Engineering Chemistry BCHY101L

Module 6

Spectroscopic, Diffraction and Microscopic Techniques

VIT®
Vellore Institute of Technology
(Deemed to be University under section 3 of UGC Act, 1956)

a) Fundamental concepts in spectroscopic and instrumental techniques
b) Principle and applications of UV-Visible Spectroscopy technique
c) Principle and applications of X-Ray Diffraction (XRD) technique (including numerical)
d) Overview of various techniques:
    i. Atomic Absorption Spectroscopy (AAS)
    ii. Infrared (IR) Spectroscopy
    iii. Nuclear Magnetic Resonance (NMR) Spectroscopy
    iv. Scanning Electron Microscopy (SEM) &
    v. Transmission Electron Microscopy (TEM)

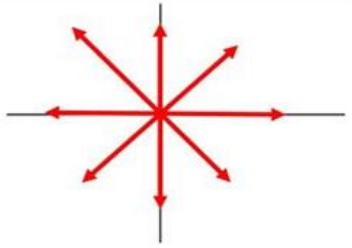
a) Fundamental concepts in spectroscopic and instrumental techniques

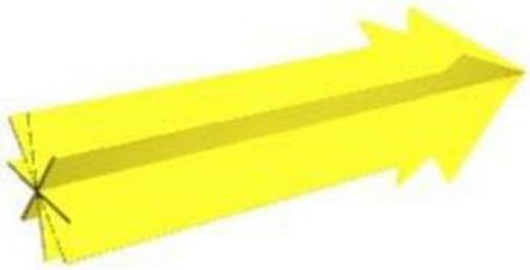
Spectroscopy Basics:

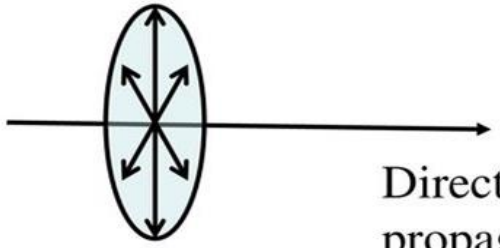
- Spectroscopy is a branch of science that studies the interaction between electromagnetic (EM) radiation and matter.
- Spectroscopy is used as a tool for studying the structures of atoms and molecules.
- The basic principle shared by all spectroscopic techniques is to shine a beam of EM radiation onto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

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.



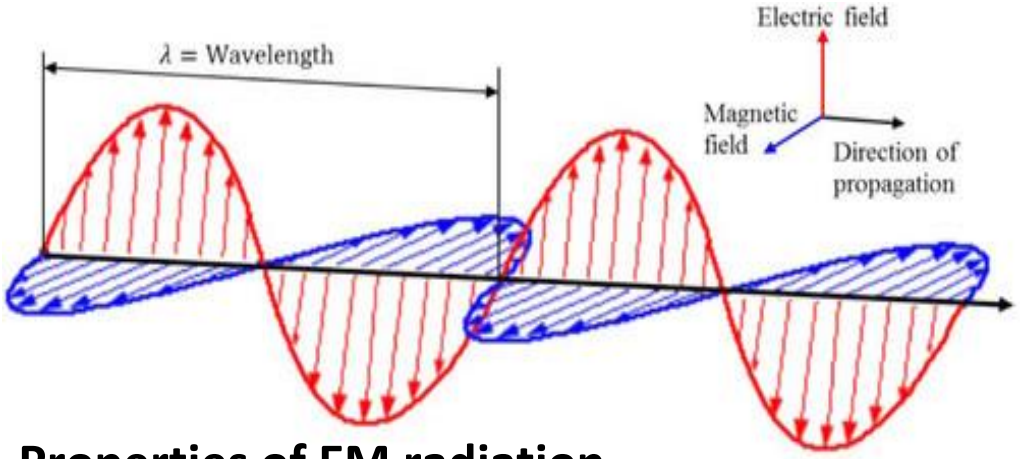




Ordinary light

EM Radiation Basics:

- EM radiation is a form of energy that has both wave and particle properties as shown in the classical sinusoidal wave model (**Fig. shown below**), and embodies parameters such as **Wavelength, λ (m), Frequency, υ (Hz)**, **Velocity (m/s)** and **Amplitude (m)**.
- Compared to other wave phenomena such as sound, EM radiation requires no supporting medium for its transmission, and thus passes readily in vacuum.
- EM radiation is represented as electric and magnetic fields that undergo in-phase, sinusoidal oscillations at right angles to each other and to the direction of propagation.



