

Module 6:

Spectroscopic and Diffraction Techniques

- **Fundamental concepts in spectroscopic and instrumental techniques**
- **Principle and applications of UV-Visible Spectroscopy technique**
- **Principle and applications of X-Ray Diffraction (XRD) technique**

a) Fundamental concepts in spectroscopic and instrumental techniques

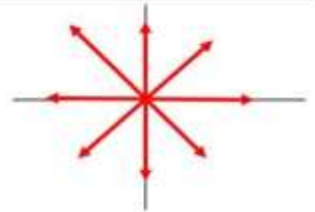
Spectroscopy Basics:

- Spectroscopy is a branch of science that studies the interaction between electromagnetic (EM) radiation and matter.
- ***Spectroscopy is used as a tool for studying the structures of atoms and molecules.***
- *The basic principle shared by all spectroscopic techniques is to shine a beam of EM radiation onto a sample, and observe how it responds to such a stimulus. The response is recorded as a function of radiation wavelength.*
- Visible light is in the form of energy and it travel in waves.

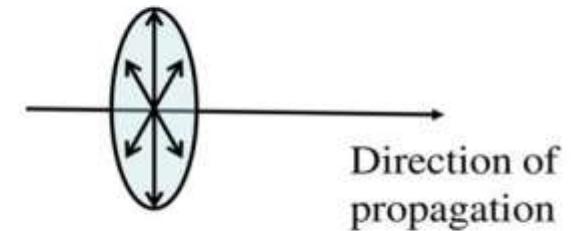
Light is an electromagnetic wave and transverse in nature.

Natural light or ordinary light is unpolarized in nature.

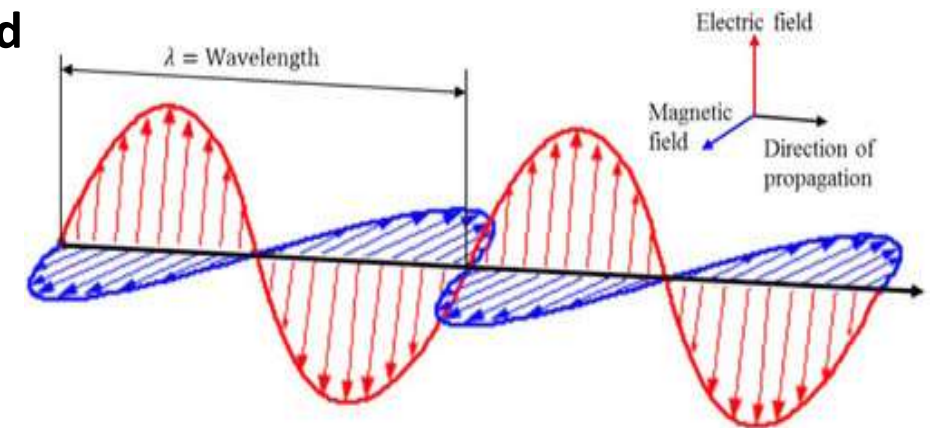
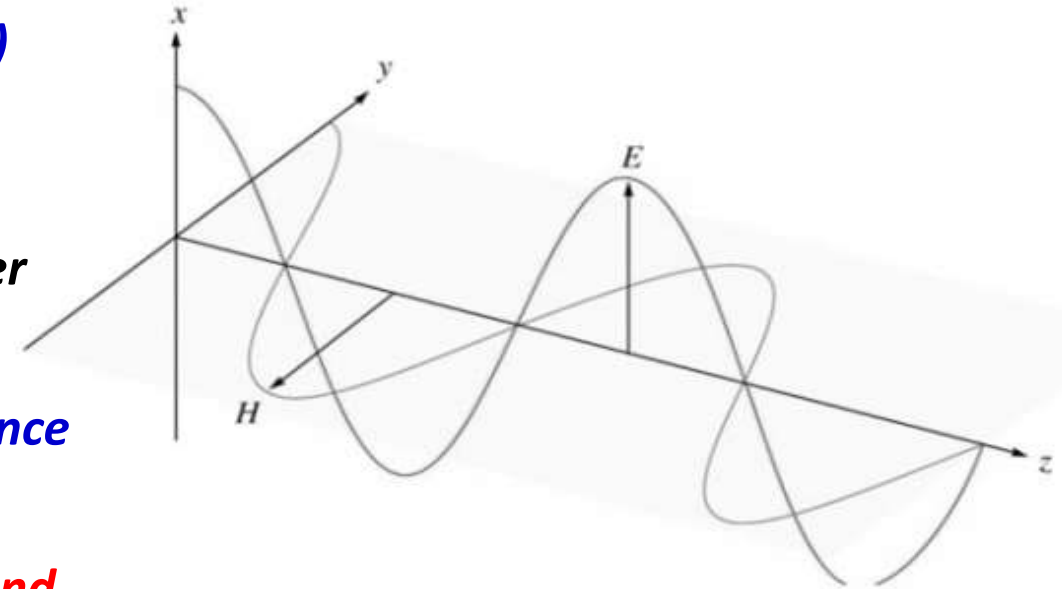
Means vibrations take place symmetrically in all directions in the plane perpendicular to the direction of propagation of light.



Ordinary light



- *As per the wave theory, light travel in the form of waves. It was believed that radiant energy is emitted by fluctuation of electric (E) charge and magnetic (H) field.*
- *Light is electromagnetic radiation, which has oscillating electric (E) and magnetic (H) field vectors in planes perpendicular to each other and to the direction of propagation.*
- *Electric field vector is of most significance for UV/Vis and fluorescence spectroscopy.*
- *The magnetic field vector is important for electron paramagnetic and nuclear magnetic resonance spectroscopy.*
- **Light is a electromagnetic radiation and has properties of a wave and a particle, although having a mass of zero.**
- As a particle, it will interact with matter by transferring its energy E:
- $E = hc/\lambda = h\nu$ (1)
- Where, '***h***' is the Planck constant ($h = 6.63 \times 10^{-34} \text{Js}$) and '***ν***' is the frequency of the radiation.



Types of EM radiation interaction with matter:

- If matter is exposed to EM radiation e.g. IR light (Fig. shown below), the *radiation can be either **absorbed, transmitted, reflected, scattered or undergo photoluminescence.***
- **Photoluminescence** is a term used to designate a number of effects including **Fluorescence, Phosphorescence and Raman scattering.**
- Complement of the absorbed light gets transmitted.
- Color of an object that we see is due to the wavelengths **transmitted or reflected.** Other wavelengths are **absorbed.** **The more absorbed, the darker the color (more concentrated solution).**
- *Interaction of EM radiation with matter is a **quantum phenomenon** and is dependent on both the **properties of radiation and appropriate structural parts of the samples** involved.*
- Origin of EM radiation is due to **energy changes within matter itself.**
- In spectrochemical methods, we measure the absorbed radiation.

