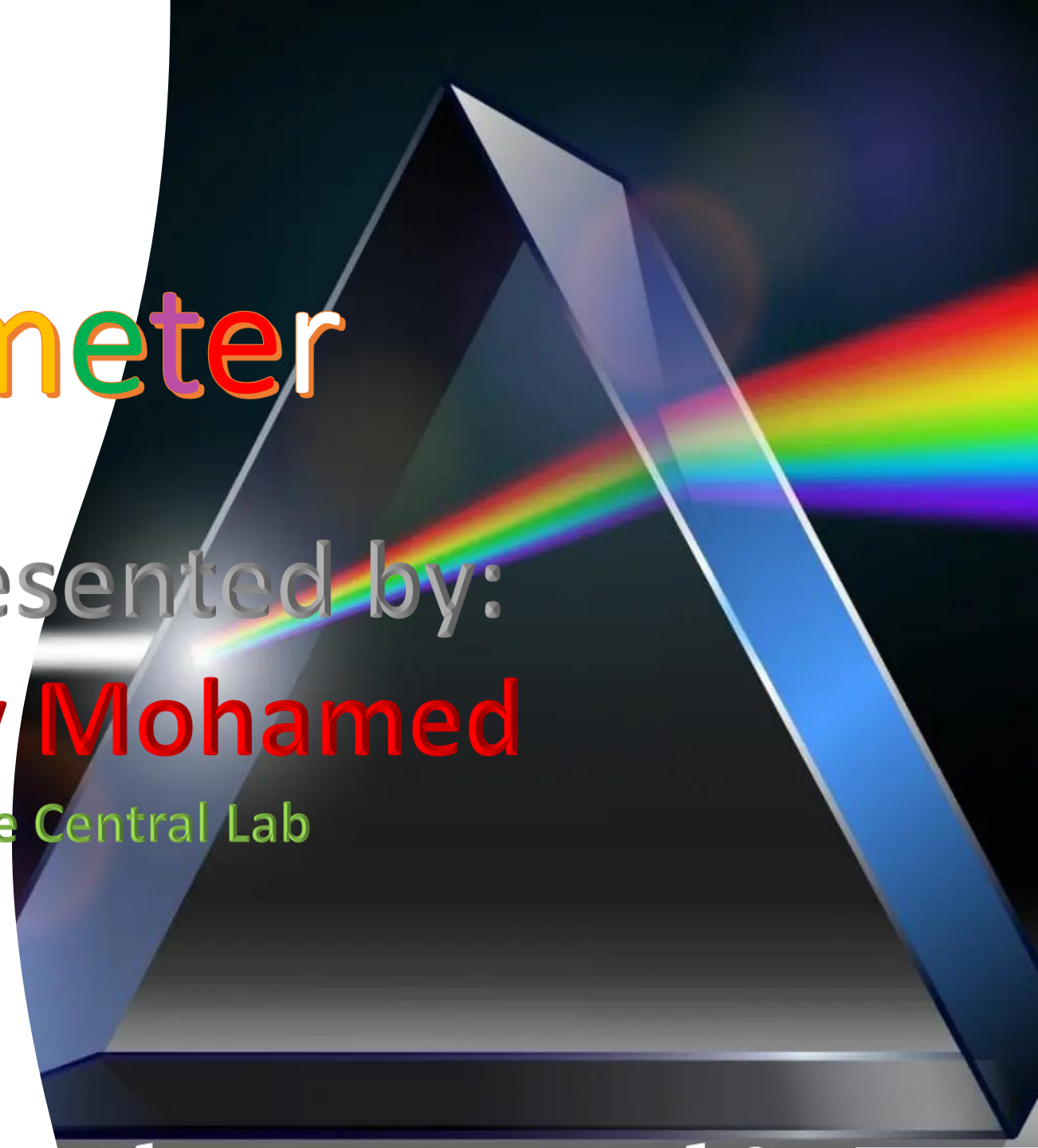


spectrophotometer

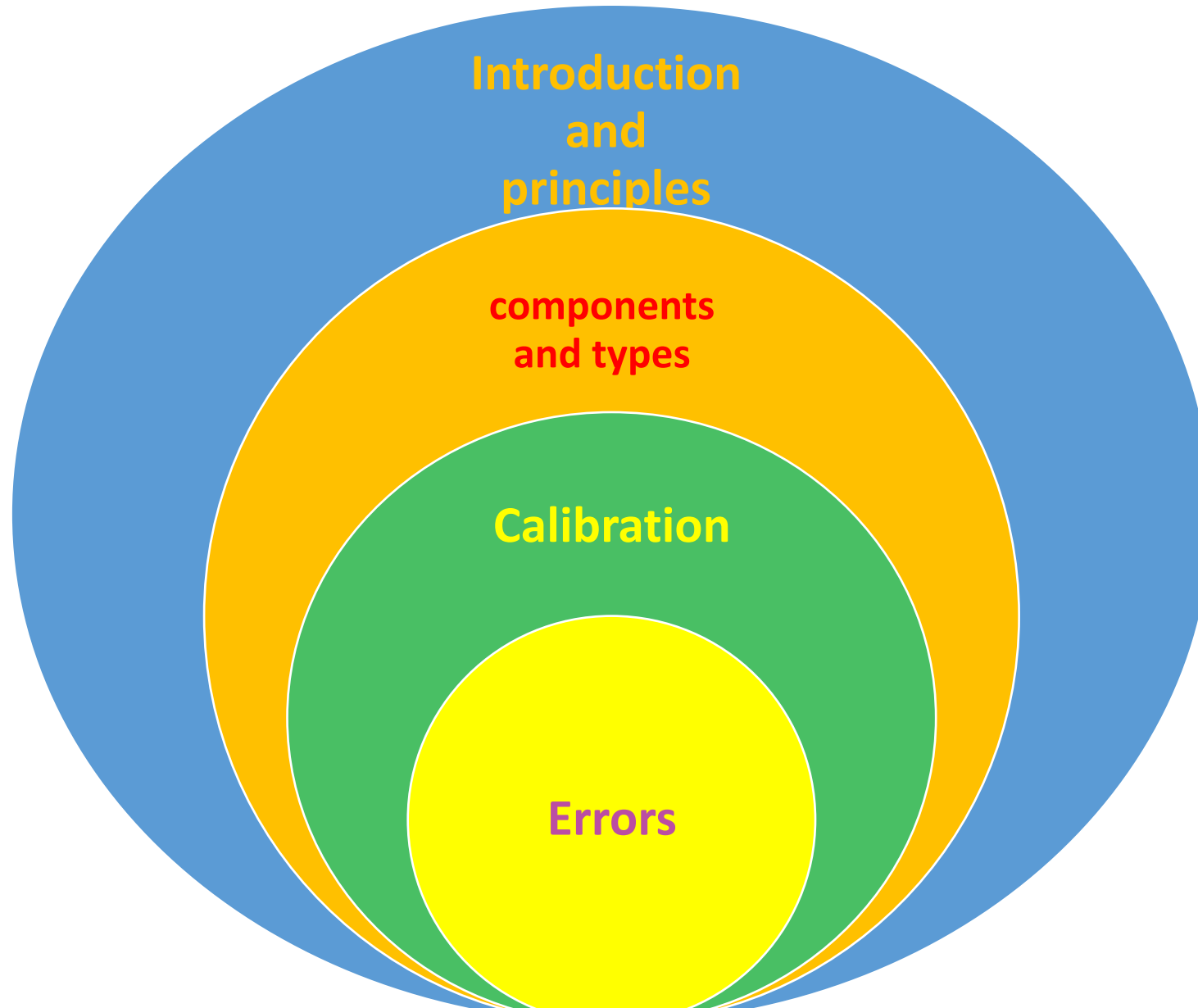
Prepared and presented by:

Dr. Abeer Hamdy Mohamed

General Manager Of The Central Lab



Aims and scope



Introduction:

إسحاق الكندي



```
graph TD; A[إسحاق الكندي] --> B[ابن الهيثم]; B --> C[Isaac Newton]; C --> D[William Wollaston]; D --> E[Joseph Von Fraunhofer];
```