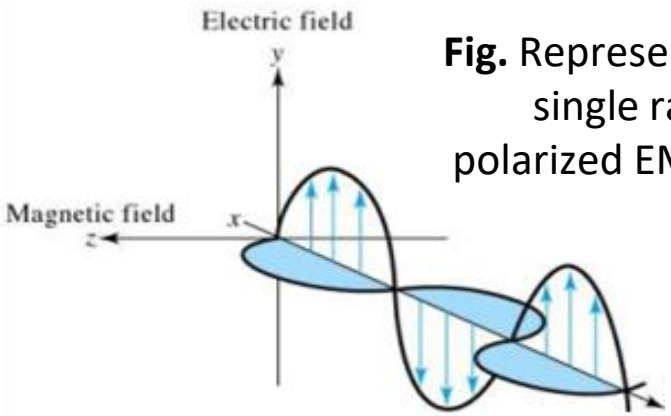


Fig. Representation of a single ray of plane-polarized EM radiation.

Properties of EM radiation

- **Wavelength (λ)** is the spatial distance between two consecutive peaks (one cycle) in the sinusoidal waveform and is measured in sub-multiples of meter, usually in nm.
- Maximum length of the vector is called **Amplitude**.
- **Frequency (υ)** of the EM radiation is the number of oscillations made by the wave within the timeframe of one second. It therefore has the units of 1 s^{-1} = 1 Hz. Frequency is related to the wavelength via the speed of light (c = 2.998x10^8 m.s^{-1}), $\upsilon = c\,\lambda^{-1}$.
- **Wavenumber (υ^{-1})** describes the number of completed wave cycles per distance and is typically measured in 1 cm^{-1}.

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

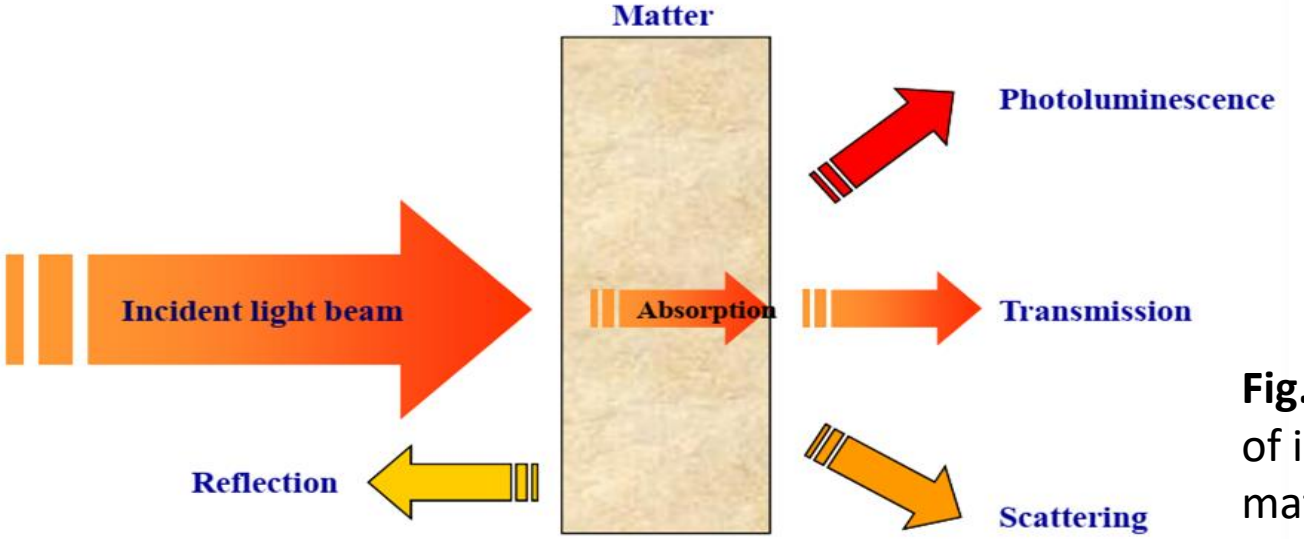


Fig. Schematic representation of interaction of radiation and matter

- 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.**

Interaction with matter:

- Photon is the elementary particle which carries the EM radiation and has properties of a wave as well as particle, albeit having a mass of zero.
- **As a particle, photon interacts with matter by transferring its energy**