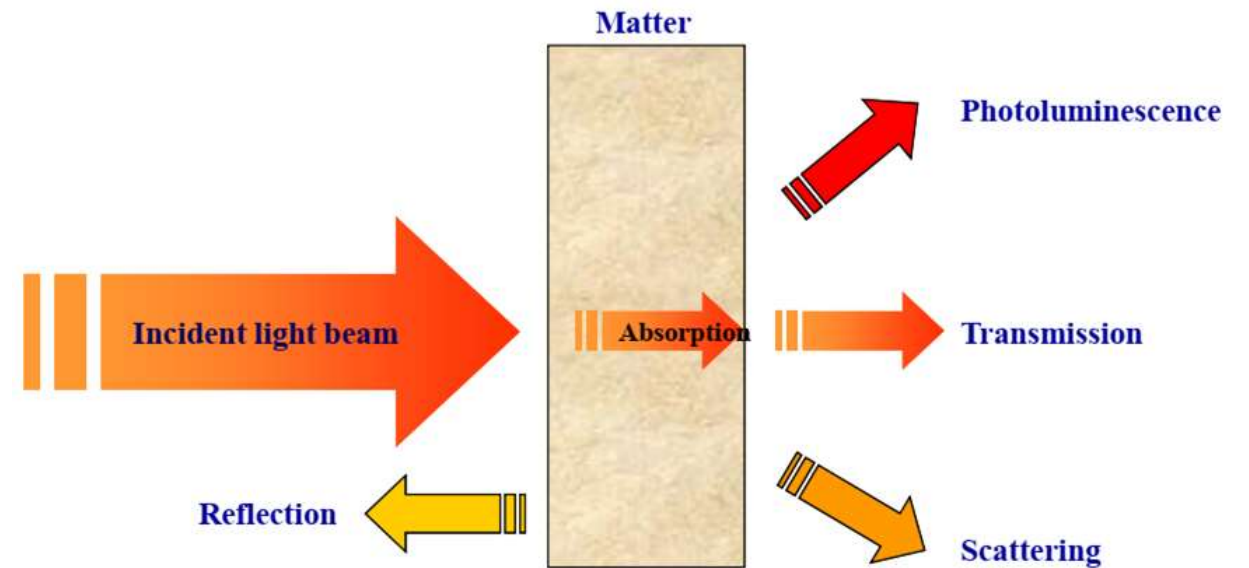
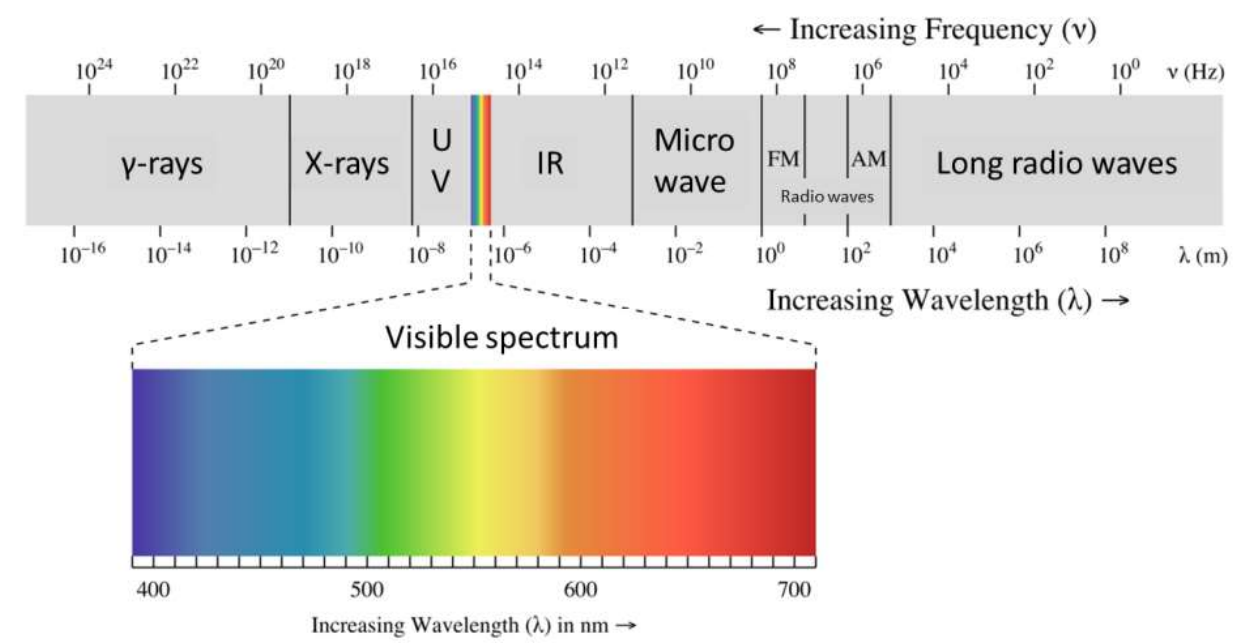


Fig. Schematic representation of interaction of radiation and matter

- Like light, there are many forms of electromagnetic radiations such as Ultra-violet (UV), Infra-red (IR), X-rays, Radio waves etc.
- Electromagnetic spectrum is the range of frequencies (the spectrum) of electromagnetic radiation and their corresponding wavelengths and photon energies.*
- Electromagnetic radiation used in spectrum covers an enormous range of frequencies from **wireless waves of lowest frequencies (or highest wavelengths)** to **γ -rays and cosmic rays which have very high frequencies.**
- The electromagnetic radiation may be divided into various regions, as shown in table, based in the wavelengths in meters.*



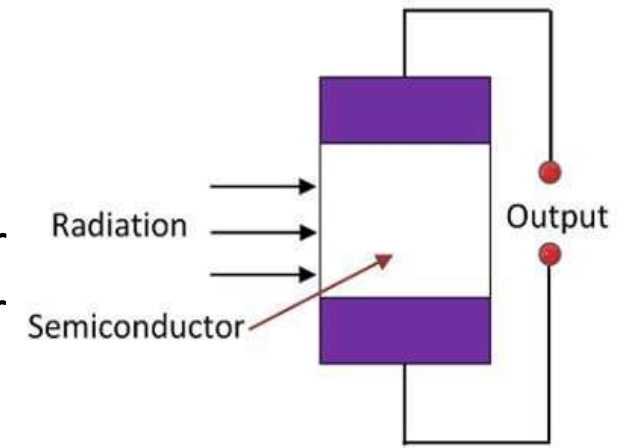
Name of the light	Frequency (Hz)	Wavelength Range	Motions probed
Microwave	$10^9 - 10^{13}$	1cm to 100μm	Rotations
Infrared	$10^{13} - 10^{14}$	100μm to 1μm	Vibrations
Visible and Ultraviolet (UV-Vis)	$10^{14} - 10^{16}$	1μm to 10nm	Electronic Transitions

Absorption and Emission spectra:

- **Absorption Spectra** are produced when light from a source that gives out a continuous range of wavelength is passed through matter (solid or liquid or gas or vapour) which is at lower temperature than the source of light.
- **Emission spectra** are produced by heating a substance directly in a flame or heating it electrically and passing the emitted radiation through a prism.
- If the *some substance under analysis is passed through under certain wavelength range of electromagnetic radiations, the radiation of certain wavelengths are absorbed by the substance.*
- *The wavelengths that are absorbed characterize particular functional groups present in the compound. The dark pattern of lines are corresponding to the wavelengths absorbed is called absorption spectrum.*
- In absorption spectrum, transmitted light is analyzed by the spectrometer relative to the incident light of given frequency.

Spectrometric Instruments:

- Ultraviolet-Visible (UV-Vis), Atomic Absorption Spectroscopy (AAS) and Atomic Emission Spectroscopy (AES) are used for **measurement of substances**.
- IR, Raman, X-ray Fluorescence (XRF), Energy-dispersive X-ray (EDX) and Nuclear Magnetic Resonance (NMR) spectroscopy techniques are mainly used for **characterization of substances**.
- **Intensity of the radiation** is mostly measured with a **photoelectric transducer**.
- *The large number of wavelengths emitted by these systems makes it possible to investigate their electron configurations of ground and various excited states.*



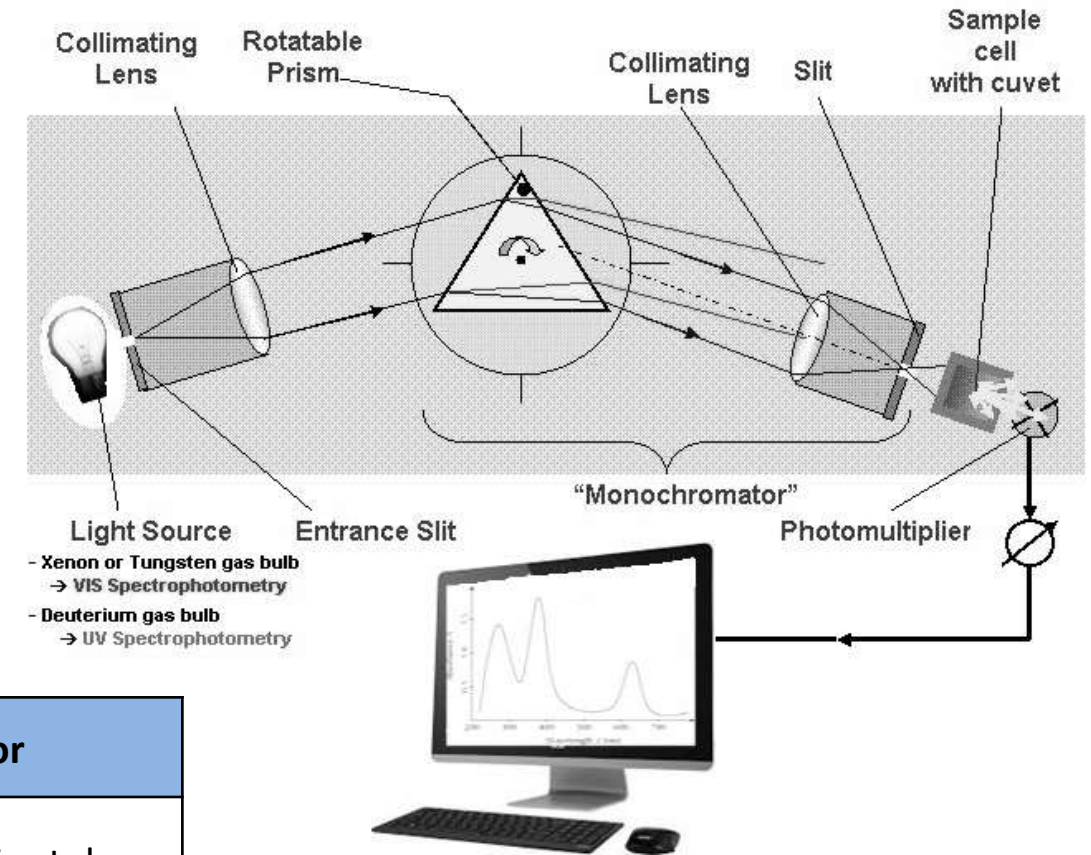
Photoelectric Transducer

Spectrometer:

It is a device that is used for producing a spectrum and making measurements (frequency and intensity measurements) on the spectrum.