ابن الهيثم

Isaac Newton

William Wollaston

Joseph Von Fraunhofer

The First Spectrophotometer

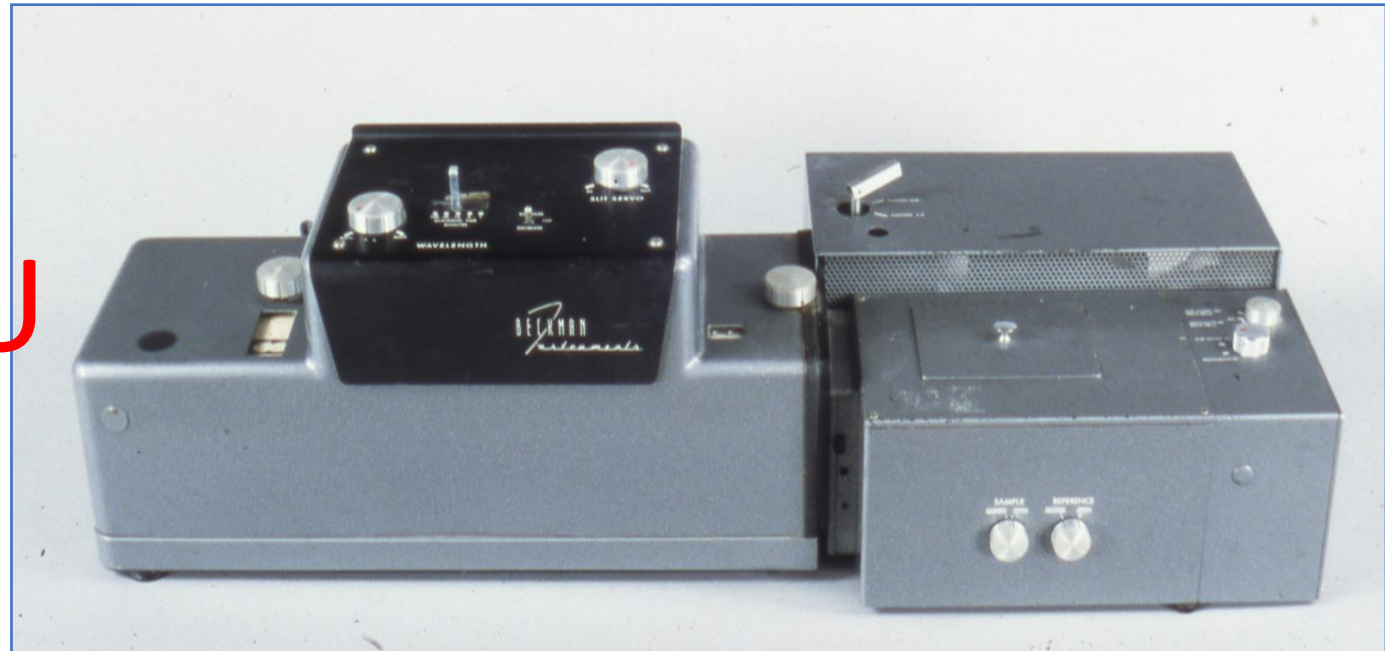
Arnold Beckman

• A model

B •

• C

DU

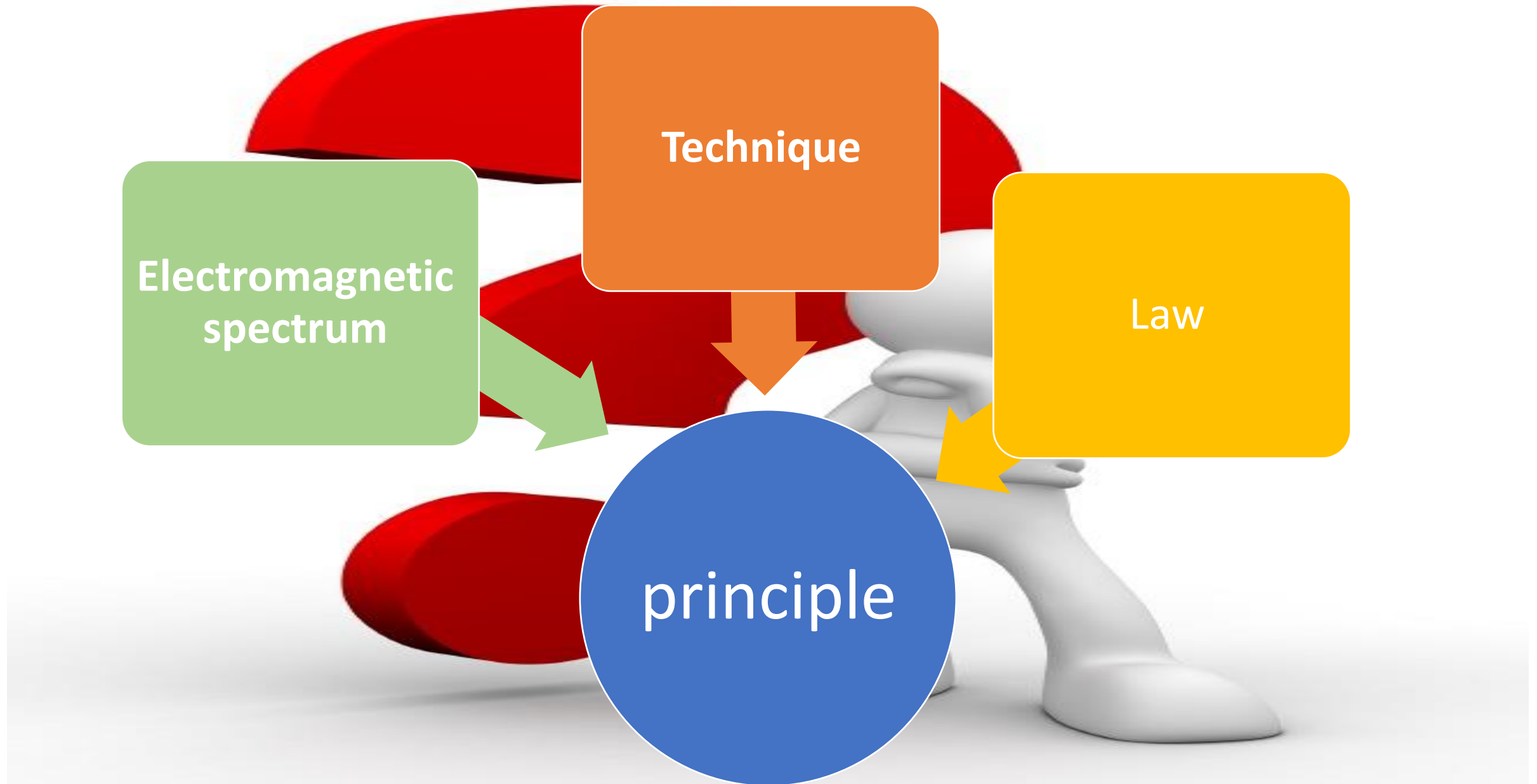


Electromagnetic
spectrum

Technique

Law

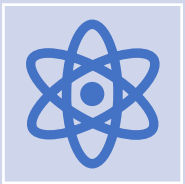
principle



Techniques



Spectrophotometric techniques are used to measure the concentration of solutes in solution by measuring the amount of light that is absorbed by the solution in a cuvette placed in the spectrophotometer.



The spectrophotometer can measure the amount of light or electromagnetic radiation (of certain frequency) transmitted or absorbed by the solution.

spectrophotometer

```
graph TD; A[spectrophotometer] --> B[spectrometer]; A --> C[photometer]; B --> D[Produce light]; C --> E[Measure light];
```

spectrometer

photometer

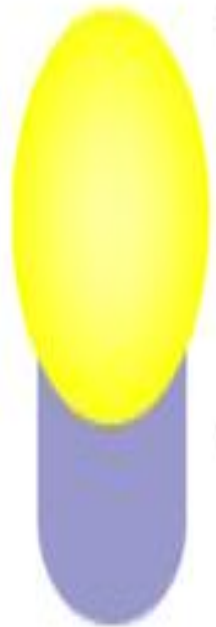
Produce light

Measure light

The basic structure of spectrophotometer

collimator

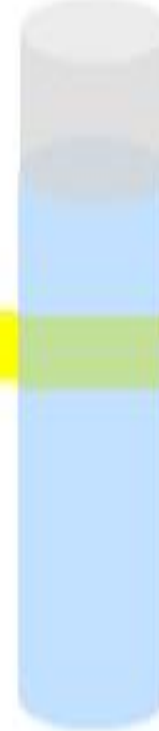
Wavelength selector



Light source



Monochromator



sample



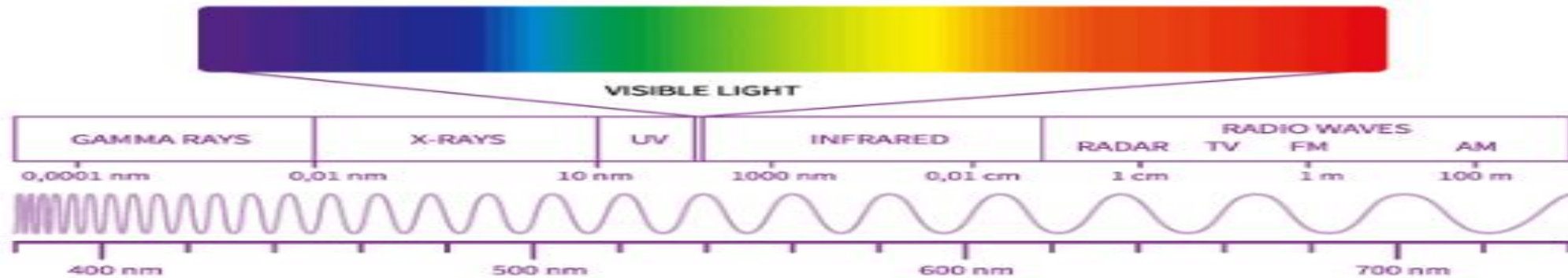
Detector

0.20

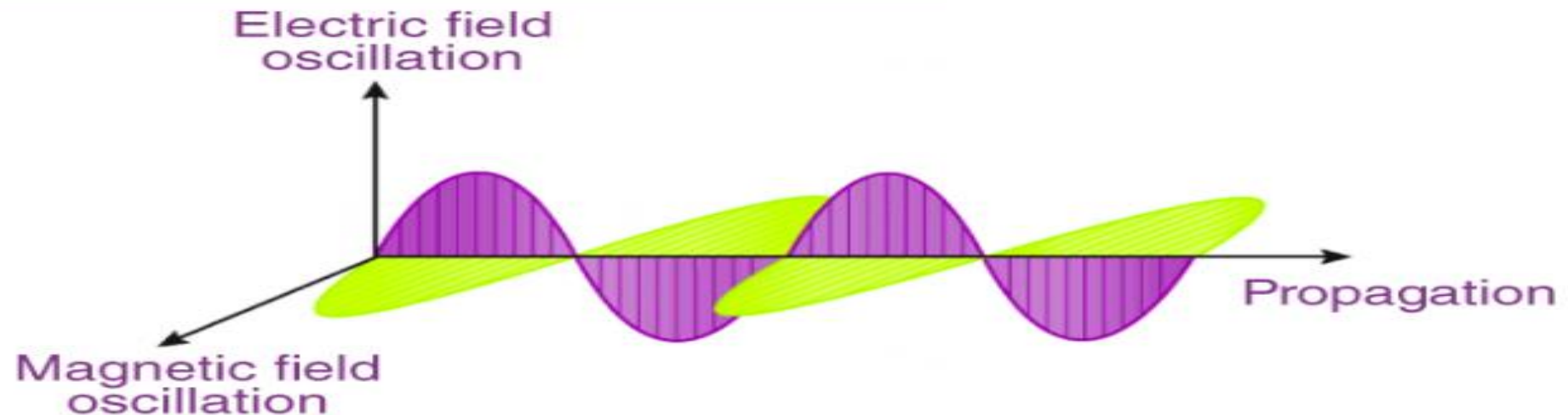
I_0

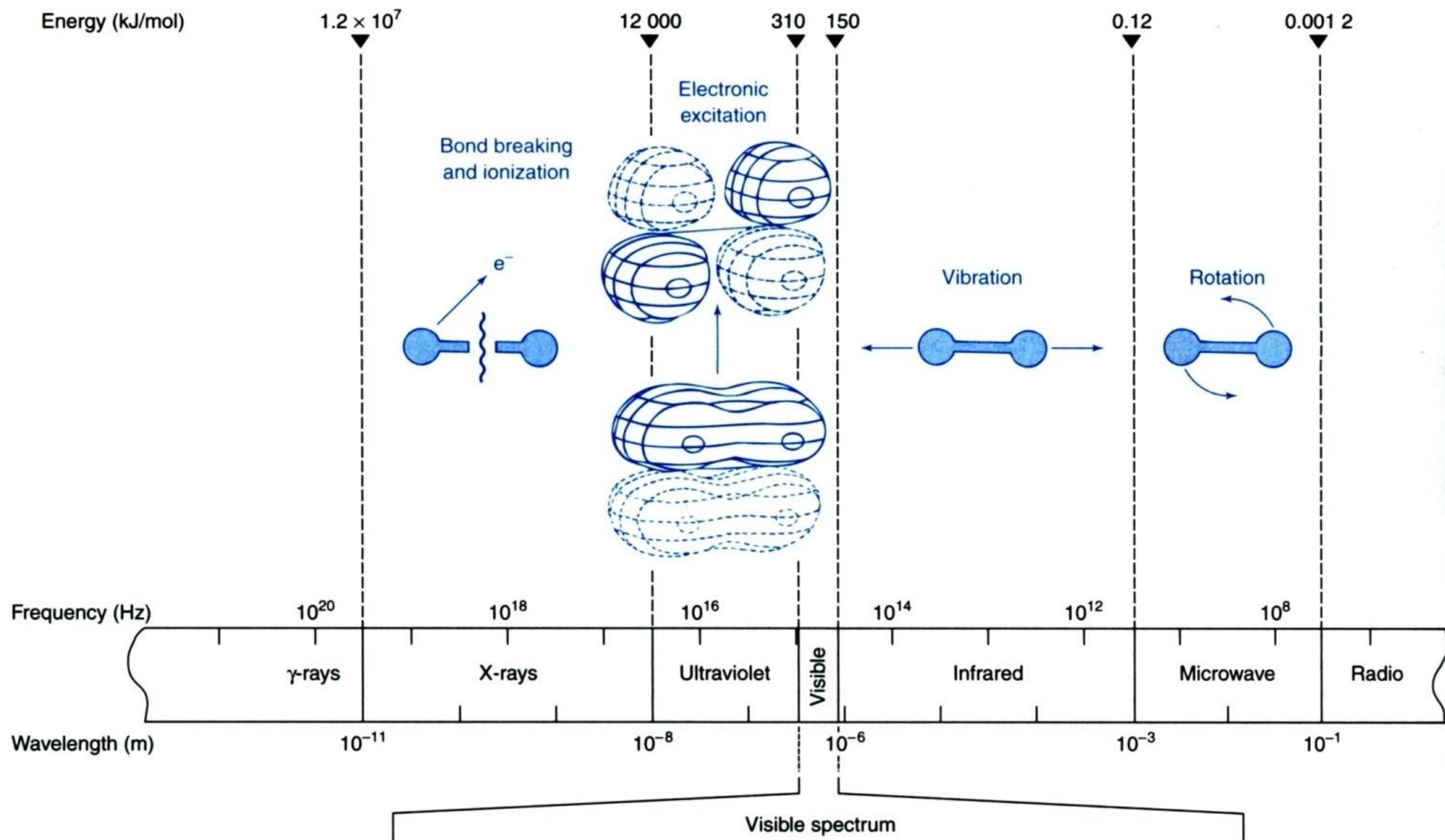
I_t

Electromagnetic Spectrum



ELECTROMAGNETIC WAVES





<u>Radiation</u>	<u>Frequency Hz</u>	<u>Wavelength</u>	<u>Transition</u>
gamma-rays	10^{20} - 10^{24}	$<10^{-12}$ m	Nuclear
x-rays	10^{17} - 10^{20}	1 nm-1 pm	inner electron
ultraviolet	10^{15} - 10^{17}	400 nm-1 nm	outer electron
visible	4 - 7.5×10^{14}	750 nm-400 nm	outer electron
near-infrared	1×10^{14} - 4×10^{14}	2.5 μ m-750 nm	outer electron molecular vibrations
infrared	10^{13} - 10^{14}	25 μ m-2.5 μ m	Molecular vibrations
microwaves	3×10^{11} - 10^{13}	1 mm-25 μ m	Molecular rotations, electron spin flips*
radio waves	$<3 \times 10^{11}$	>1 mm	nuclear spin flips*

Types of spectroscopy and uses

IR

Organic and inorganic comp

chemical composition of the material.