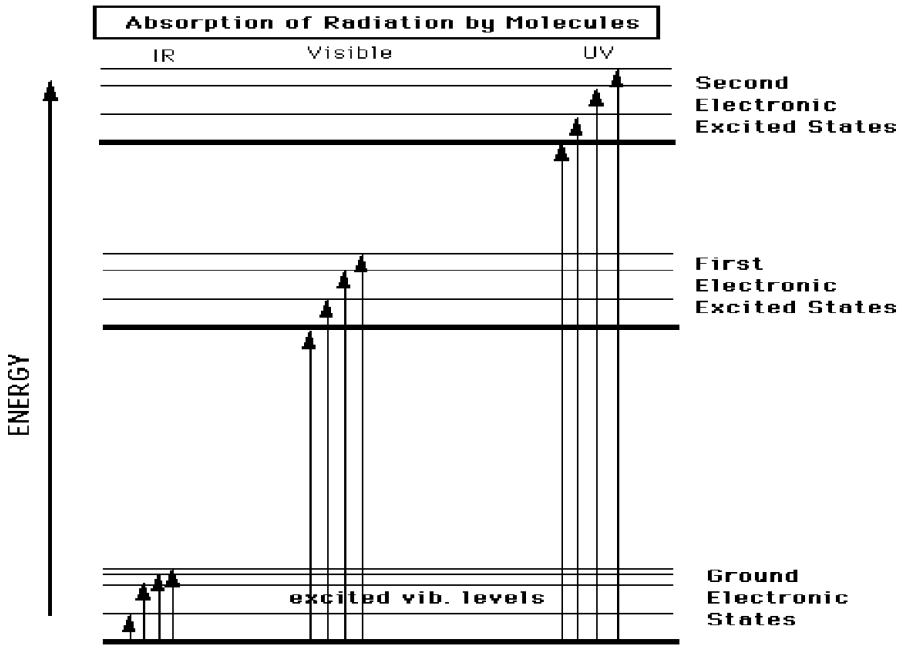
$$E = \frac{hc}{\lambda} = h\upsilon$$

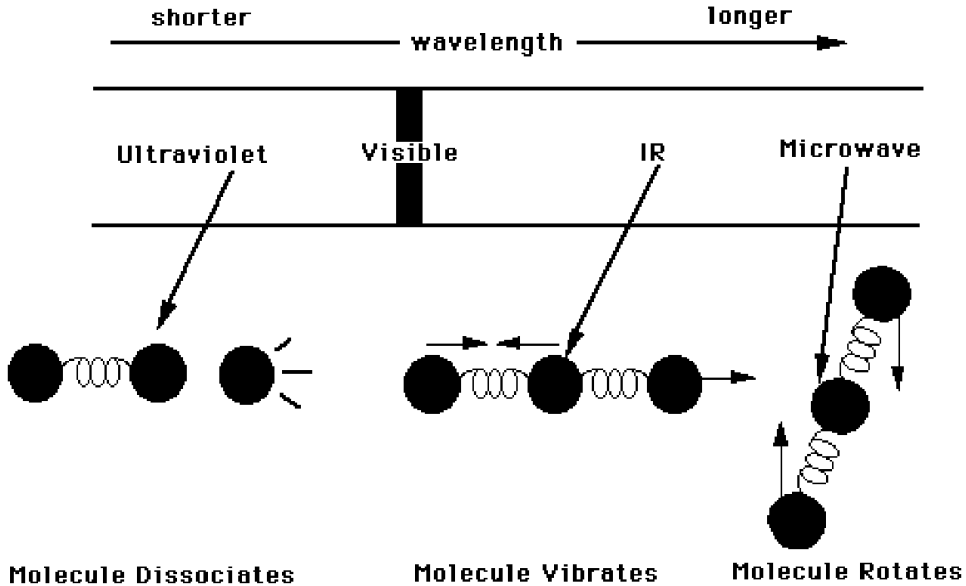
where, h is Planck constant ($h = 6.63\ 10^{-34}$ Js) and υ is the frequency of the radiation.

- Considering a diatomic molecule, each electronic state of a molecule possesses its own set of rotational and vibrational levels. In order for a transition to occur in the system, energy must be absorbed. The energy change (ΔE) needed is defined in quantum terms by the difference in absolute energies between the final and the starting states as