It comprises of...

- a source of radiation
- a dispersing unit (prism) and
- a detector



UV-Vis Spectrophotometer

Spectral region	Source	Dispersing element	Detector
Ultraviolet (UV)	Hydrogen discharge tube	Quartz prism or grating	Photomultiplier tube
Visible	Tungsten lamp	Glass prism or grating	Photomultiplier
Infrared (IR)	Heated ceramic element	Rock salt prism or grating	Thermocouple
Microwave	Klystron tube	-	Quartz crystal detector

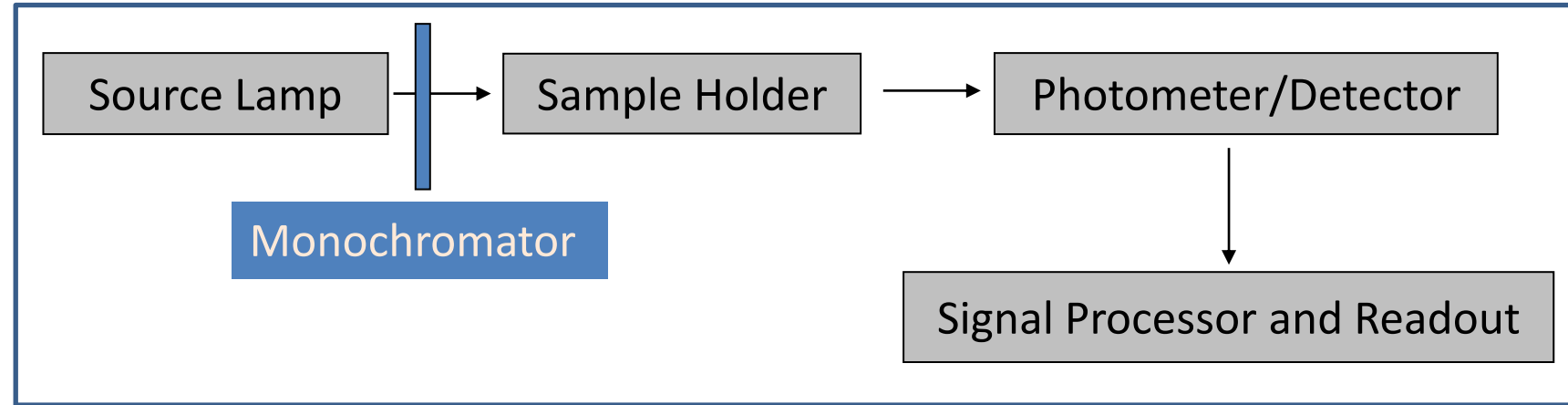
Components of a UV-Vis Spectrophotometer

Source lamp

- *Tungsten filament* incandescent lamp used in *Visible and adjacent parts of UV and IR regions*.
- Hydrogen or deuterium discharge lamps are used in 160~360 nm (UV region).
- *Deuterium lamps provide maximum intensity.*
- Source used in UV- Vis spectroscopy should meet the following criteria:
 - (i). *Beam produced should be in the detectable and measurable range,*
 - (ii). *Should serve as a continuous source of energy and*
 - (iii). *Should be stable.*

Monochromator

- Filter the energy source so that a limited portion is allowed to be incident on the sample.
- Gratings are normally used as monochromators.
- A particular wavelength can be selected using monochromator.



Sample holder

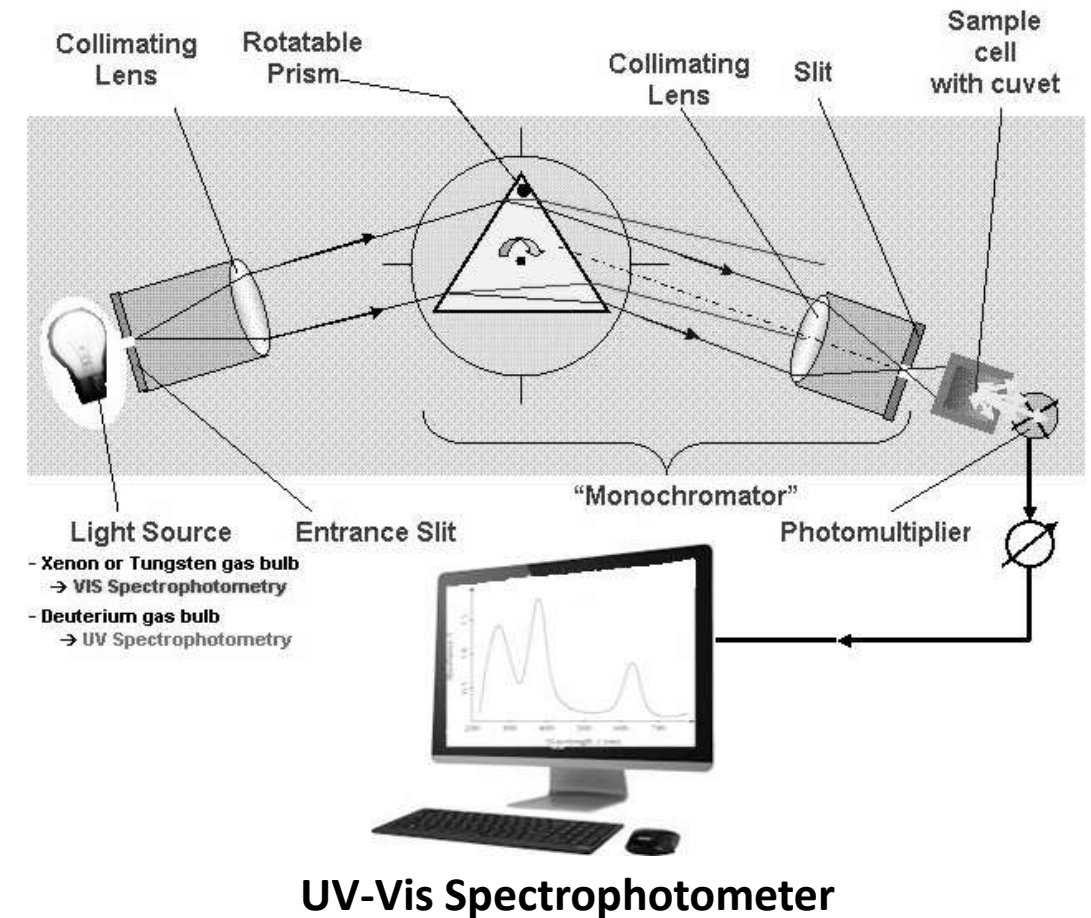
- The selection of material used for constructing the *cuvette* is based on the selected range of measurement.
- Cuvette thickness depends on the absorption intensity. Cuvettes with varied shapes are used (rectangular, cylindrical or cylindrical with flat ends).
- *Cell thickness: 1, 2 and 5 cm.*
- Main factor is that the cuvette window should be normal to the beam direction.
- Requirement of cuvettes in terms of its make and thickness: *UV region – quartz and Visible region – glass or quartz cells.*

Photometer/Detector

Mechanism behind the photoelectric devices is the **conversion of radiant energy to electrical signal**. Basically, 3 types of photometers are used: **(a). Photovoltaic cells, (b). Phototubes and (c). Photoconductive cells.**