UV-visible

Transition element (analytical chemistry)

drug identification and to check nucleic acid purity

Mass

Identifying isotopes

identifying biomolecules or proteins present in biological samples.

Raman

Have 5 type

Fluorescence

The fluorescence of a sample

Lambert law

$$T = \frac{I}{I^{\circ}}$$

$$A = -\log T$$

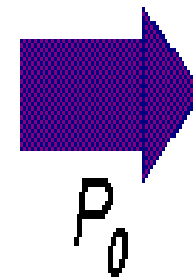
$$A = -\log \frac{I}{I^{\circ}}$$

$$A = -\log \frac{I}{I^{\circ}} = kb$$

Beer law

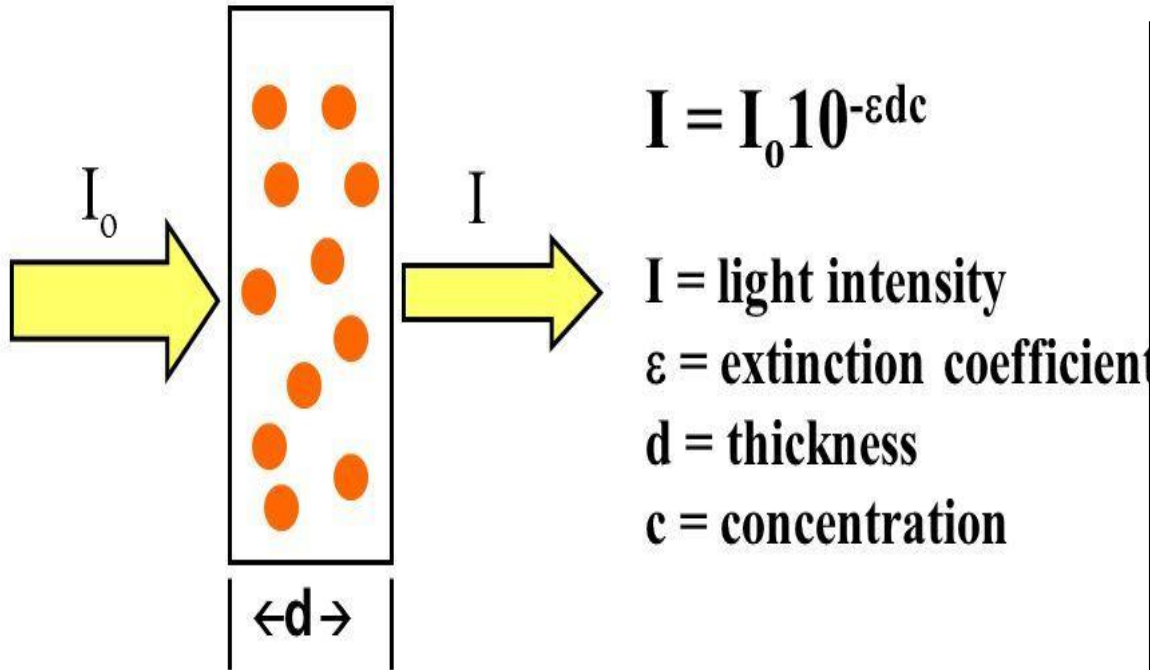
$$A = \epsilon bc$$

where: A = absorbance (no units)
 ϵ = molar absorptivity (L/mole-cm)
 b = cell pathlength (cm)
 c = concentration of analyte (mol/L)

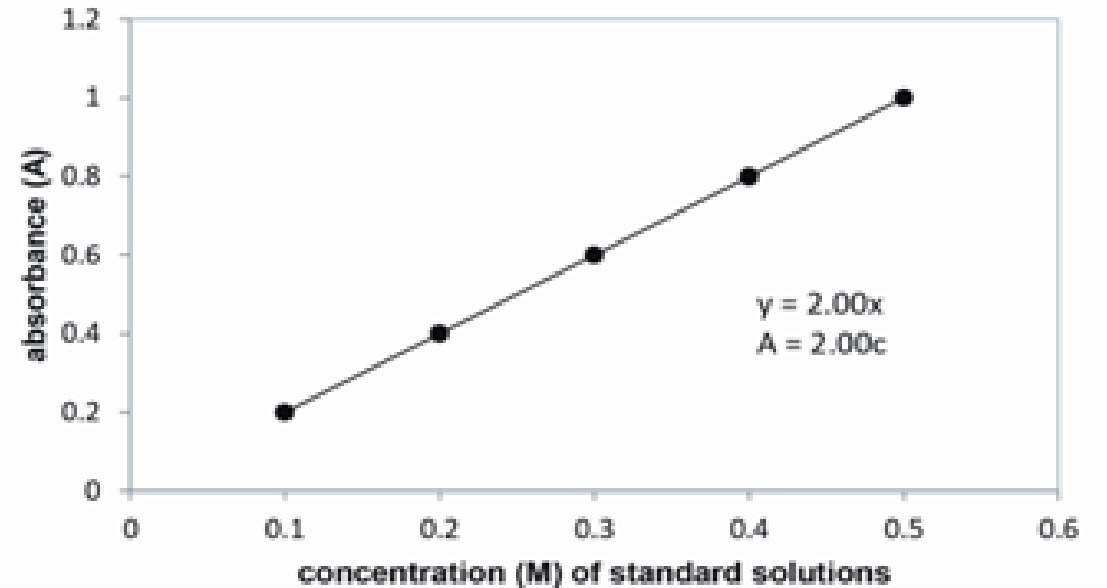


$\leftarrow b \rightarrow$

Beer-Lambert Law



$$\text{Absorption (A)} = -\log(I/I_0) = \epsilon dc$$



Colour wheel



Wavelength of maximum absorption (nm)	Color absorbed	Color observed
380—420	Violet	Green-yellow
420—440	Violet-blue	Yellow
440—470	Blue	Orange
470—500	Blue-green	Red
500—520	Green	Purple
520—550	Yellow-green	Violet
550—580	Yellow	Violet-blue
580—620	Orange	Blue
620—680	Red	Blue-green
680—780	Red	Green