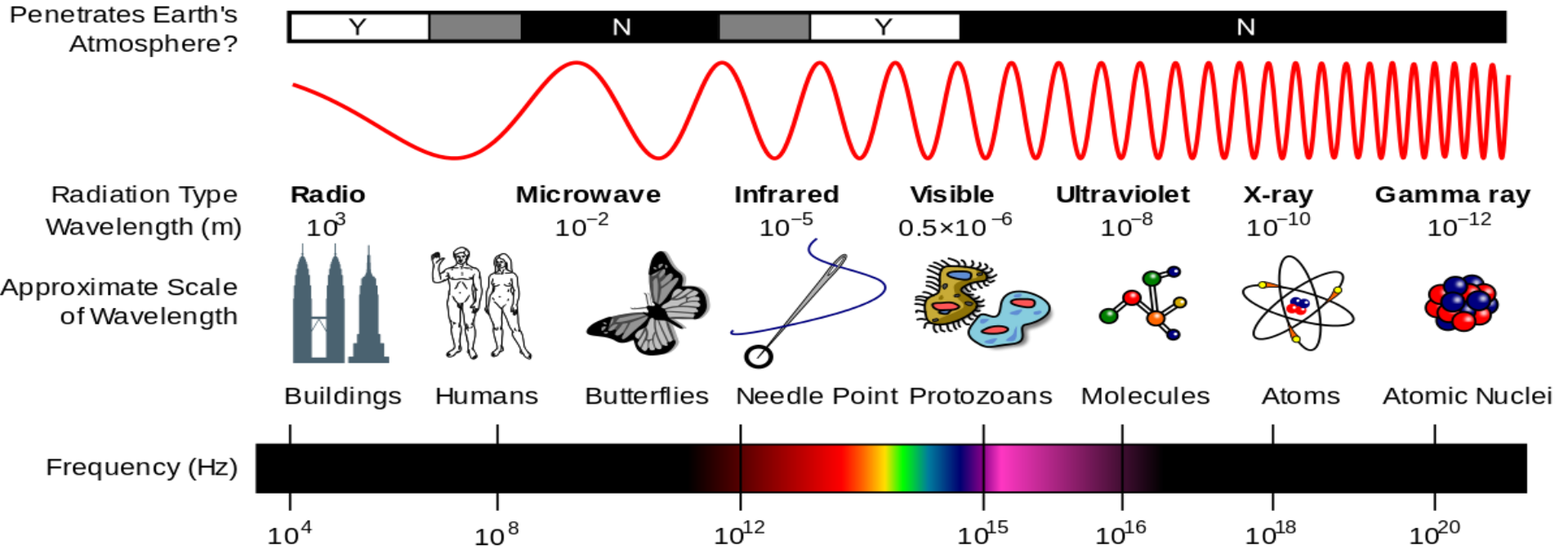
$$\Delta E = E_{final} - E_{start} = h\upsilon.$$

- **Electrons in either atoms or molecules** may be distributed between several energy levels but **principally reside in the lowest levels (ground state).** In order for an electron to be promoted to a higher level (**excited state**), energy must be put into the system.
- **If this energy $E = h\upsilon$ is derived from EM radiation, this gives rise to an absorption spectrum,** and an electron is transferred from the electronic ground state (S_0) into the first electronic excited state (S_1).
- The molecule will also be in an excited vibrational and rotational state. Subsequent relaxation of the molecule into the vibrational ground state of the first electronic excited state will occur. The electron can then revert back to the electronic ground state.
- **For non-fluorescent molecules, this is accompanied by the emission of heat (ΔH).**





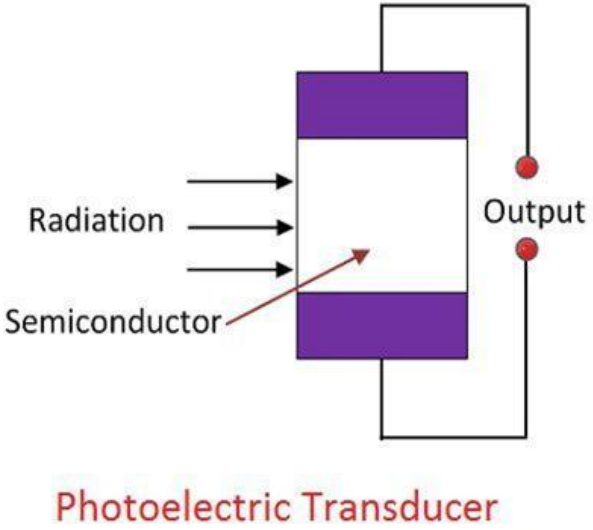
Frequency range of each transition during the spectroscopic analysis



Type of radiation	Frequency range (Hz)	Wavelength range	Type of transition
Radio waves	$<3x10^{11}$	>1 mm	excitement of nucleus to a higher spin state
Microwaves	$3x10^{11}$-10^{13}	1 mm-25 mm	molecular rotations, electron spin flips
Infrared	10^{13}-10^{14}	25 mm-2.5 mm	molecular vibrations
Near-infrared	$1\sim4x10^{14}$	2.5 mm-750 nm	outer e^- molecular vibrations
Visible	$4\sim7.5x10^{14}$	750 nm-400 nm	outer electron
Ultraviolet	10^{15}-10^{17}	400 nm-1 nm	outer electron
X-rays	10^{17}-10^{20}	1 nm-1 pm	inner electron
Gamma-rays	10^{20}-10^{24}	$<10^{-12}$ m	Nuclear

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**.



Photoelectric Transducer

- 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.