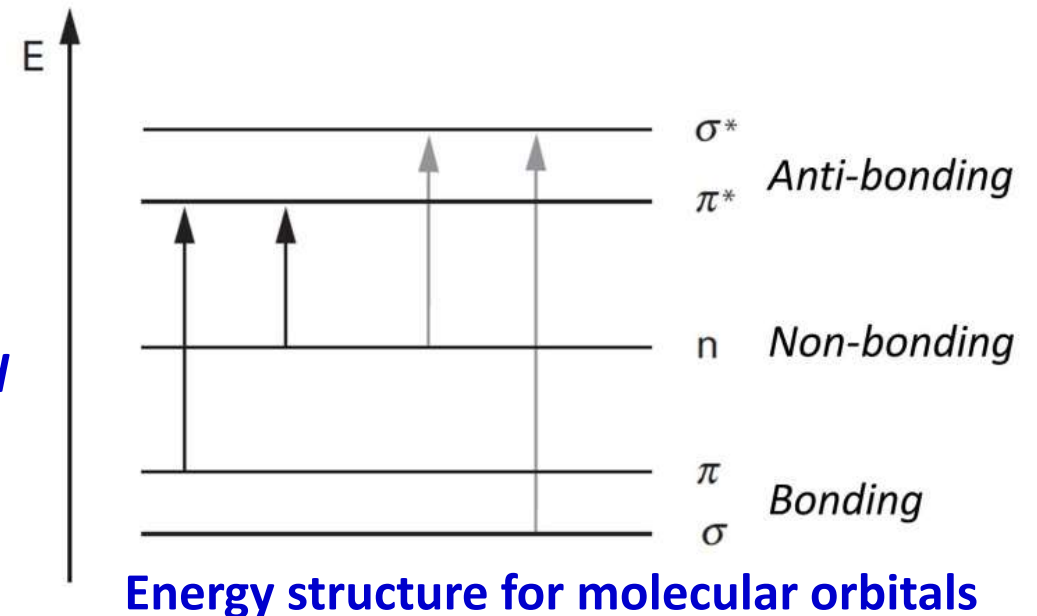
Signal processing

Electrical signal generated by the transducer is sent to the **signal processor**, where it is displayed in a more convenient form for the analyst.



Absorption spectrum

- The *plot of absorption probability against wavelength* is called **absorption spectrum**.
- In the simpler instance of **single atoms**, electronic transitions lead to the occurrence of **line spectra**.
- **An excited state is result of the energetically favoured electron elevation from the highest occupied molecular orbital (HOMO) to the lowest unoccupied molecular orbital (LUMO).**
- Since the **existence of more different kinds of energy levels**, **molecular spectra are usually observed as band spectra which are molecule-specific (due to the distinctive vibration states).**
- **Ultraviolet and visible (UV-Vis) radiation interacts with matter which causes electronic transitions (elevation of electrons from the ground state to a high energy state).**
- The range of **ultraviolet region** is between **$\approx 190\text{-}380\text{ nm}$** and the **visible region** is **between $\approx 380\text{-}750\text{ nm}$** .
- Arrows specify the possible electronic transitions.
- The length of the arrows specifies the energy required to be given into the system in order to enable the transition.
- **Black arrows represent the probable transitions with energies from the UV-Vis spectrum for some organic and biological molecules.**
- **The grey arrows transitions require higher energies (e.g. X-rays).**



UV – Visible spectroscopy

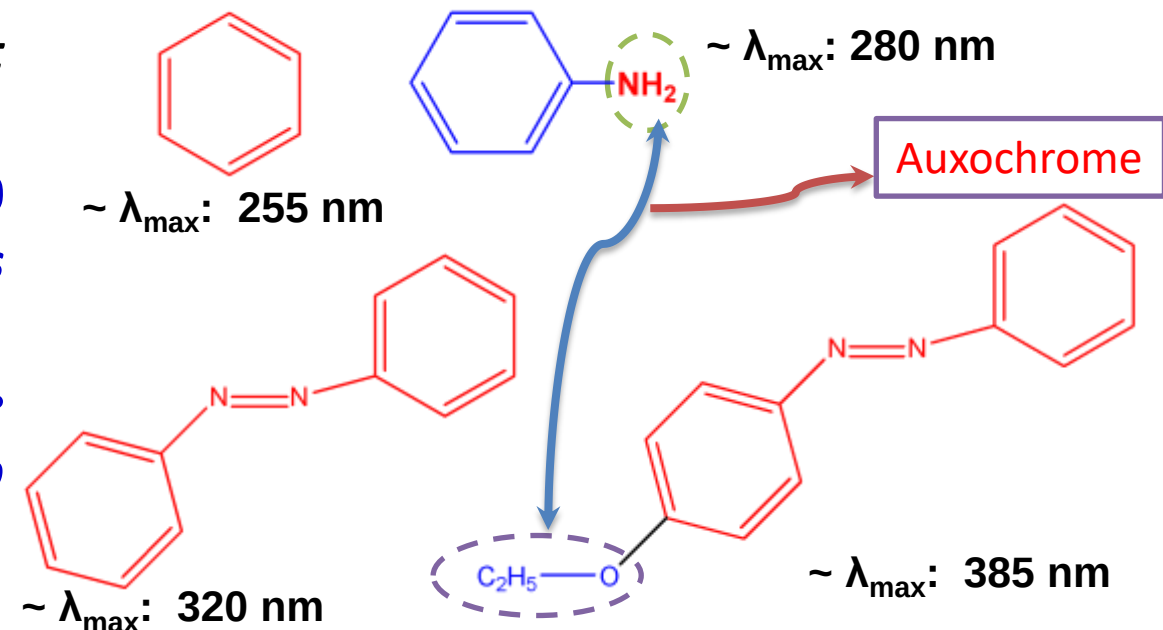
- UV-Visible regions of the electromagnetic spectrum and their associated techniques are probably the **most widely used for analytical work and research into chemical/biological problems**.
- The electronic transitions in molecules can be classified according to the participating molecular orbitals.
- There are four possible transitions ($n \rightarrow \pi^*$, $\pi \rightarrow \pi^*$, $n \rightarrow \sigma^*$, $\sigma \rightarrow \sigma^*$), from which **only two can be elicited** with light from the **UV-Vis spectrum** for some organic/biological molecules: **$n \rightarrow \pi^*$ and $\pi \rightarrow \pi^*$** .
- The **$n \rightarrow \sigma^*$ and $\sigma \rightarrow \sigma^*$ transitions require higher energies** and are **energetically not within the range of UV-Vis spectroscopy**.
- Molecular (sub-) structures accountable for interaction with electromagnetic radiation are called chromophores.

Chromophore: Any isolated covalently bonded group that shows a characteristic absorption in the UV-Vis. region.

The only molecular moieties likely to absorb light in the **200 to 800 nm region** are **π -electron functions and hetero atoms having non-bonding electron pairs**.

Auxochrome: Group of atoms attached to a chromophore which modifies the ability of that chromophore to absorb light.

Ex. COOH , $-\text{OH}$, $-\text{SO}_3\text{H}$, $-\text{NH}_2$, $-\text{NH-R}$, $-\text{N-R}_2$

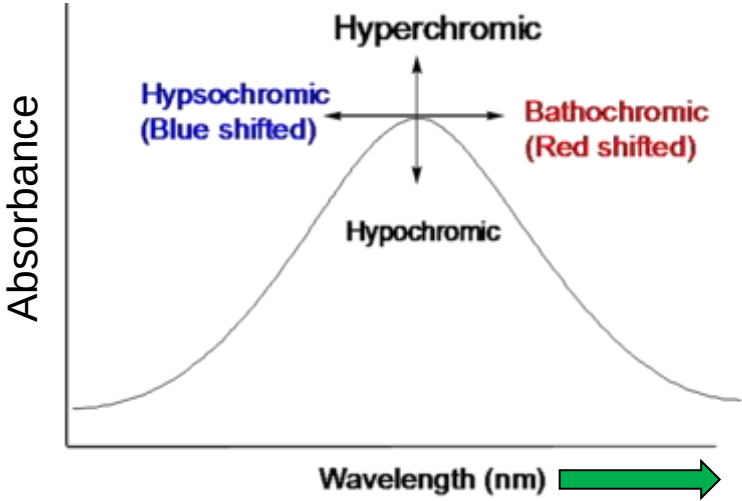


- Chromophores in the table, it can be noticed that the only molecular moieties with π -electron functions and hetero atoms having non-bonding valence-shell electron pairs are likely to absorb light in the 200 to 800 nm region.
- In alcohols and ethers, the oxygen non-bonding electrons do not give rise to absorption above 160 nm. *Hence, pure alcohol and ether solvents can be used for spectroscopic studies.***