parameter

nitrate

silica

sulfate

ammonia

color

iron

Mn

Al

nitrite

fluoride

phosphate

Wavelength

220-275

410

420

425

456

510

525

535

543

570

690



ما على الصلاة

فاصل للصلاة ته نعهد

ياذن الله

٢٠٠٠
١٠٠٠
١٠٠٠
١٠٠٠



**Spectro
photom
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visible

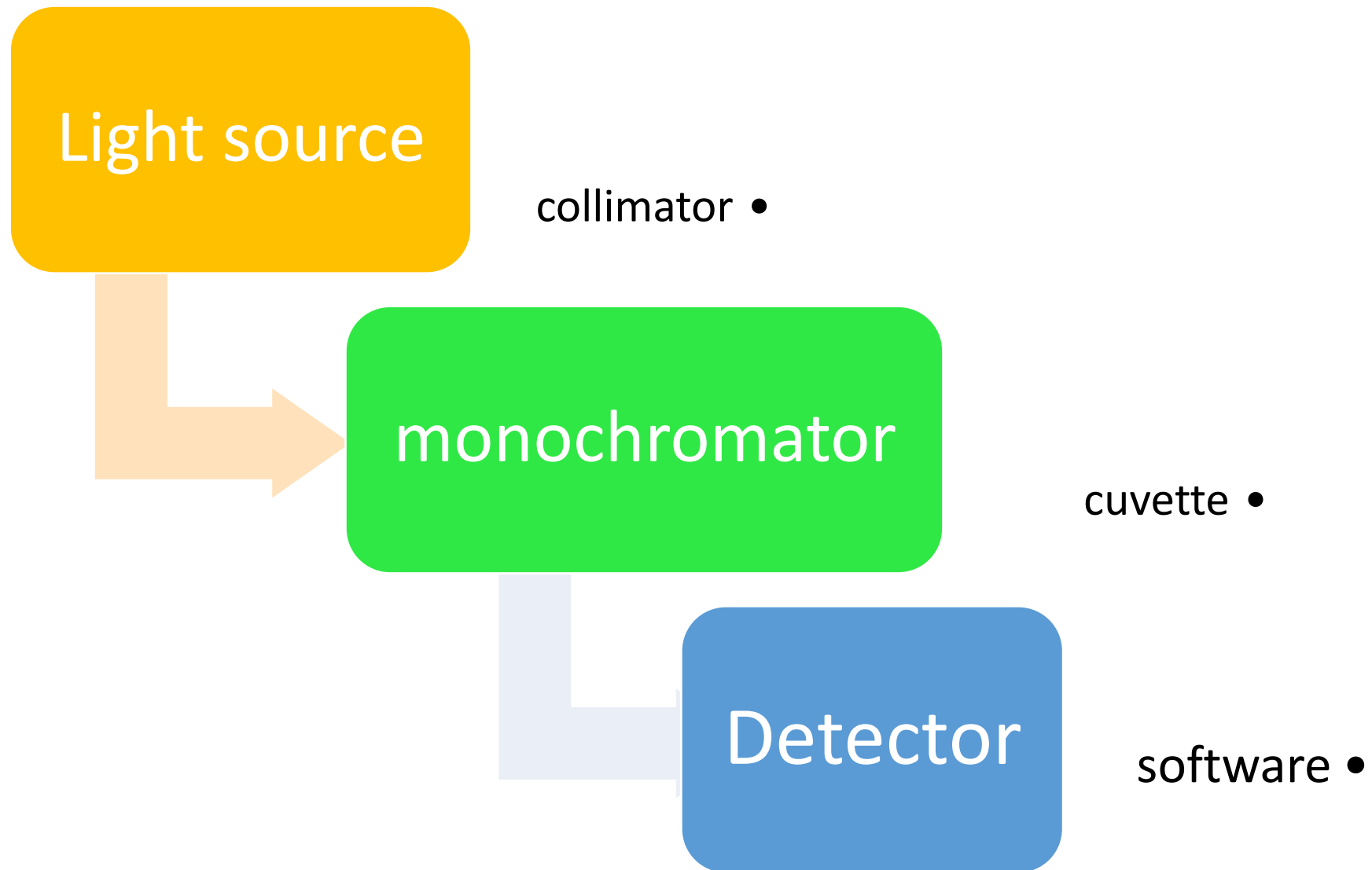
UV

UV-Visible

IR

Atomic

Spectrophotometer primary components





Light Source Requirements

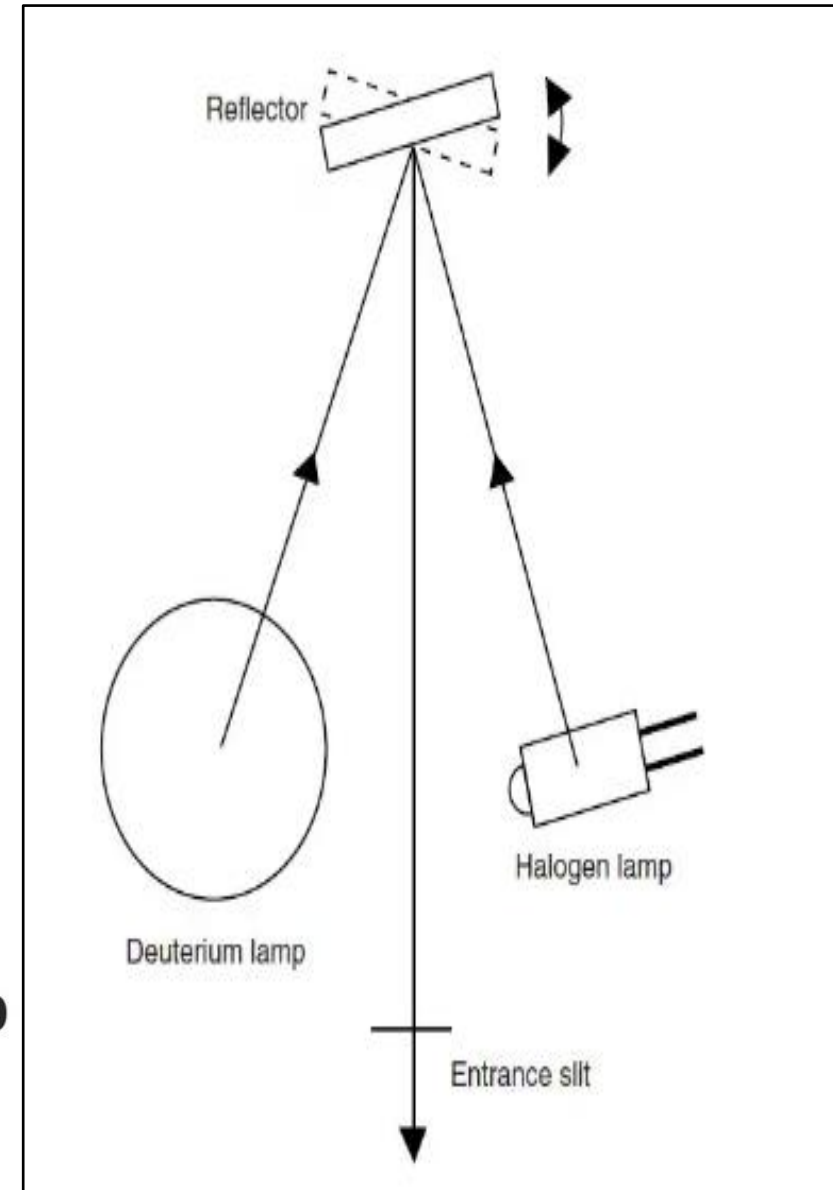
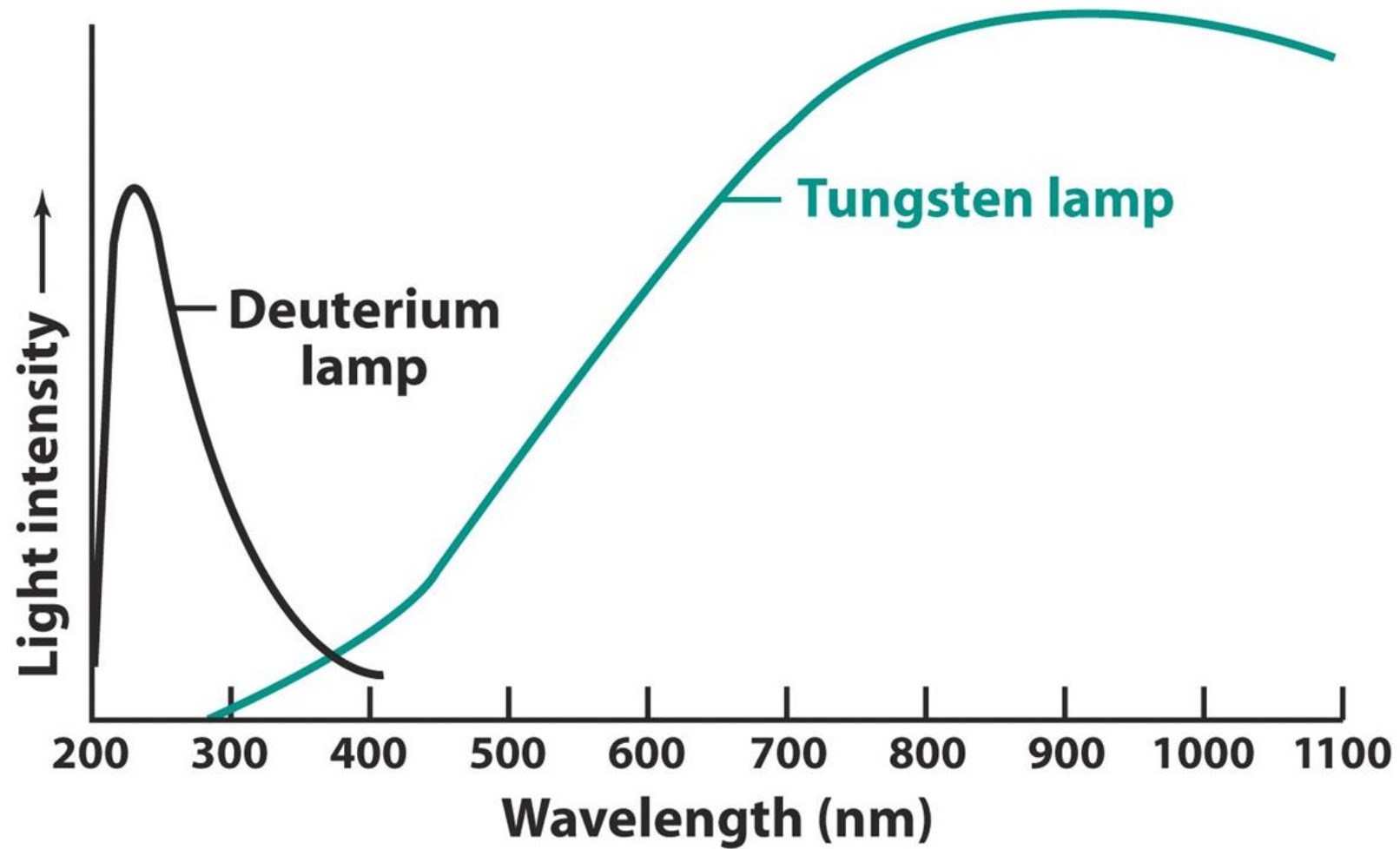


The requirements for a spectrophotometer light source include:

- a) bright across a wide wavelength range
- b) stable over time
- c) long service life
- d) low cost.