(b). Principle and applications of UV-Visible Spectroscopy technique

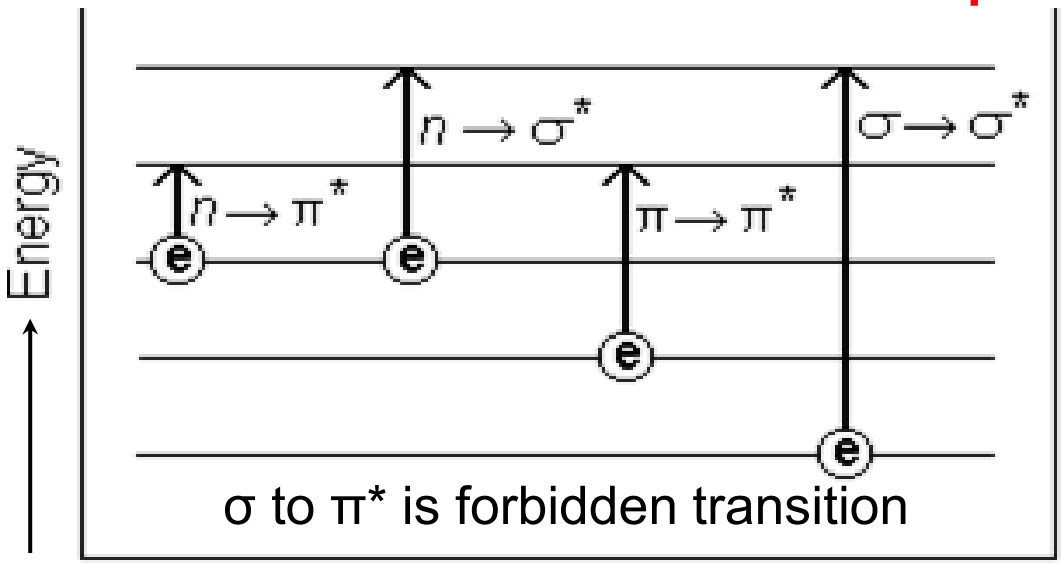
- In UV-Vis spectroscopy, energy is absorbed by a molecule in the UV region (1 nm-400 nm) or visible region (400 nm-750 nm) resulting in electronic transition of valence electrons.
- Different molecules absorb radiation of different wavelengths depending on their structure. An absorption spectrum will show a number of absorption bands corresponding to structural (functional) groups within the molecule.
- For ex. absorption by carbonyl group in acetone is of the same wavelength as the absorption by carbonyl group in diethyl ketone.

Three types of electronic transitions involving:

(i). π, σ and *n* electrons; (ii). charge-transfer electrons and (iii). *d* and *f* electrons.

- Inorganic species show charge-transfer absorption and are called *charge-transfer complexes*.
- For a complex to demonstrate charge-transfer behaviour, one of its components must be able to donate electrons and other component must be able to accept electrons.
- Absorption of radiation then involves the transfer of an electron from the donor to an orbital associated with the acceptor (ε will be very high > 10,000 $dm^3mol^{-1}cm^{-1}$).
- Absorption of UV-Vis radiation in organic molecules is restricted to certain functional groups (***chromophores***) that contain valence electrons of low excitation energy. The spectrum of a molecule containing these chromophores is complex and broad.

Electronic excitations in UV- Visible spectroscopy



σ to σ* transitions: Electron in a bonding σ orbital is excited to the corresponding antibonding **σ*** orbital. Energy required is large. For ex. methane having only C-H bonds can undergo only σ to σ* transitions showing abs. maximum at 125 nm. Abs. maxima due to σ to σ* transitions are not seen in typical UV-Vis. spectra (200-700 nm).