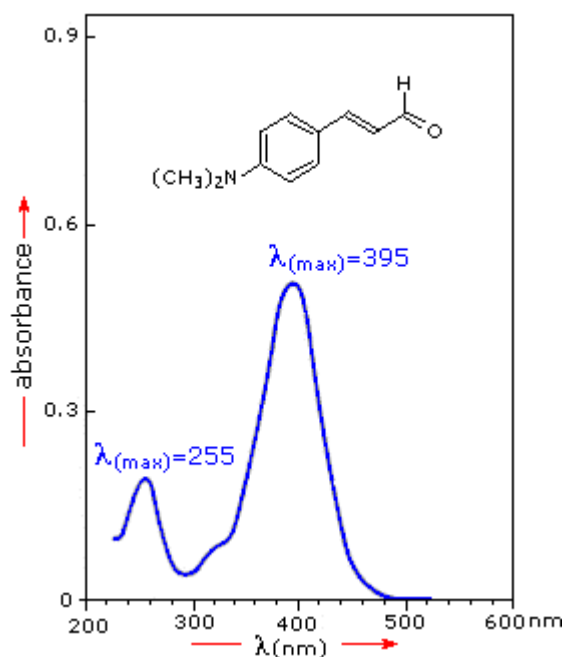
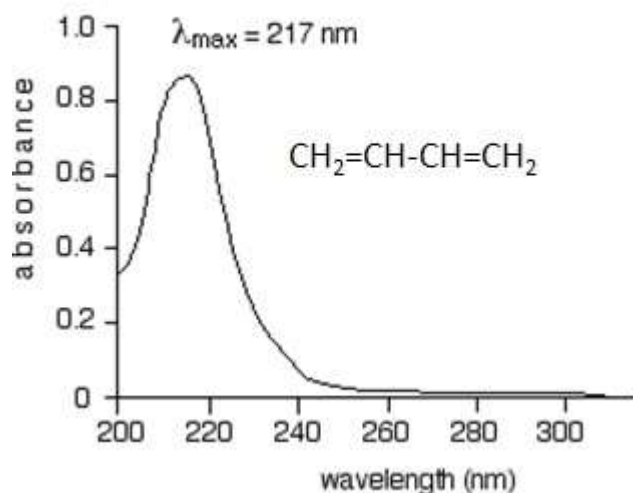
Chromophore	Example	Excitation	λ_{max} (nm)	ϵ	Solvent
C=C	Ethene	$\pi \rightarrow \pi^*$	171	15,000	Hexane
C $\equiv$ C	1-Hexyne	$\pi \rightarrow \pi^*$	180	10,000	Hexane
C=O	Ethanal	$n \rightarrow \pi^*$	290	15	Hexane
		$\pi \rightarrow \pi^*$	180	10,000	Hexane
N=O	Nitromethane	$n \rightarrow \pi^*$	275	17	Ethanol
		$\pi \rightarrow \pi^*$	200	5,000	Ethanol
C-X: X = Br or I	Methyl bromide	$n \rightarrow \sigma^*$	205	200	Hexane
	Methyl iodide	$n \rightarrow \sigma^*$	255	360	Hexane

Based on the functional group present and attached to chromophores...

- Bathochromic shift: absorption maximum shifted to longer wavelength (Blue to Red [**Red shift**]).
- Hypsochromic shift: absorption maximum shifted to shorter wavelength (Red to Blue [**Blue shift**]).
- Hyperchromism: increase in molar absorptivity
- Hypochromism: decrease in molar absorptivity



Applications of UV-Vis spectrophotometer:

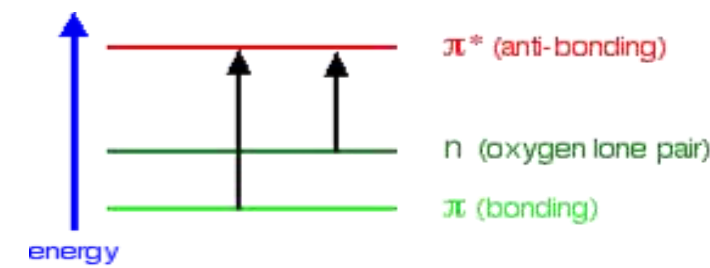


Qualitative analysis:

- Identification of chromophores by scanning the absorbance at each wavelength.
- *In UV-Visible spectroscopy, the presence of chromophores in a molecule is accurately identified, but **most instruments fail to provide absorption data for wavelengths below 200 nm**, thus detection of isolated chromophores is problematic. Fortunately, due to conjugation, the absorption maxima generally move to longer wavelengths.*
- In the Fig. absorbance in **UV-Visible absorption spectrum** for 1,3-butadiene is just a **measure of the amount of light absorbed**, **there are no non-bonding electrons. Indicates that the only electron moves from π bonding to π anti-bonding ($\pi \rightarrow \pi^*$) orbitals.**
- In the case of **unsaturated aldehyde**, the conjugation becomes the major structural feature identified by this technique.
- **The absorption extends into the visible region of the spectrum, and this compound is orange colored.**

Quantitative analysis:

- ❖ Absorbance at a particular λ_{max} can be measured using photometry mode.
- ❖ For structure–function studies of haem proteins, these changes can be used.
- ❖ In **nucleic acids** [adenine, guanine (purine) and cytosine, thymine, uracil (pyrimidine) bases], the UV light absorption is observed between 260 nm and 275 nm is due to $n \rightarrow \pi^*$ and $\pi \rightarrow \pi^*$ transitions.
- ❖ These bases *absorption spectra* in polymers are *sensitive to pH* and *highly influenced due to the electronic interactions between bases*.
- ❖ Determining the *reaction rate and pK_a values (dissociation constants) of weak acids or bases*.
- ❖ Determining the *percentages of keto and enol forms*.
- ❖ To analyze *metals in waste water*.
- ❖ Determining *total serum protein, serum cholesterol*, etc.
- ❖ Characterizing *pharmaceuticals, food, paint, glass and metals*.



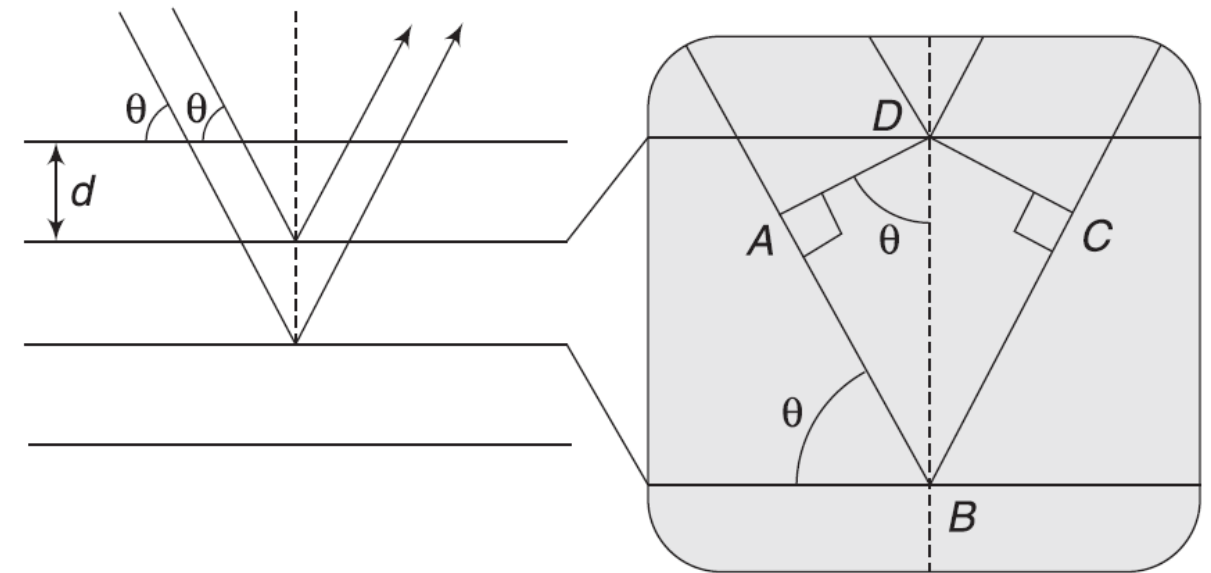
Example	λ_{max}	Transition
$\text{C}_6\text{H}_{13}\text{CH}=\text{CH}_2$	177	$n \rightarrow \pi^*$
$\text{C}_5\text{H}_{11}\text{C}\equiv\text{C}\cdot\text{CH}_3$	178	$n \rightarrow \pi^*$
	196	-
	225	-
$\text{CH}_3\text{-CO-CH}_3$	186	$n \rightarrow \sigma^*$
	280	$n \rightarrow \pi^*$
$\text{CH}_3\text{-CHO}$	180	$n \rightarrow \sigma^*$
	293	$n \rightarrow \pi^*$
$\text{CH}_3\text{-COOH}$	240	$n \rightarrow \pi^*$
$\text{CH}_3\text{-CO-NH}_2$	214	$n \rightarrow \pi^*$
$\text{CH}_3\text{N}=\text{NCH}_3$	339	$n \rightarrow \pi^*$
CH_3NO_2	280	$n \rightarrow \pi^*$
$\text{C}_4\text{H}_9\text{NO}$	300	-
	665	$n \rightarrow \pi^*$
$\text{C}_2\text{H}_5\text{NO}_2$	270	$n \rightarrow \pi^*$

Diffraction and Scattering

- Diffraction refers to a phenomena which occur when a wave encounters an obstacle.
- Diffraction is a slight modification of light as it passes by edges of opaque bodies or through narrow slits or reflecting from ruled surfaces.
- The amount of diffraction depends on the wavelength of light and the opening's size.