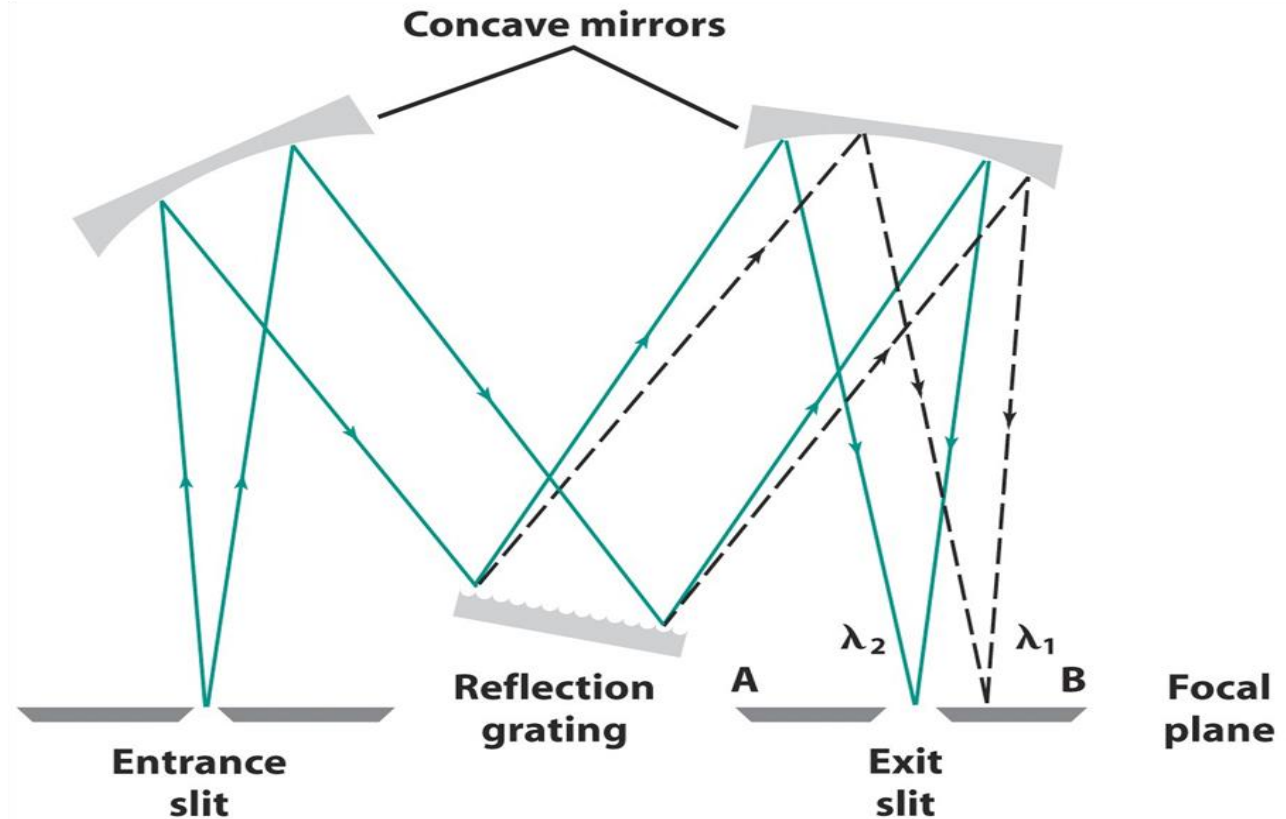
1- Light source

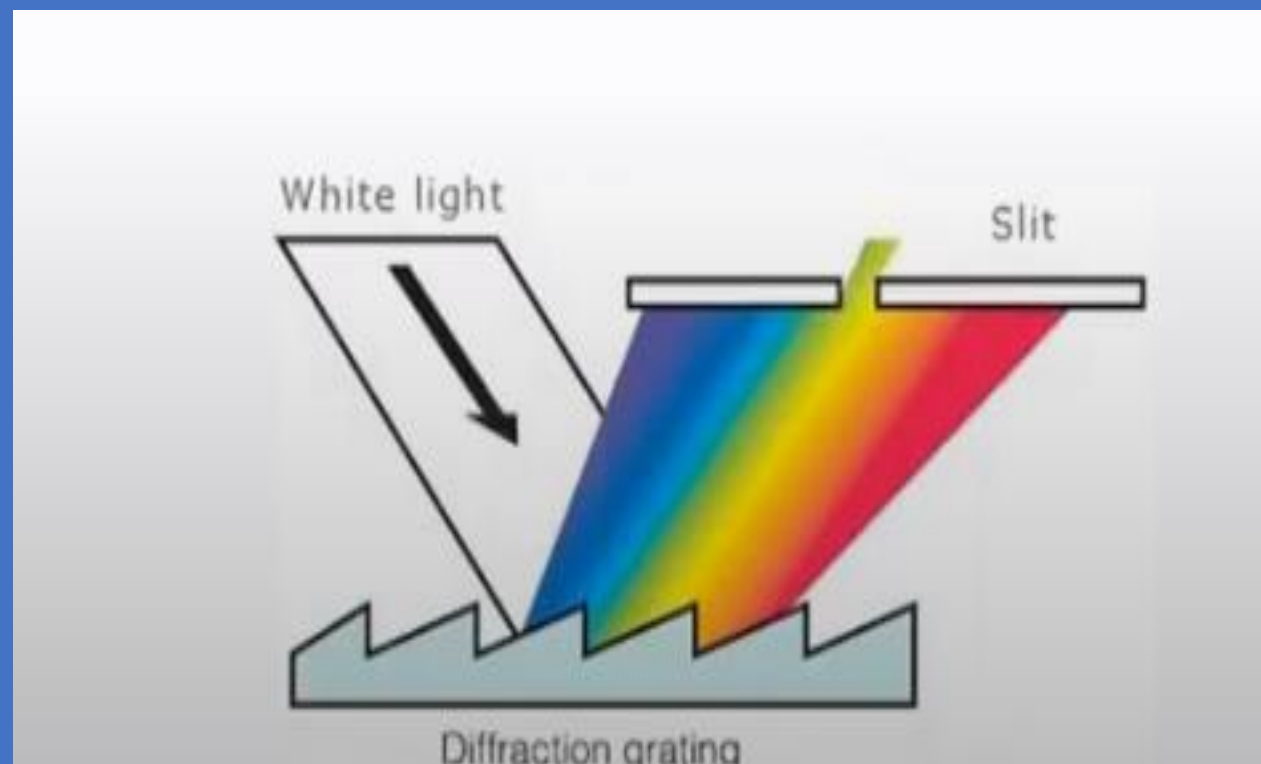
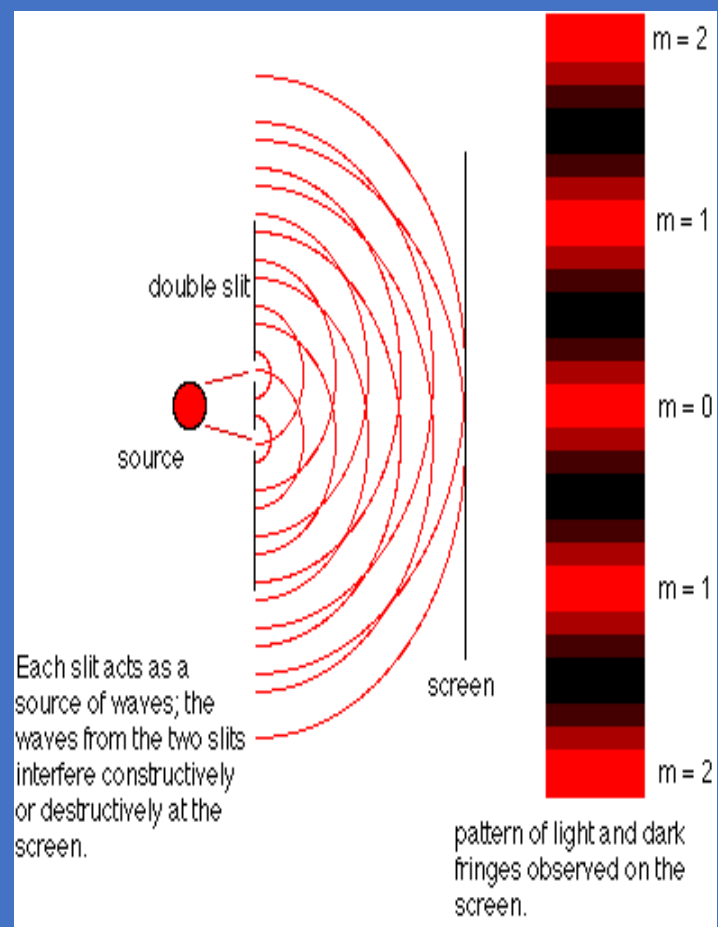
- Tungsten lamp: Vis. near IR (320 nm~2500 nm)
- Deuterium arc lamp: UV (200~400 nm)
- Electric discharge lamp ($\text{Hg}_{(g)}$ or Xenon): Vis & UV and fluorescence
- Glowbar (silicon carbide rod): IR (5000~200 cm^{-1})
- Laser: intense monochromatic sources.



2) Monochromator

- a. entrance slit
- b. collimating mirror or lens
- c. a prism or grating
- d. focal plane
- e. exit slit





Cuvette

Sample Cell



3-Detectors

Convert radiant energy (photons) into an electrical signal

Ideal detector :

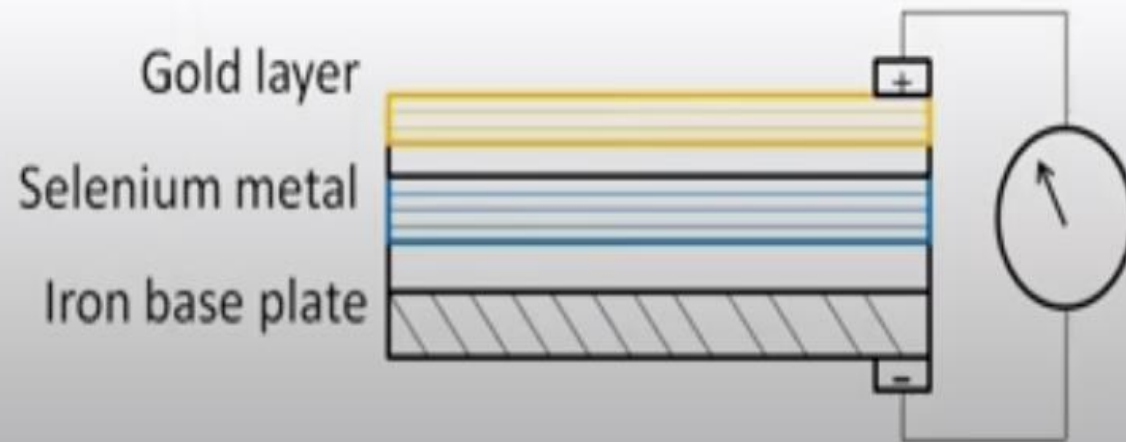
- high sensitivity
- high signal/ low noise
- constant response for wavelengths
- and fast response with time

1- Barrier layer Cell

It does not require a power supply but gives out the current which is directly proportional to incident light.

It has three metal layers –

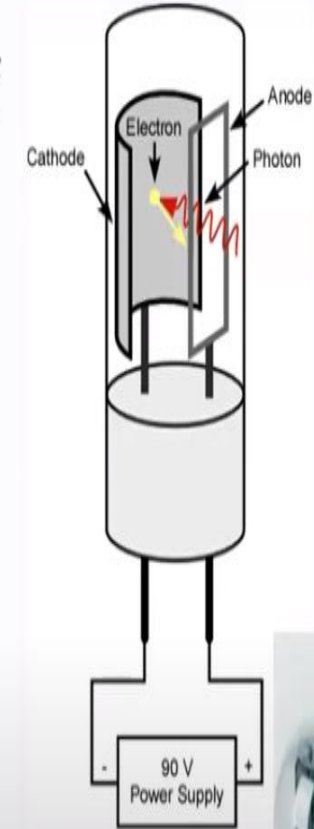
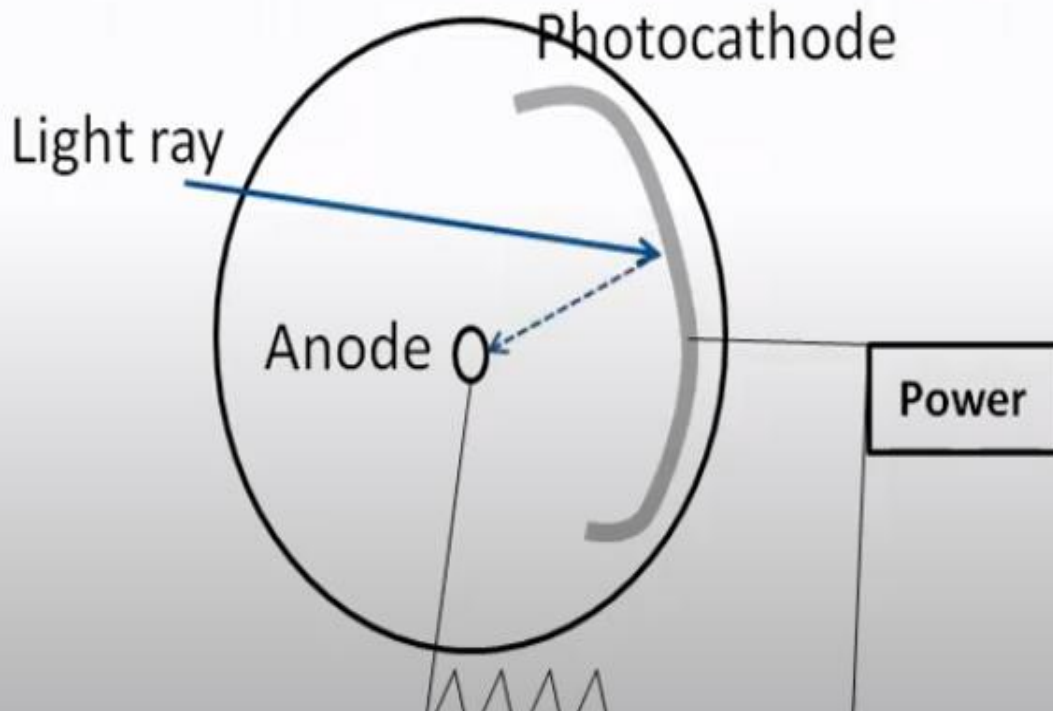
- i) metal base plate (Iron),
- ii) Selenium metal (Semiconductor),
- iii) thin layer of gold or silver.



Device defect
LESS SENSITIVE

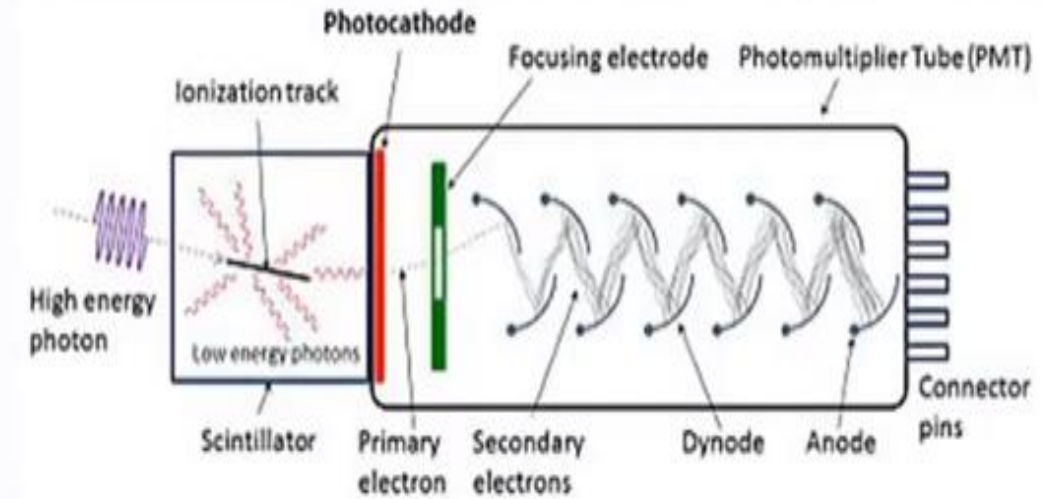
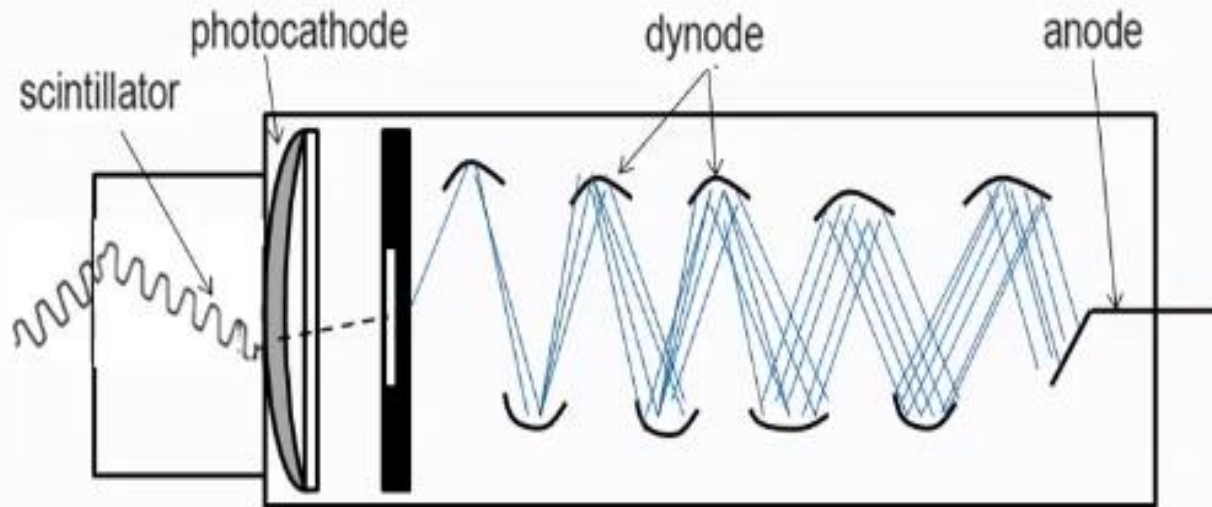
2- photo-emissive cell

- It is a evacuated glass bulb.
- It has a half cylindrical metal cathode. The inner surface of cathode is coated with a light sensitive layer of cesium or potassium oxide or silver oxide.
- A metal ring at the center of bulb, act as an anode.