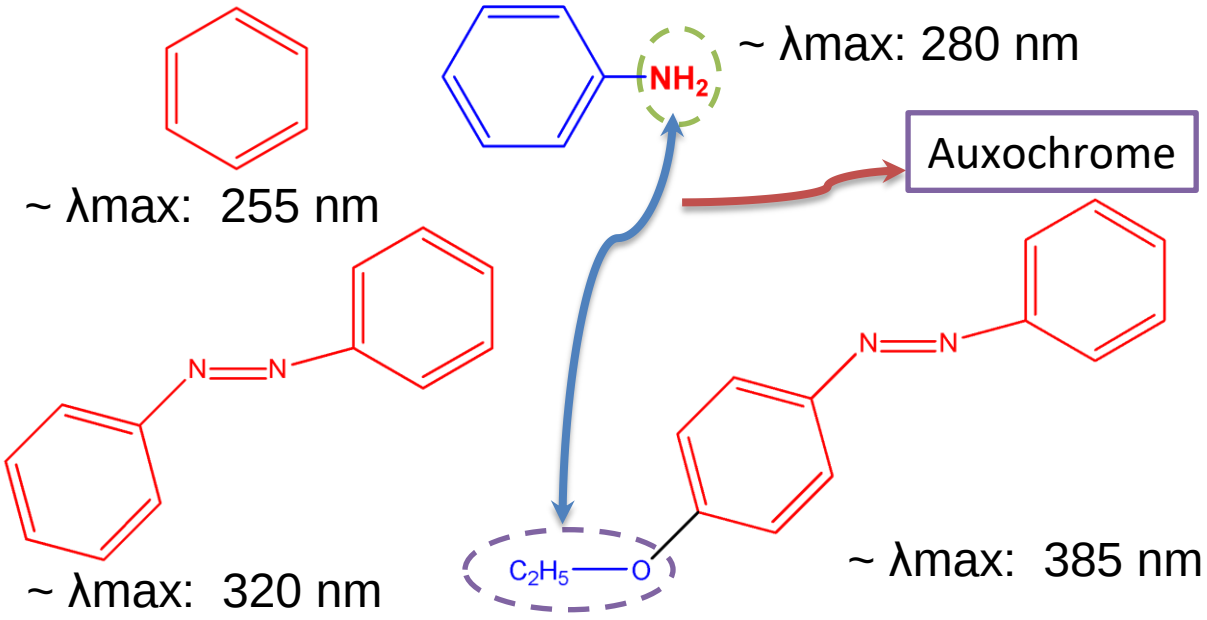
n* to σ * transitions:** Saturated compounds containing atoms with lone pairs (non-bonding electrons) are capable of ***n* to σ *** transitions. These transitions usually need lesser energy than **σ to σ transitions. They can be initiated by light whose wavelength is in the range 150 - 250 nm. The number of organic functional groups with ***n* to σ *** peaks in the UV region is small.

***n* to π* and π to π * transitions:** need an unsaturated group in the molecule to provide the **π** electrons. Most absorption spectroscopy of organic compounds is based on these transitions, since their absorption peaks fall in the experimentally convenient spectral region between 200 - 700 nm.

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.

Chromophore	Example	Excitation	λ_{max}, nm	ε	Solvent
C=C	Ethene	Π —> Π*	171	15,000	hexane
C≡C	1-Hexyne	Π —> Π*	180	10,000	hexane
C=O	Ethanal	n —> Π* Π —> Π*	290 180	15 10,000	hexane hexane
N=O	Nitromethane	n —> Π* Π —> Π*	275 200	17 5,000	ethanol ethanol
C-X; X=Br X=I	Methyl bromide Methyl Iodide	n —> σ* n —> σ*	205 255	200 360	hexane hexane

Auxochrome: group of atoms attached to a chromophore which modifies the ability of that chromophore to absorb light.
Ex. COOH, -OH, -SO_3H, -NH_2, -NH-R, -N-R_2



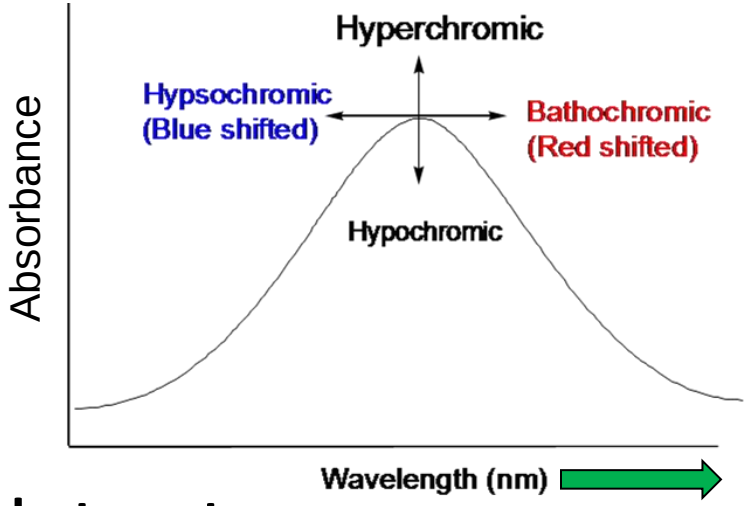
Based on the functional group present and attached to chromophores…

Bathochromic shift: absorption maximum shifted to longer wavelength (Blue to Red [Red shift]).

