500h.This lamp generates continuous or discontinuous spectral.

III) Xenon flash lamps

Xenon flash lamps have several advantages as the following :

1)Their range between (190nm - 1000 nm)

2) Emit both UV and visible wavelengths

3) Long life

4) Do not heat up the instrument

5) Reduce warm up time

Dispersion devices

Dispersion devices causes a different wavelength of light to be dispersion at different angles.

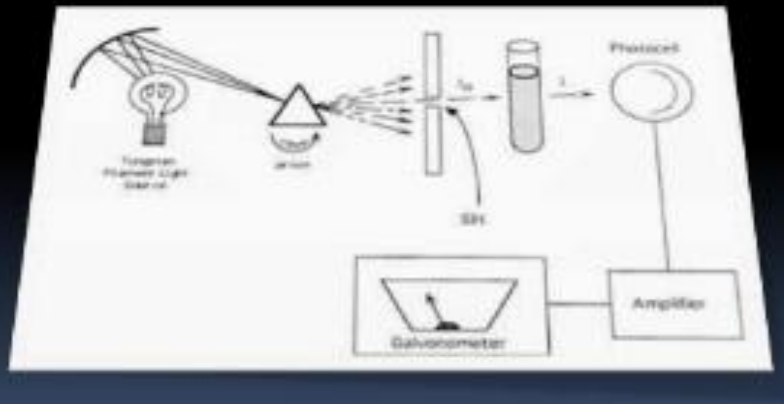
Types of Dispersion devices used are

- Prism
- Filters

Prism

Prism is used to isolate different wavelength

Prism may be made of glass or quartz.



Filters

Filters separate different parts of the electromagnetic spectrum by absorbing or reflecting certain wavelengths and transmitting other wavelengths.

- **Absorption filters** are glass substrates containing absorbing species that absorb certain wavelength. A typical example is a cut on color filter, which blocks short

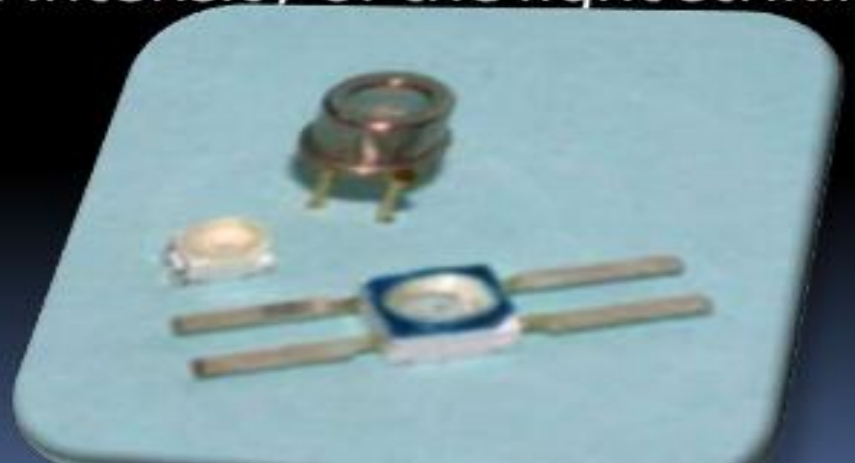
wavelength light, and transmits longer wavelength.

Absorption cells(Cuvettes)

A cuvette is a kind of cell (usually a small square tube) sealed at one end, made of Plastic, glass or optical grade quartz and designed to hold samples for spectroscopic experiments.

Detectors

Any photosensitive device can be used as a detector of radiant energy .The photocell and phototube are the simplest photodetectors, producing current proportional to the intensity of the light striking them.



Display devices

The data from a detector are displayed by a readout device, such as an analog meter, a light beam reflected on a scale, or a digital display , Or liquid crystal display(LCD) .The output can also be transmitted to a computer.