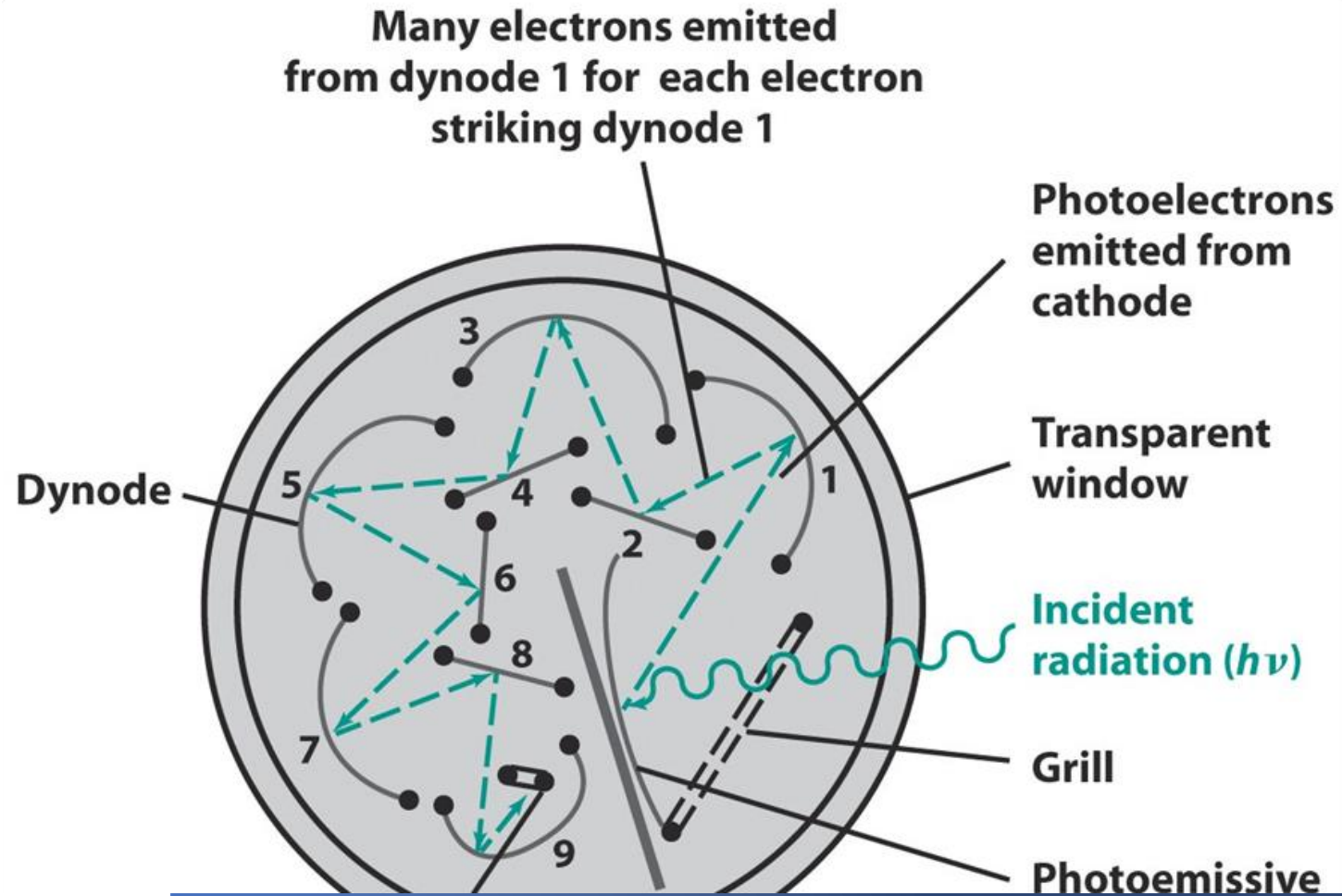


3- photomultiplier tube

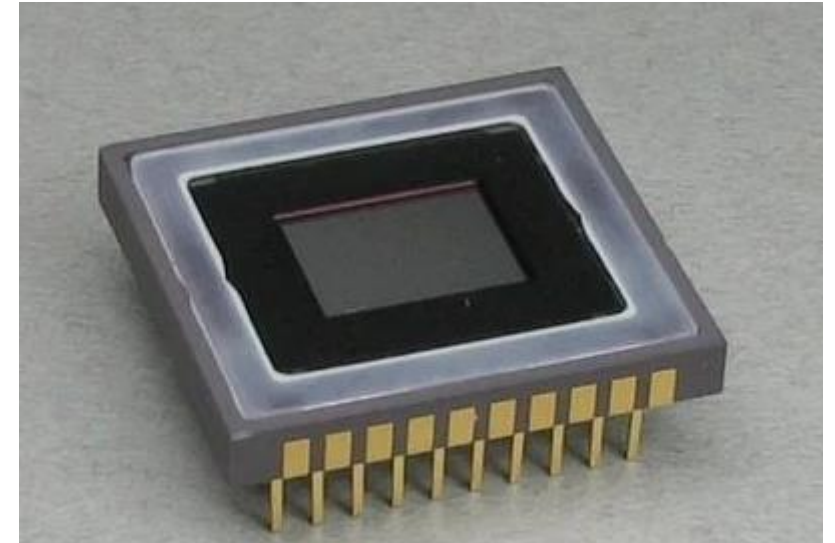
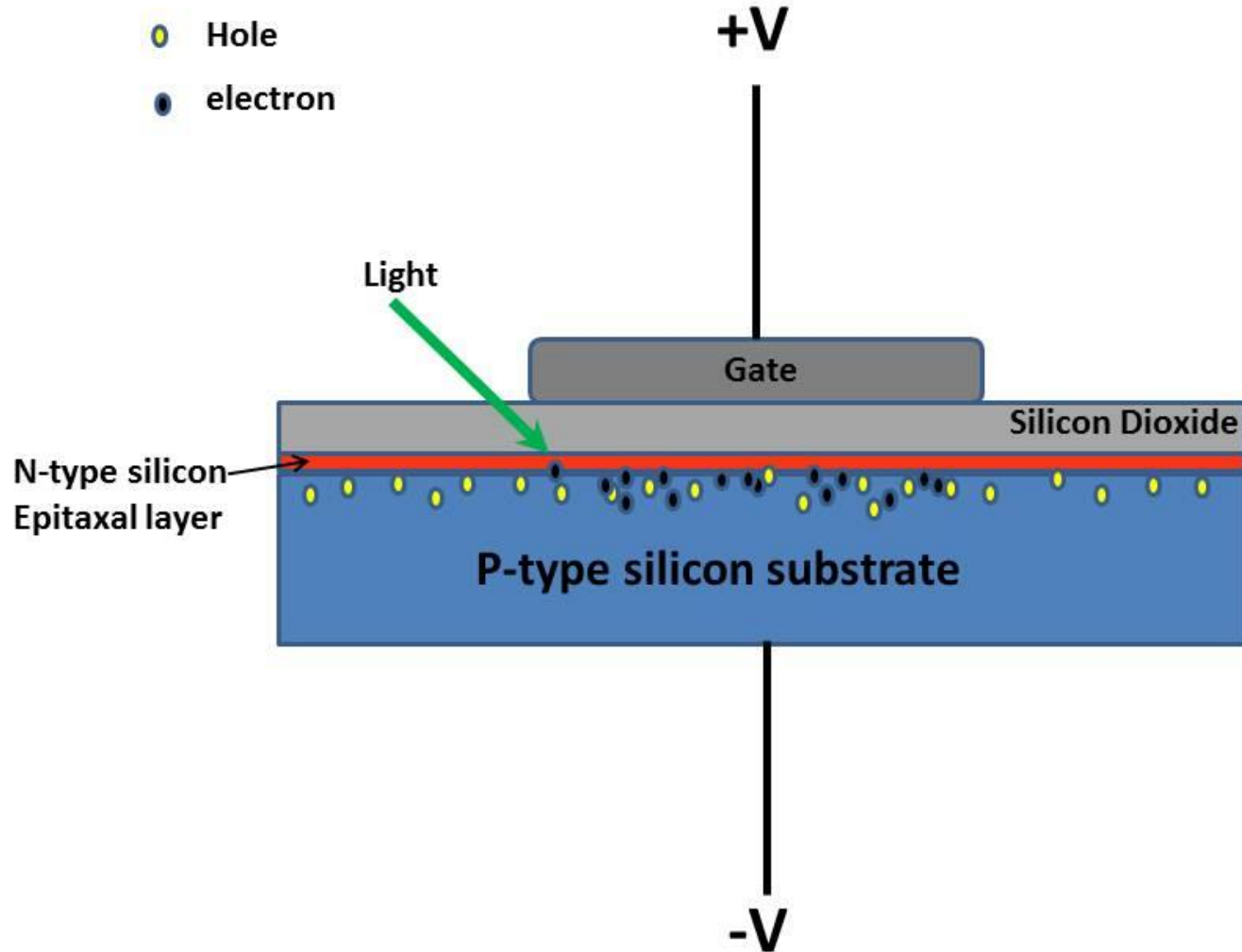
- It has a photo emissive cathode.
- It has 8 – 10 photo emissive 'dynodes'.
- It has a collector anode.
- When light radiation striking to the cathode surface, it emits 2-5 electrons. These electrons are attracted towards first dynode and it emits 2-5 electrons for each striking electrons.