X-Ray diffraction

- The diffraction pattern provides structural information of the crystalline substance, when **X-rays are scattered by the atoms of a crystal that creates interference effect.**
- Diffraction of X-ray event on a crystal is shown in Figure.
- In a crystal with the periodic arrangement atoms, a set of planes are arranged in a one dimensional out of the three-dimensional grating and X-ray incident at a grazing angle of θ as shown in Figure.



A depiction of Bragg's law. Reflection of X-rays from parallel lattice planes.

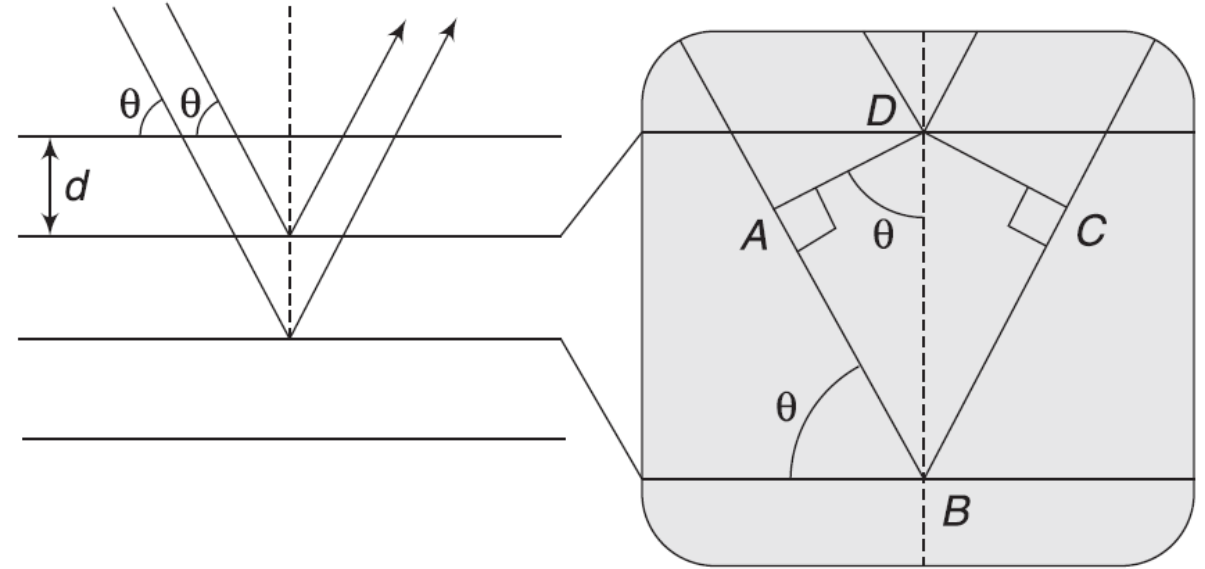
- The phase difference between the waves reflected from two successive planes is λ or its integer multiple, then the reflected X-ray intensity will be maximum.

This occurs when,

$$2 d \sin \theta = n \lambda$$

where, n is a positive integer,
 d is the lattice spacing,
 2θ the glancing angle.

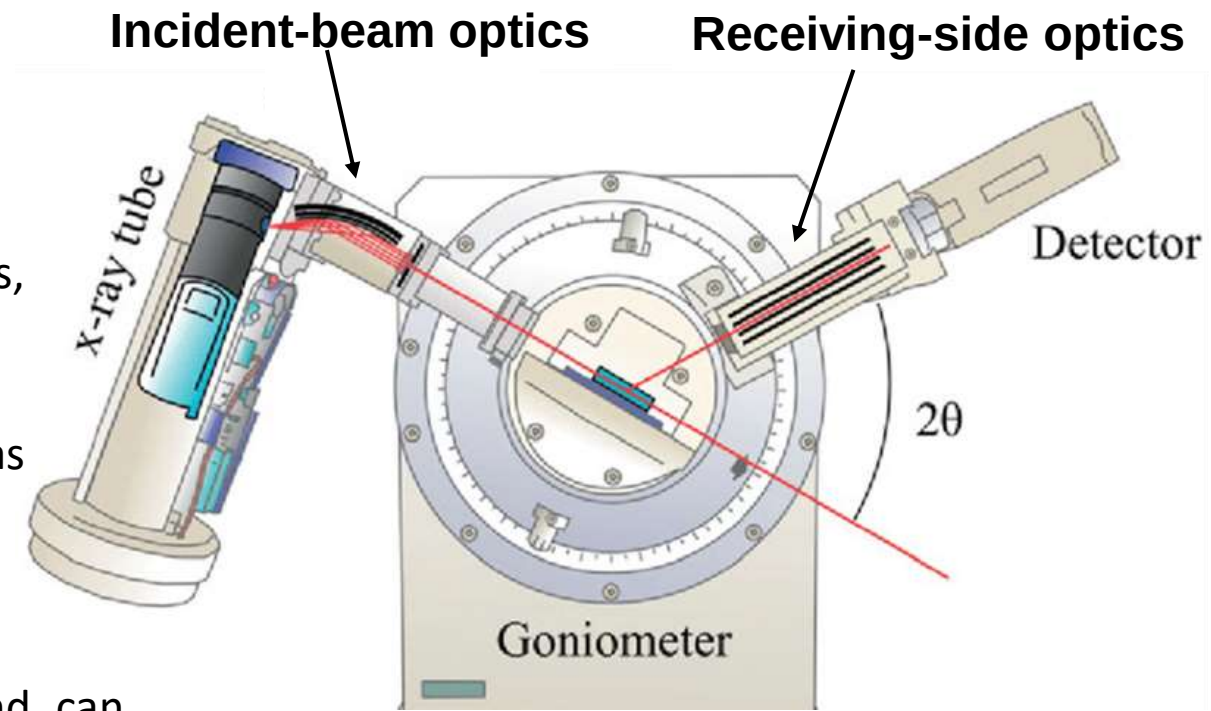
- This equation is referred as Bragg's Law.
- The diffraction also can occur from other planes as shown in Figure .



A depiction of Bragg's law. Reflection of X-rays from parallel lattice planes.

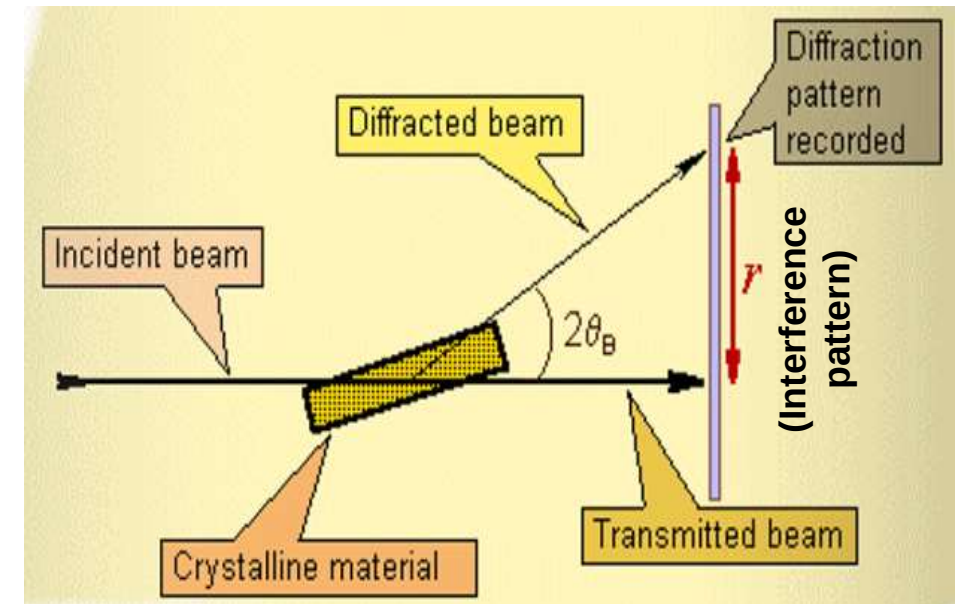
Components of X-Ray Diffraction (XRD) technique

- **X-ray tube:** source of X-rays
- **Incident-beam optics:** condition the X-ray beam before it hits the sample.
- **Goniometer:** platform that holds and moves the sample, optics, detector, and/or tube.
- **Sample holder**
- **Receiving-side optics:** condition the X-ray beam after it has encountered the sample.
- **Detector:** count the number of X-rays scattered by the sample.