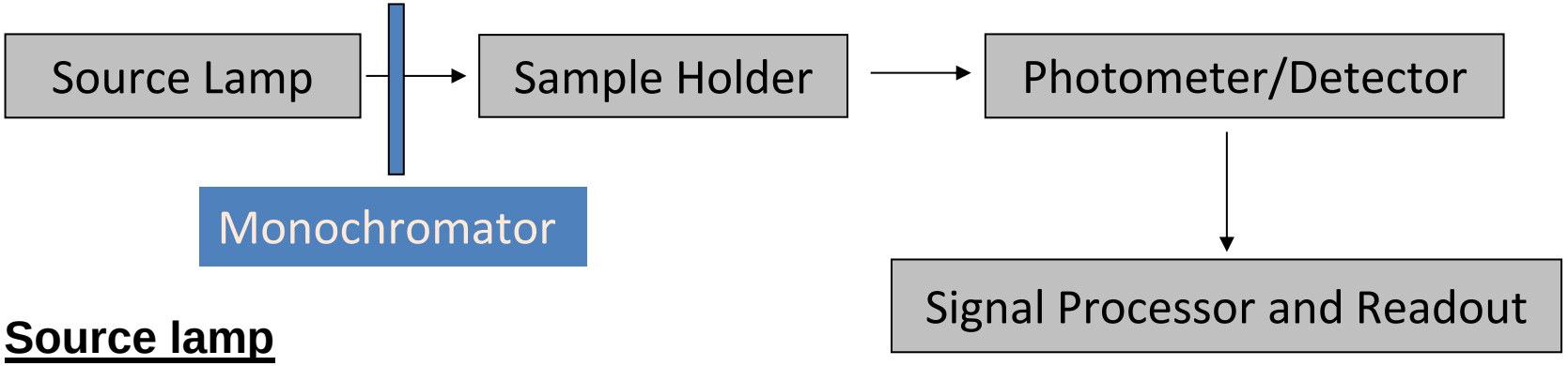
Hypsochromic shift: absorption maximum shifted to shorter wavelength (Red to Blue [**Blue shift**]).

Hyperchromism: increase in molar absorptivity

Hypochromism: decrease in molar absorptivity



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

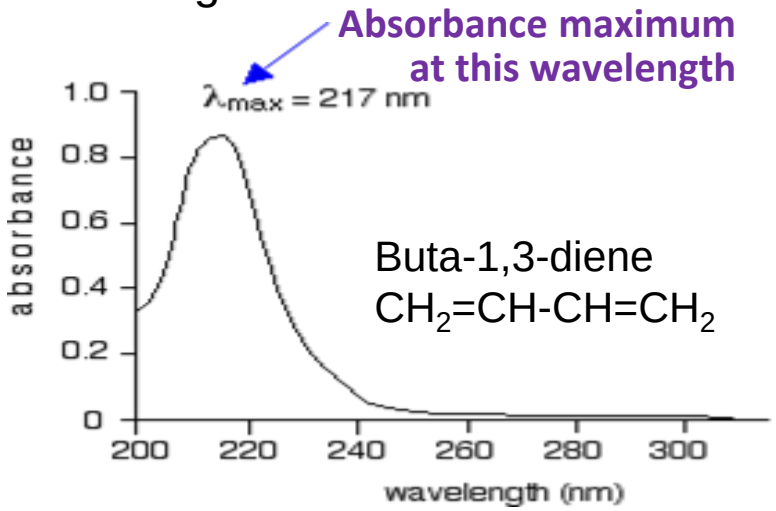
Mechanism behind the photoelectric devices is the conversion of radiant energy to electrical signal. Basically, 3 types of photometers are used: (a). Photovoltaic cells, (b). Phototubes and (c). Photoconductive cells.

Signal processing

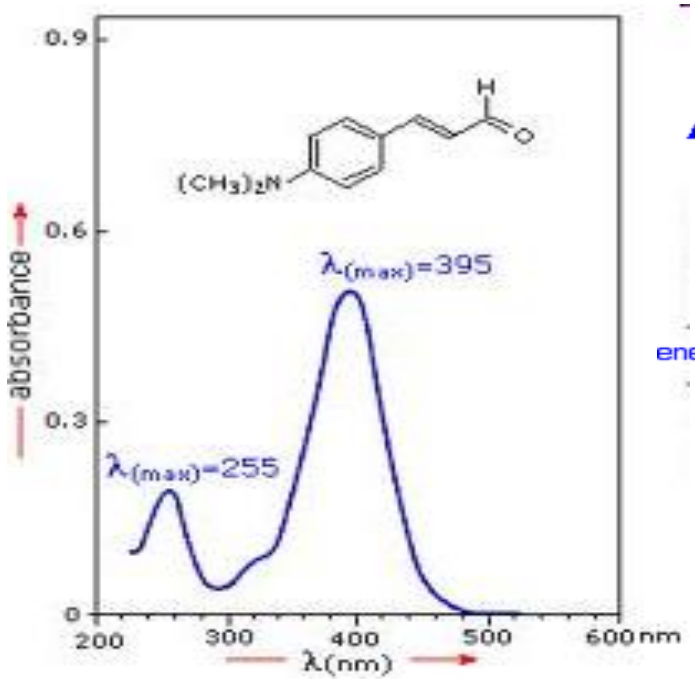
Electrical signal generated by the transducer is sent to the signal processor, where it is displayed in a more convenient form for the analyst.