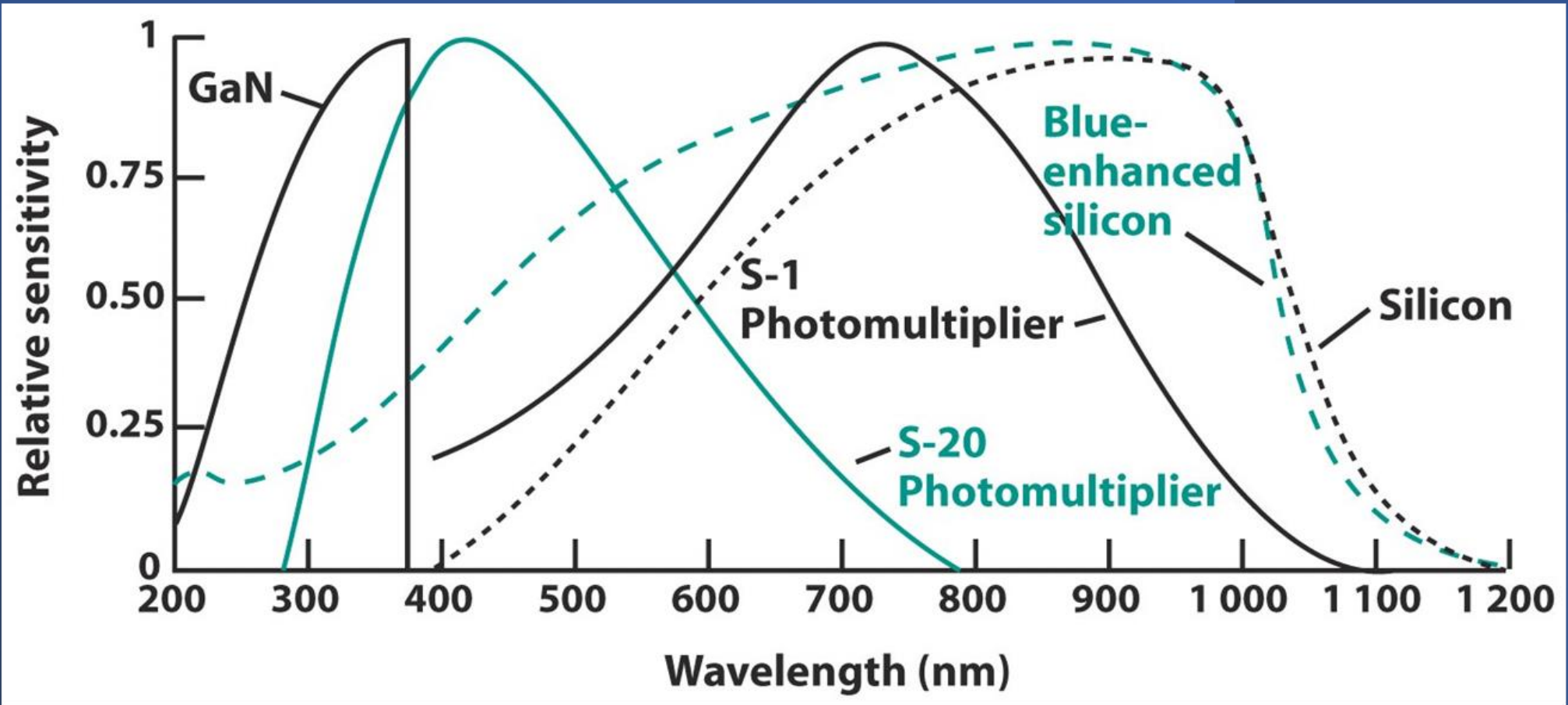


Photomultiplier tube: very sensitive detector



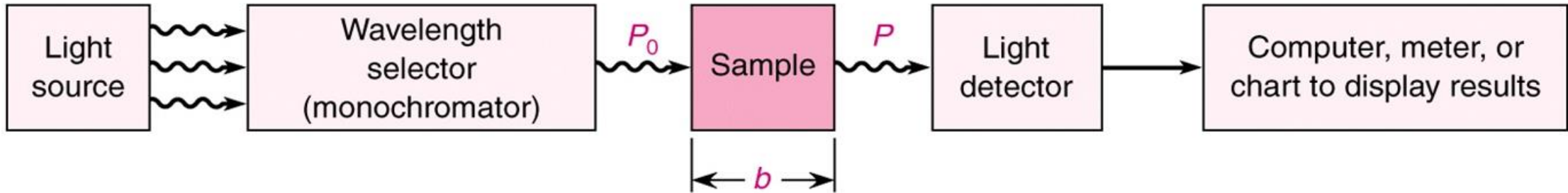
4-Charge Couple Device Detector



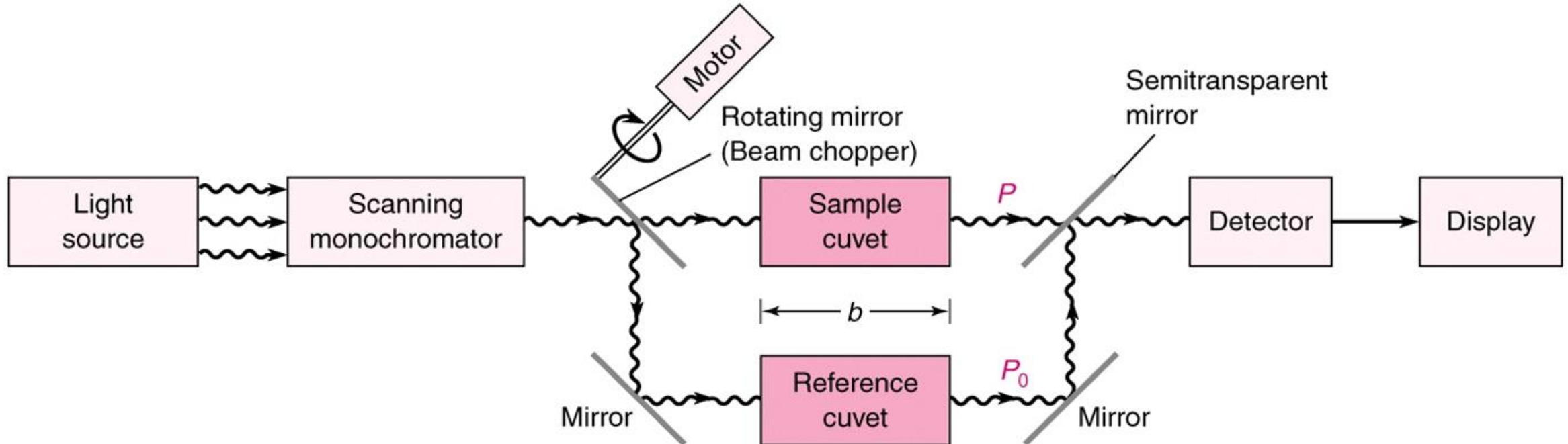


Detector response depends on the λ of the incident photons.

a) Single-beam



b) Double-beam



Calibration of Spectrophotometers

wavelength accuracy

photometric accuracy

stray light

spectral resolution

1-Calibration Standards for Checking the Wavelength Accuracy

Didymium filters

- This glass filter is designed for checking the wavelength calibration of spectrophotometers in both the visible and near infrared regions of the spectrum. The usable range is 430nm to 890nm, instruments of SBW of less than 10nm.

Holmium filters

- This filter is intended exclusively for checking the wavelength of moderate to high resolution spectrophotometers. They are custom made to order to required size and supplied either un-mounted or in anodized holders.



2-Calibration Standards to Check the Photometric Accuracy

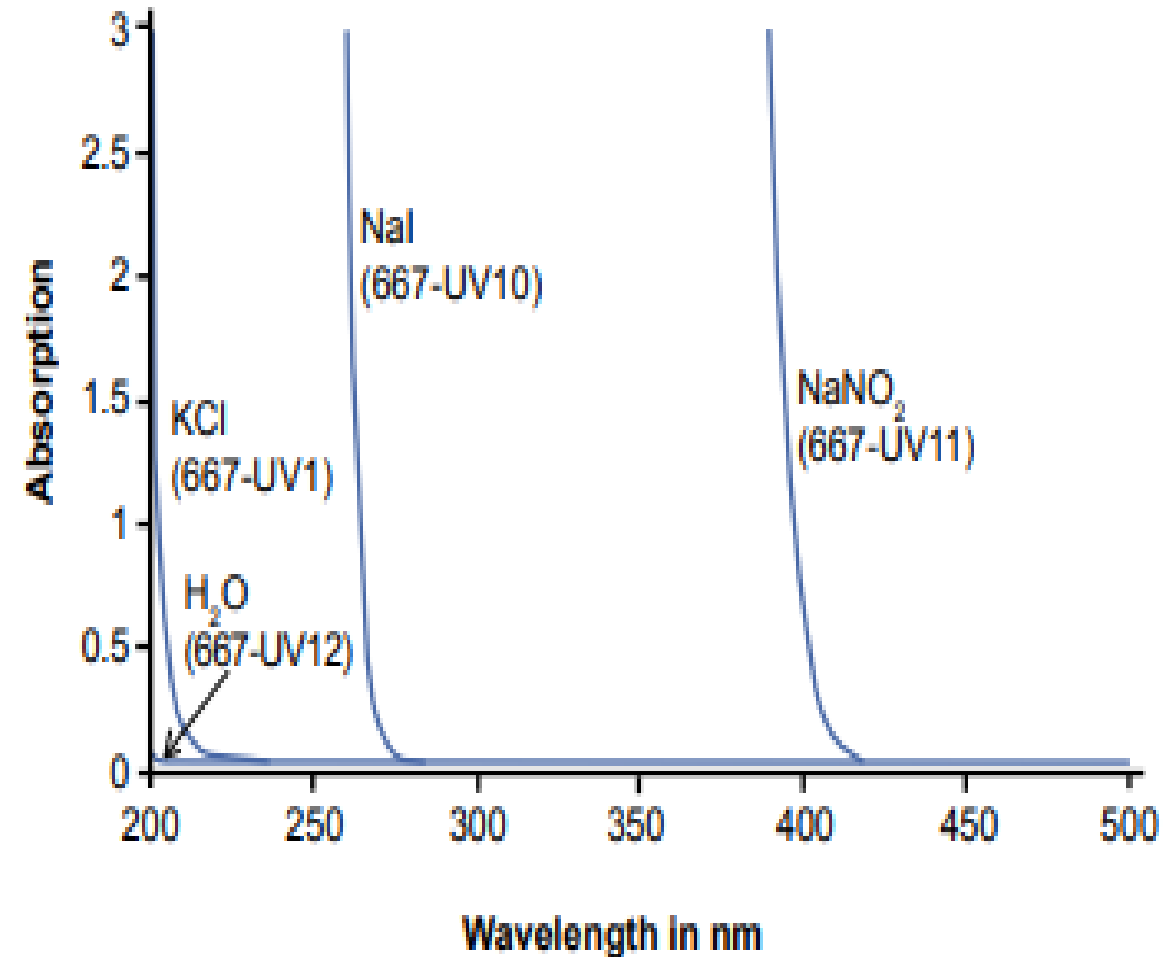
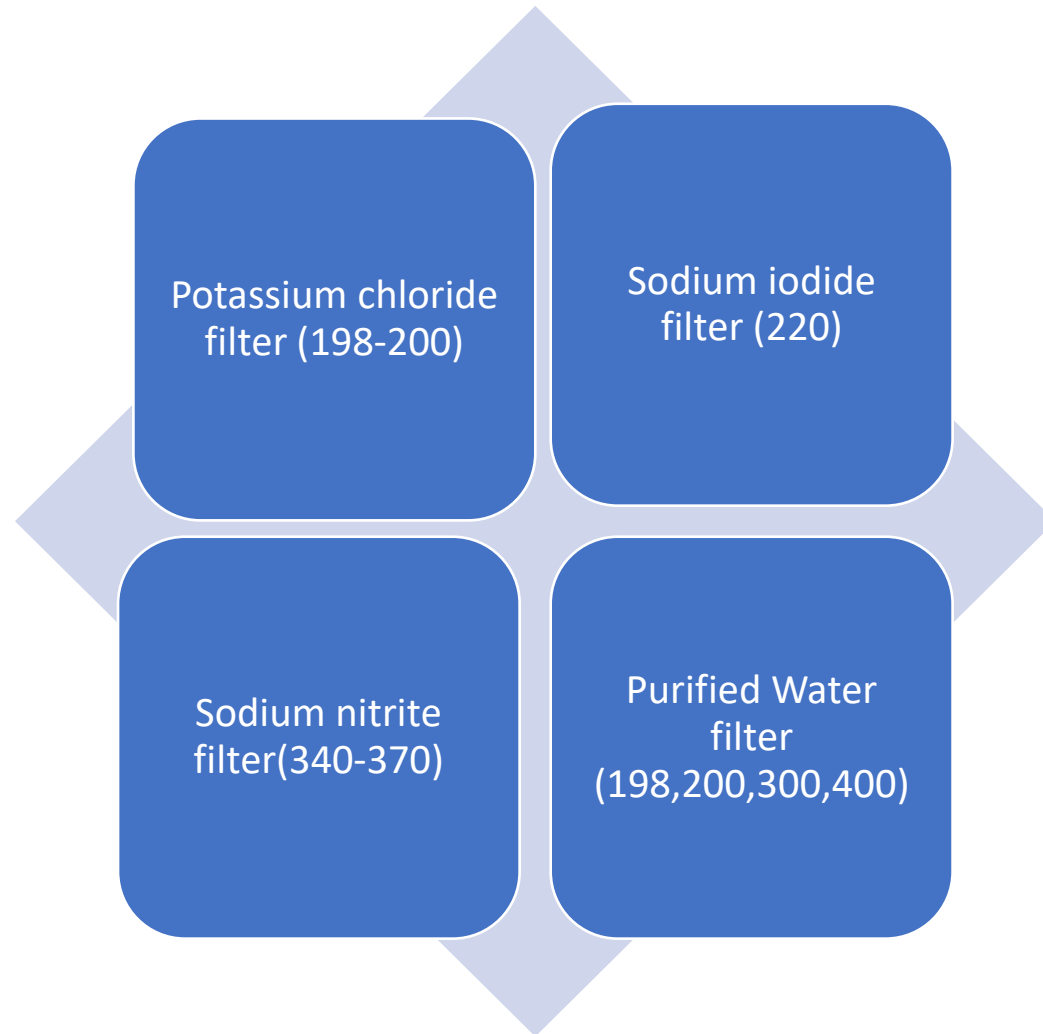
1- Potassium dichromate filter, liquid filter