Uses of Spectrophotometer

- To determines the absorbance or transmission of characteristic wavelengths of radiant energy (light) by a chemical species in solution.
- Identify organic compounds by determining the absorption maximum.
- Used for color determination within the spectral range

Types of Spectrophotometer

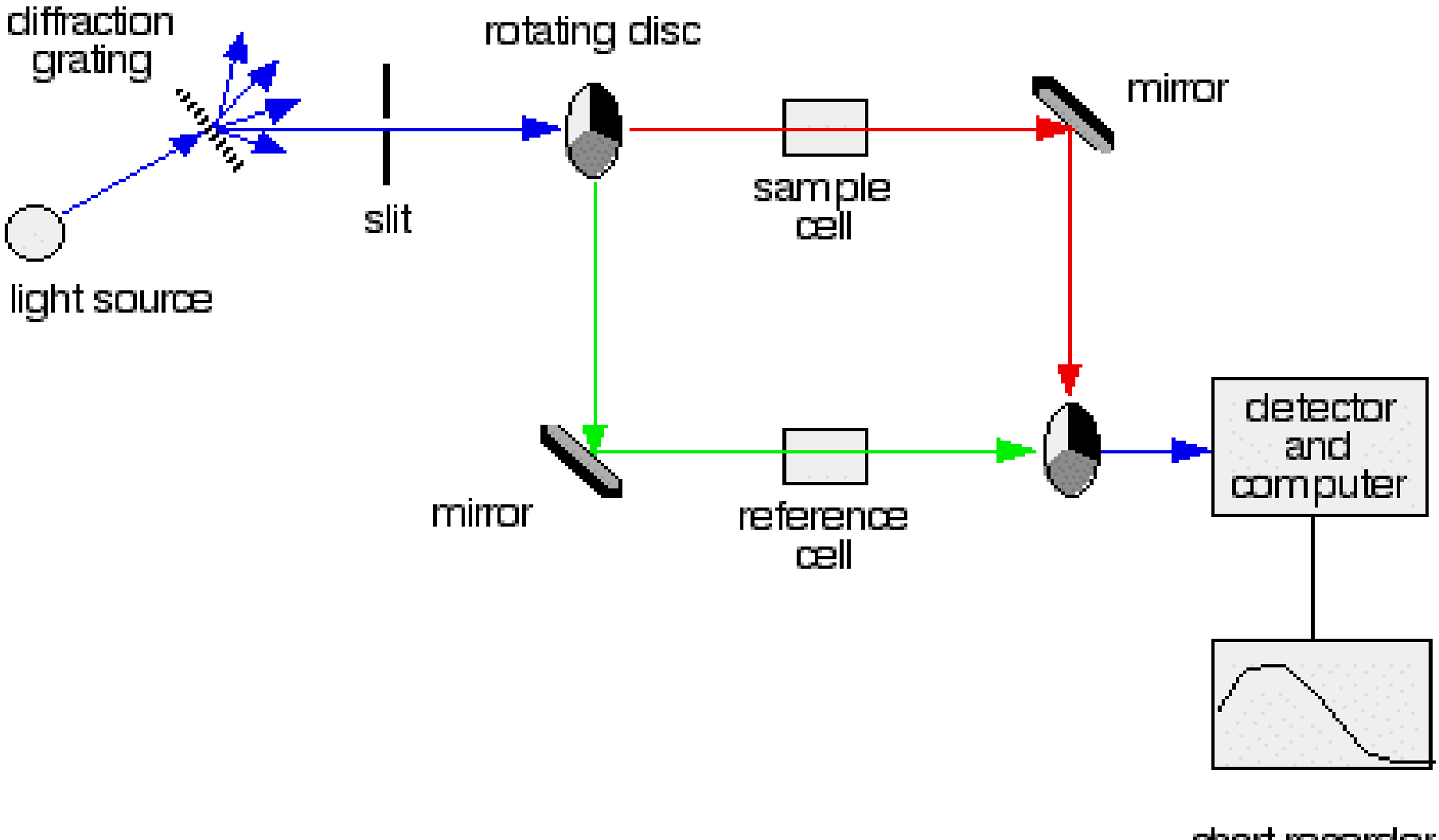
- Single Beam
- Double Beam
- Split Beam