How XRD pattern produced?

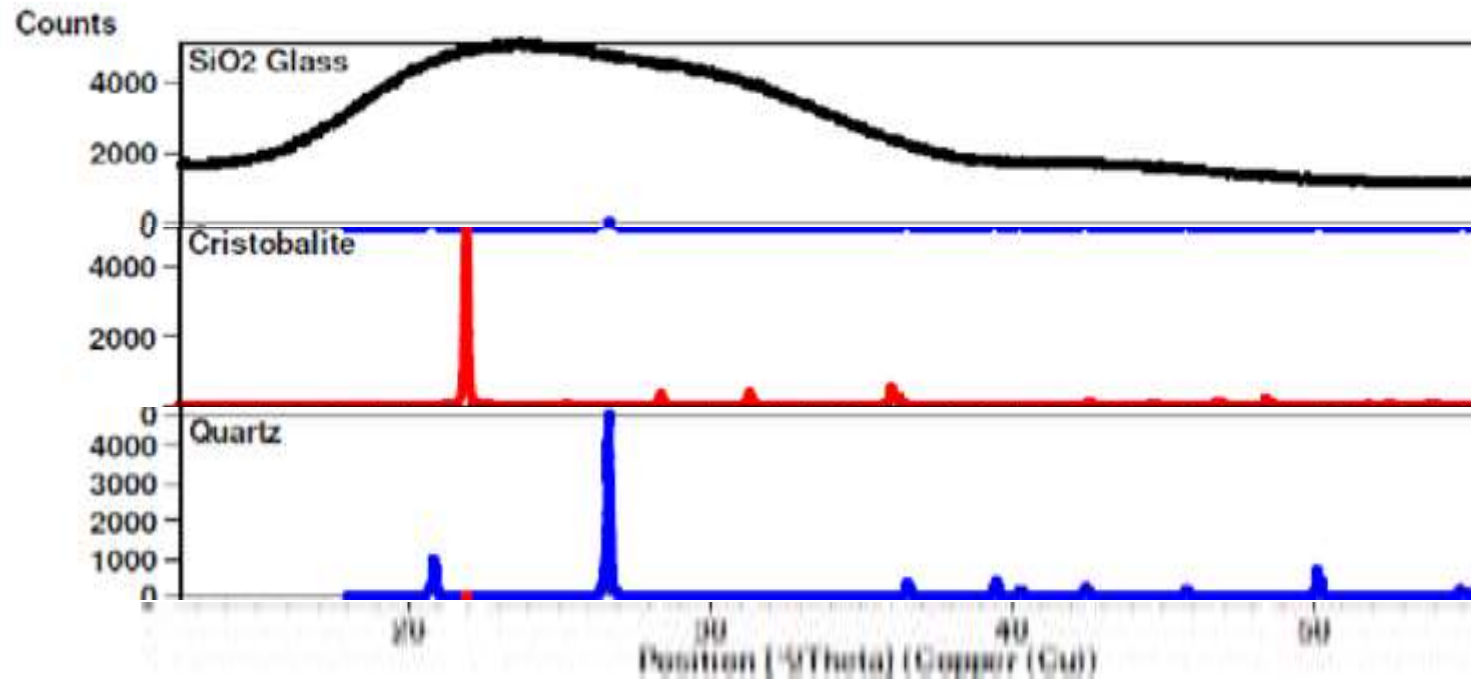
- Crystalline atoms are a periodic array of coherent scatterers and can diffract X-rays.
- The wavelength of X-rays are similar to the distance between atoms.
- Diffraction from different planes of atoms produces a diffraction pattern, which contains information about the atomic arrangement within the crystal.
- When X-ray hits the crystal planes at specific angles and the diffracted waves are in the same phase, then constructive interference (a peak) will be produced.
- Based on structure of the crystal (and crystal planes), the angles at which diffraction occurs may vary.



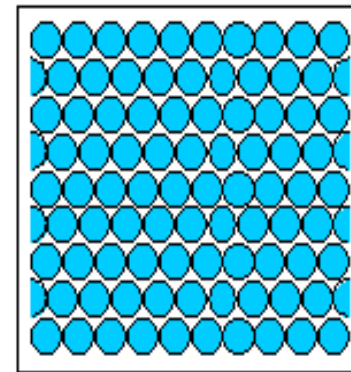
Applications of X-Ray Diffraction (XRD) technique

- XRD is a **versatile, non-destructive characterization technique** widely used in materials science and engineering for identifying unknown crystalline materials.
- XRD works by irradiating a material with incident X-rays, and then measuring the intensities and scattering angles of the X-rays that leave the material.
- XRD is used to study the **structure and function of many biological molecules, including vitamins, drugs, proteins and nucleic acids such as DNA.**
- XRD is also used to determine structural properties like....
 - lattice parameters, strain, grain size,*
 - epitaxy, phase composition, preferred orientation,*
 - to measure thickness of thin films and multi-layers*
 - to determine atomic arrangement*
- XRD also yields information on **how the actual structure deviates from the ideal one, owing to internal stresses and defects.**

XRD patterns for 3 different forms of SiO_2

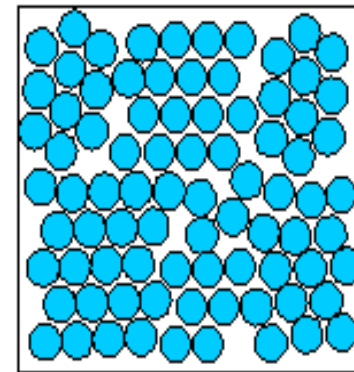


- These three phases of SiO_2 are chemically identical.
- The **amorphous glass** does not have long-range atomic order and therefore produces only broad pattern.
- **Cristobalite form polycrystalline** structure.
- **Quartz form single crystal** structure.



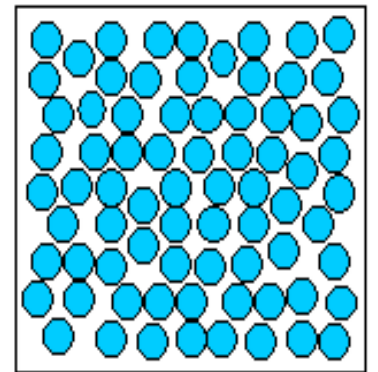
Single crystal

Periodic across the whole volume.



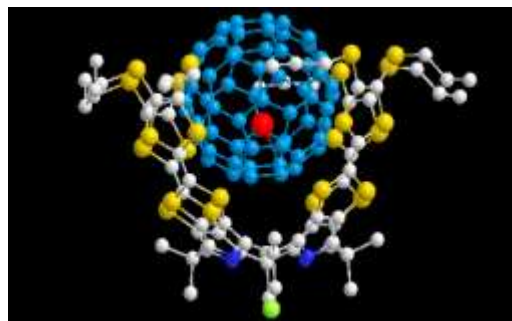
Polycrystal

Periodic across each grain.

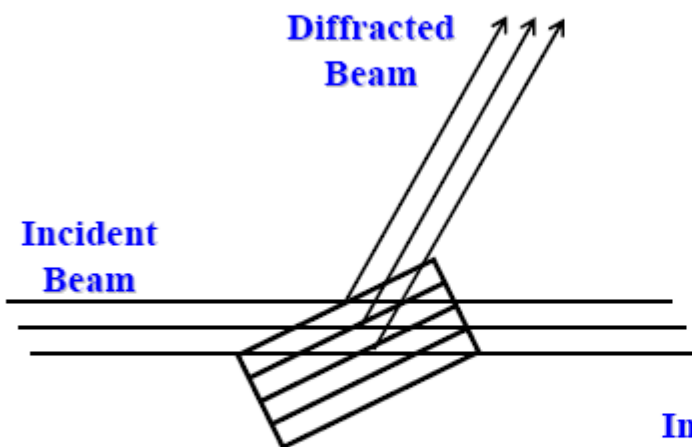


Amorphous solid

Not periodic.