2- Neutral Density Glass Filters (visible)

3- Didymium Glass Filter (uv)

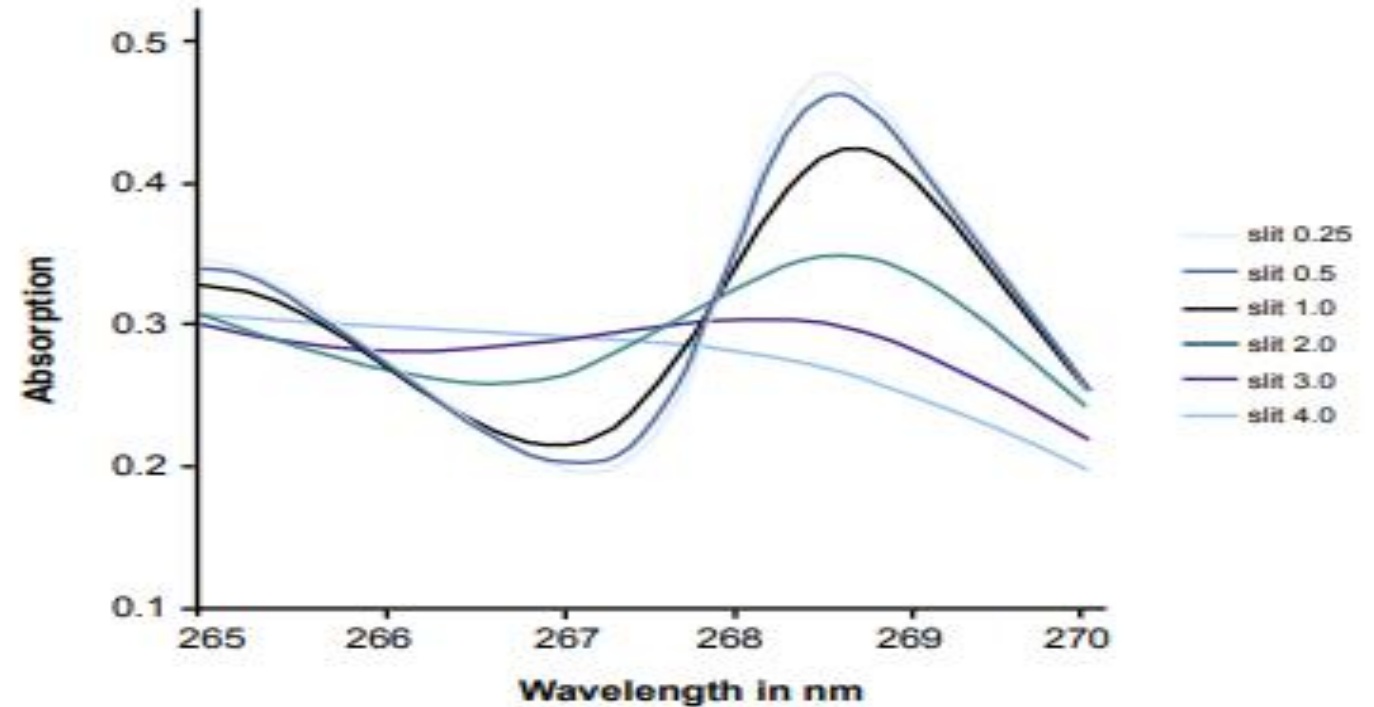


3-Calibration Standards for Checking for Stray Light



4-Calibration Standard to Check the Spectral Resolution

- Toluene Liquid Filter (266-269)

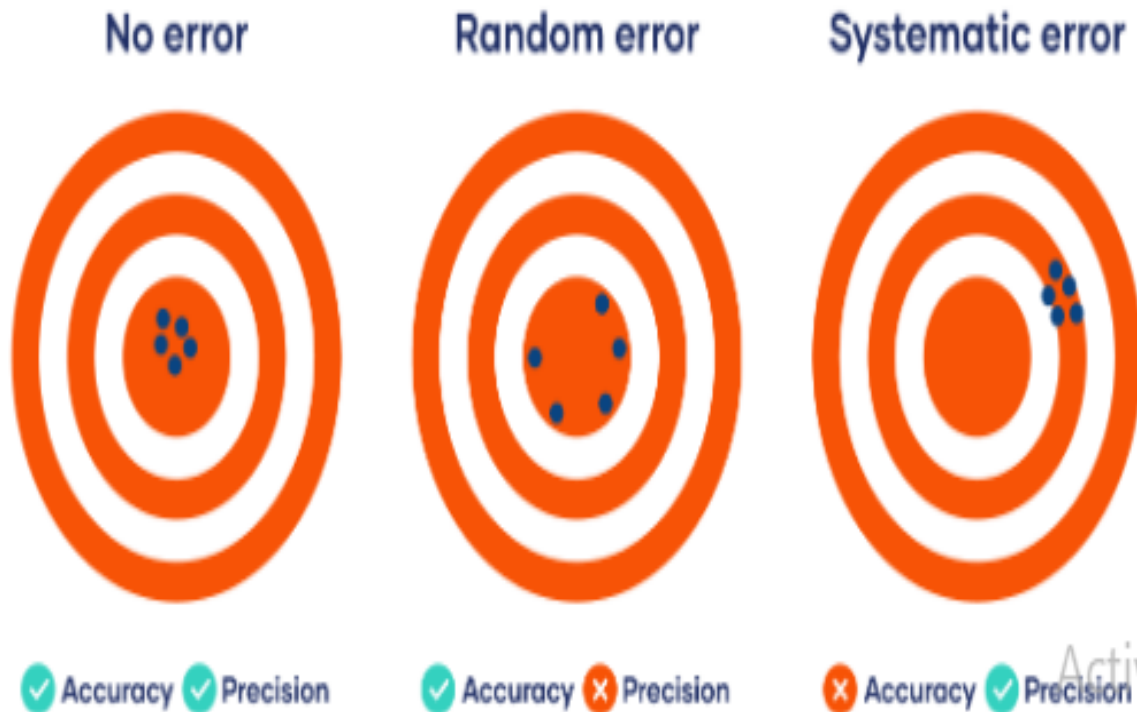


Typical spectra of a toluene filter at different slit widths

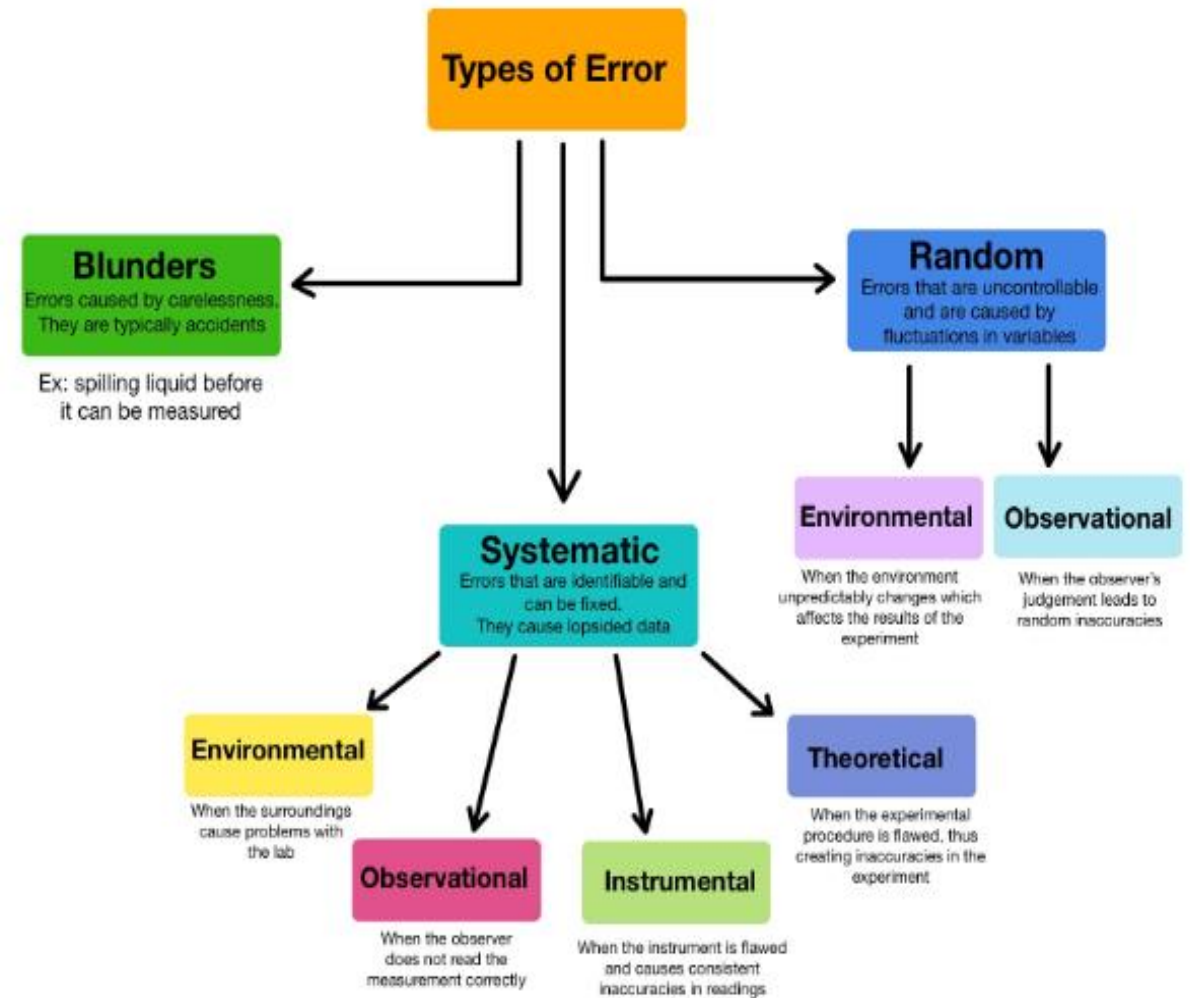
Slit Width	0.25	0.5	1.0	2.0	3.0	4.0
Ratio (Recommended Values)	2.3	2.2	2.0	1.4	1.1	1.0

Errors

Random vs. systematic error



Activat
Go to Se



How to Reduce Systematic error?

Regular maintenance

Regular calibration

Quality control