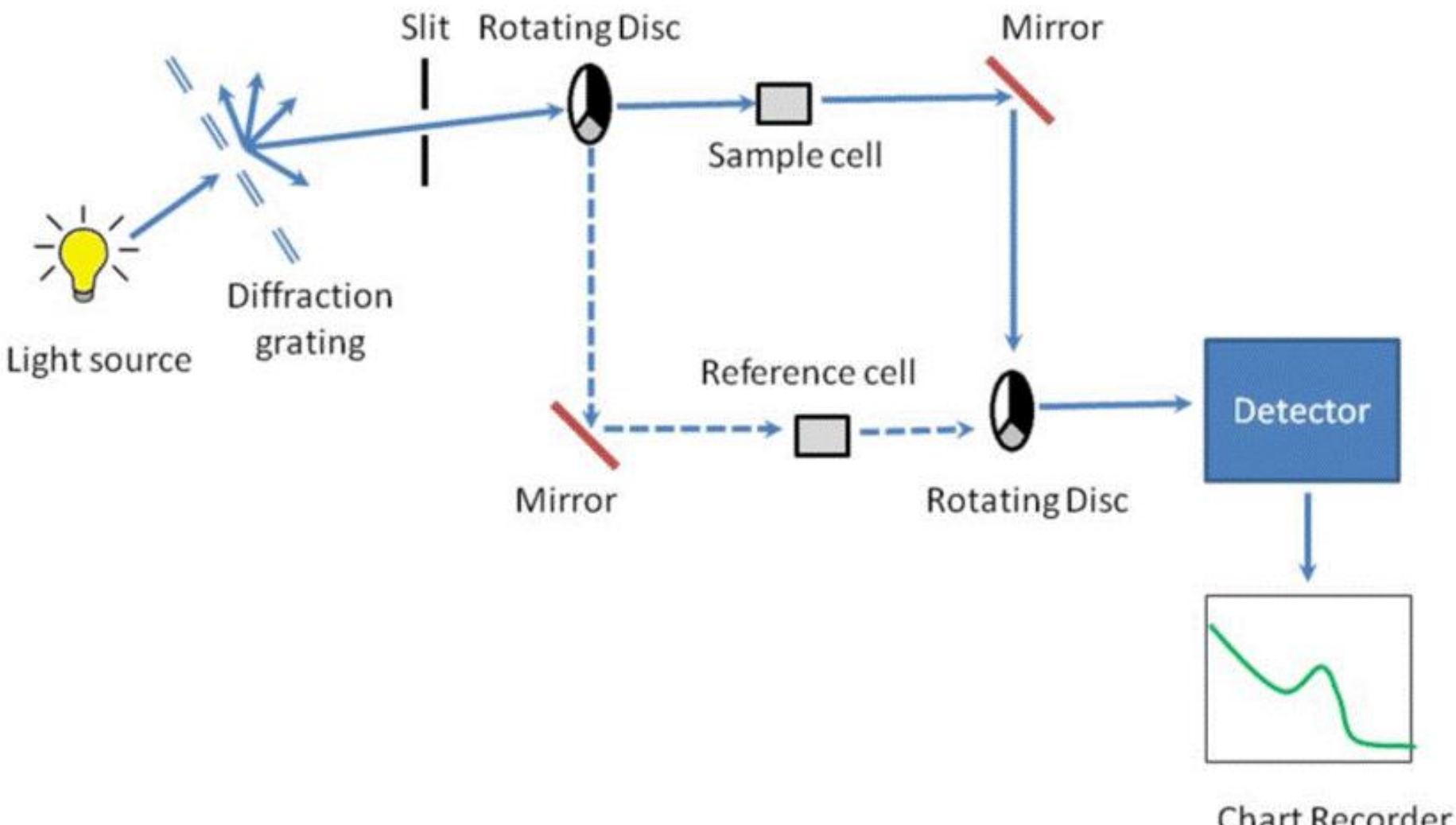
Single beam

The single beam spectrophotometer was the first invented, and all the light passes through the sample.

To measure the intensity of the incident light, the sample must be removed so all the light can pass through.

- cheaper because there are less parts and the
- system is less complicated.
- low cost


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


Slit
Rotating Disc
Mirror
Sample cell
Light source
Diffraction grating
Reference cell
Detector
Mirror
Rotating Disc
Chart Recorder