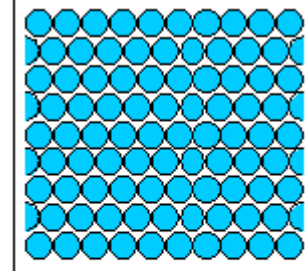
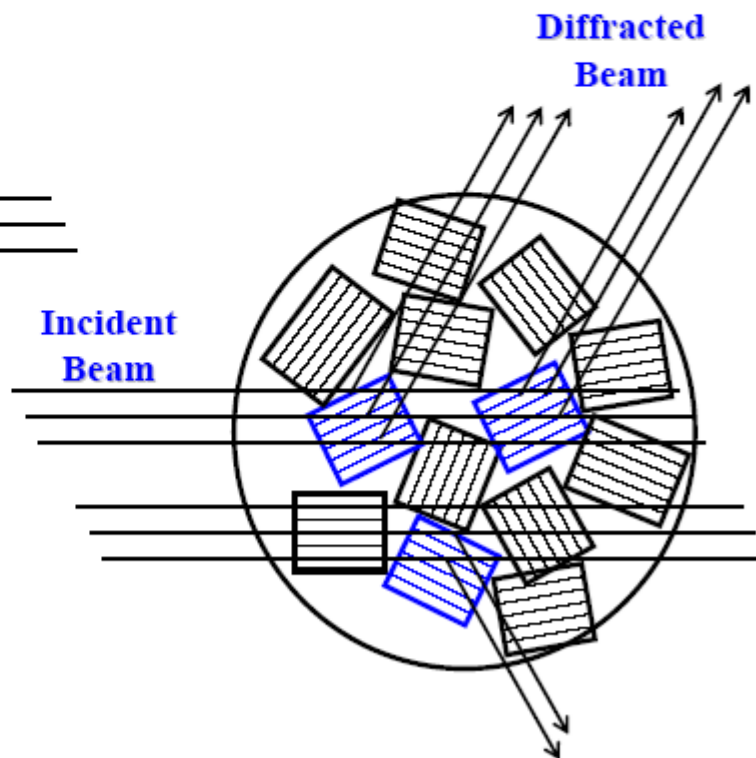


Single Crystal Diffraction

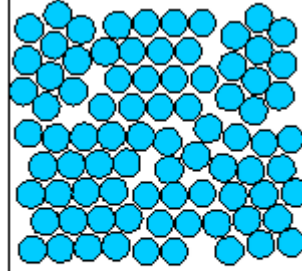


In powder diffraction only a small fraction of the crystals (shown in blue) are correctly oriented to diffract.

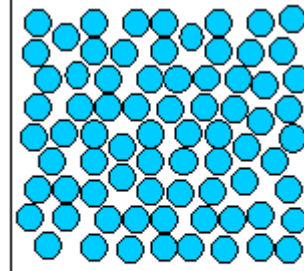
Powder Diffraction



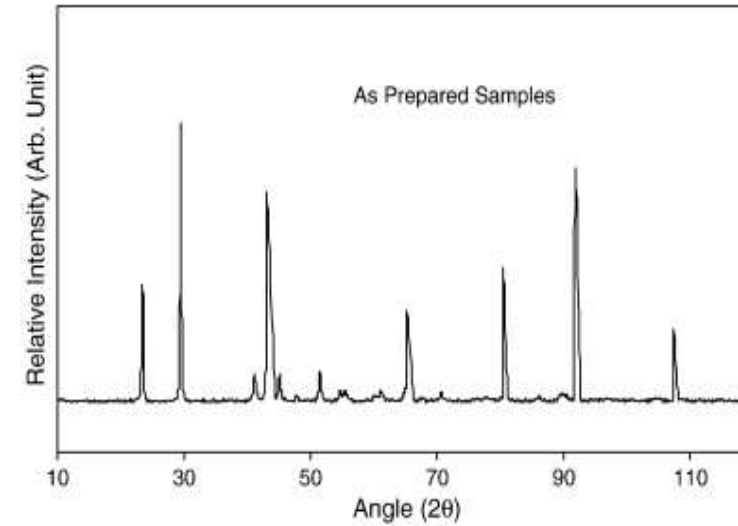
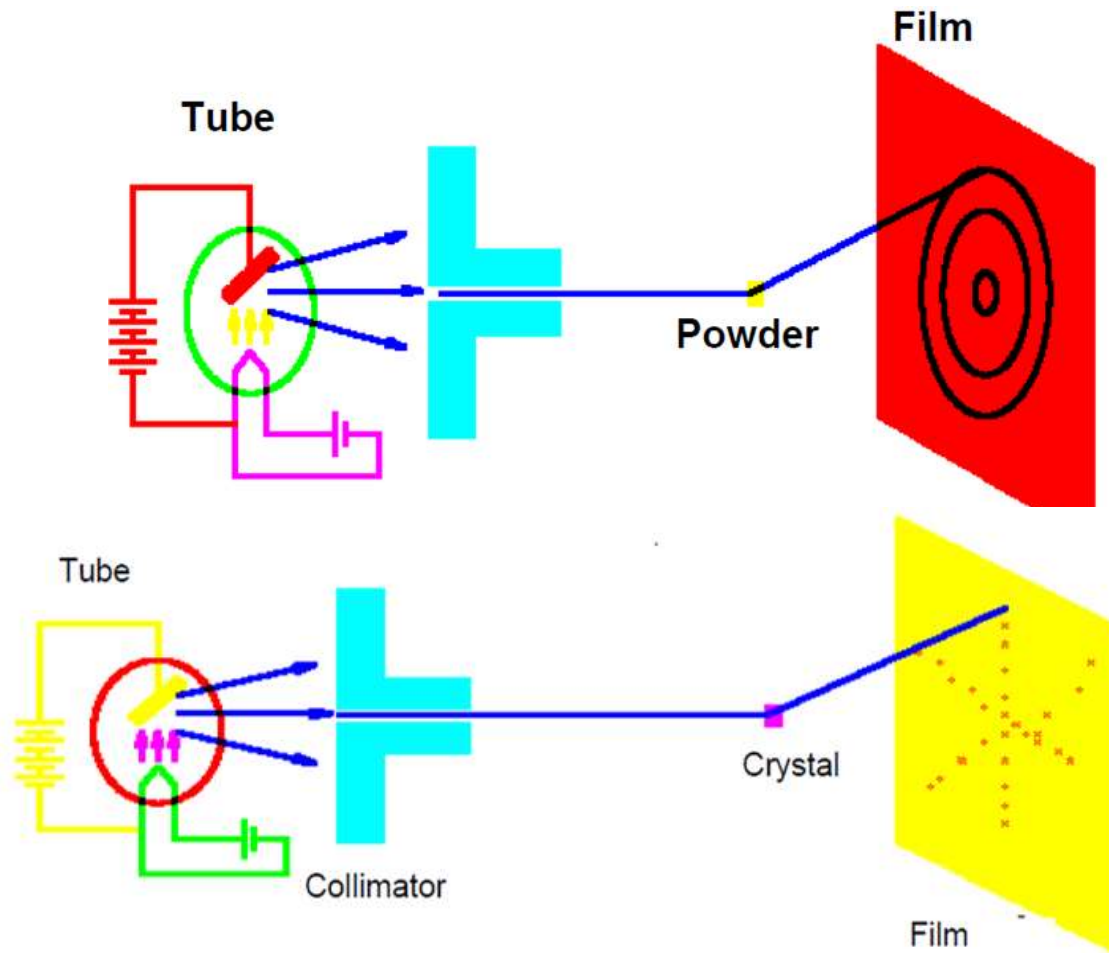
Single crystal
Periodic across the whole volume.



Polycrystal
Periodic across each grain.



Amorphous solid
Not periodic.



- **Powder X-Ray diffraction data** consists of a record of photon intensity vs detector angle 2θ
- Diffraction data can be reduced to a list of peak positions and intensities.
- **Single X-Ray Diffraction patterns are best reported using d_{hkl} and relative intensity rather than 2θ and absolute intensity.**
- Each d_{hkl} corresponds to a **family** of atomic planes $\{hkl\}$
- Individual planes cannot be resolved. This is a limitation of p-XRD vs single crystal diffraction.

Calculation of Crystallite Size using p-XRD data

- Crystallites smaller than ~120 nm create broadening of diffraction peaks. This peak broadening can be used to quantify the average crystallite size of nanoparticles using the Scherrer equation - used for nanoparticles characterization.
- Must know the contribution of peak width from the instrument by using a calibration curve

Scherrer equation can be written as:
$$\tau = \frac{K\lambda}{\beta \cos \theta}$$

- τ is mean size of ordered (crystalline) domains, which may be smaller or equal to grain size;
- K is a dimensionless shape factor, with a value close to unity. The shape factor has a typical value of about 0.9, but varies with the actual shape of the crystallite;
- β is the line broadening at half the maximum intensity (FWHM), after subtracting the instrumental line broadening, given in radians
- λ is the X-ray wavelength
- θ is the Bragg angle.
- This quantity is also denoted as $\Delta(2\theta)$

Fig. Simulated p-XRD patterns for wurtzite CdS spherical particles of different sizes from 1 μm to 1 nm.

The inset shows the 1, 2, and 5 nm XRD patterns on an expanded y-axis scale for clarity.