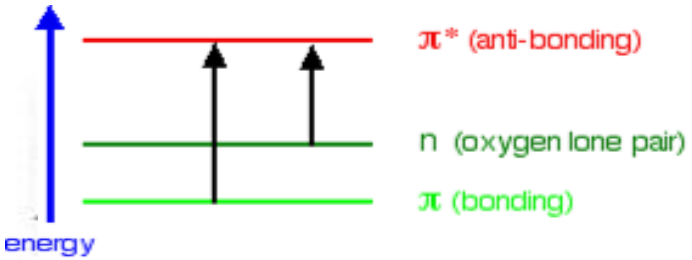
Applications of UV-Vis spectrophotometer:

Qualitative analysis: Identification of chromophores by scanning the absorbance at each wavelength.



Example	λ_{max}	Transition
$C_6H_{13}CH=CH_2$	177	$n \rightarrow \pi^*$
$C_5H_{11}C\equiv C.CH_3$	178	$n \rightarrow \pi^*$
	196	-
	225	-
CH_3-CO-CH_3	186	$n \rightarrow \sigma^*$
	280	$n \rightarrow \pi^*$
CH_3-CHO	180	$n \rightarrow \sigma^*$
	293	$n \rightarrow \pi^*$
CH_3-COOH	240	$n \rightarrow \pi^*$
CH_3-CO-NH_2	214	$n \rightarrow \pi^*$
$CH_3N=NCH_3$	339	$n \rightarrow \pi^*$
CH_3NO_2	280	$n \rightarrow \pi^*$
C_4H_9NO	300	-
	665	$n \rightarrow \pi^*$
$C_2H_5NO_2$	270	$n \rightarrow \pi^*$





Quantitative analysis:

- Absorbance at a particular λ_{max} can be measured using photometry mode.

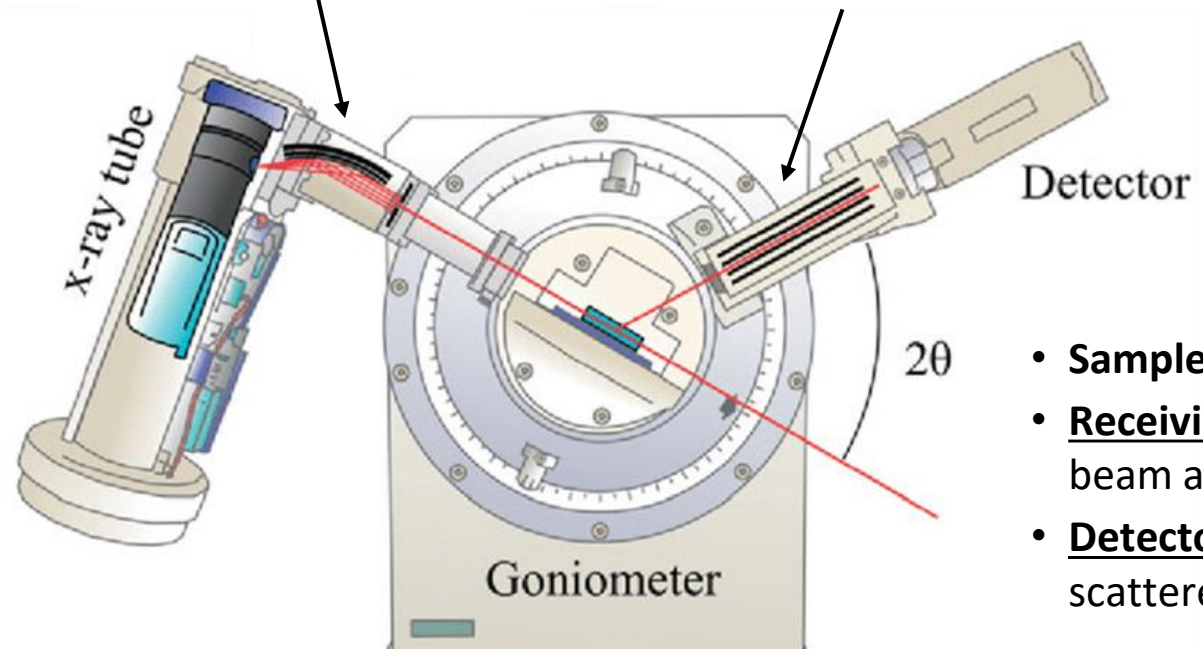
- Determining the reaction rate and pK_a values (dissociation constants) of weak acids or bases.
- Determining the percentages of keto and enol forms.
- To analyze metals in waste water.
- Determining total serum protein, serum cholesterol, etc.
- Characterizing pharmaceuticals, food, paint, glass and metals

(c). Principle and applications of X-Ray Diffraction (XRD) technique

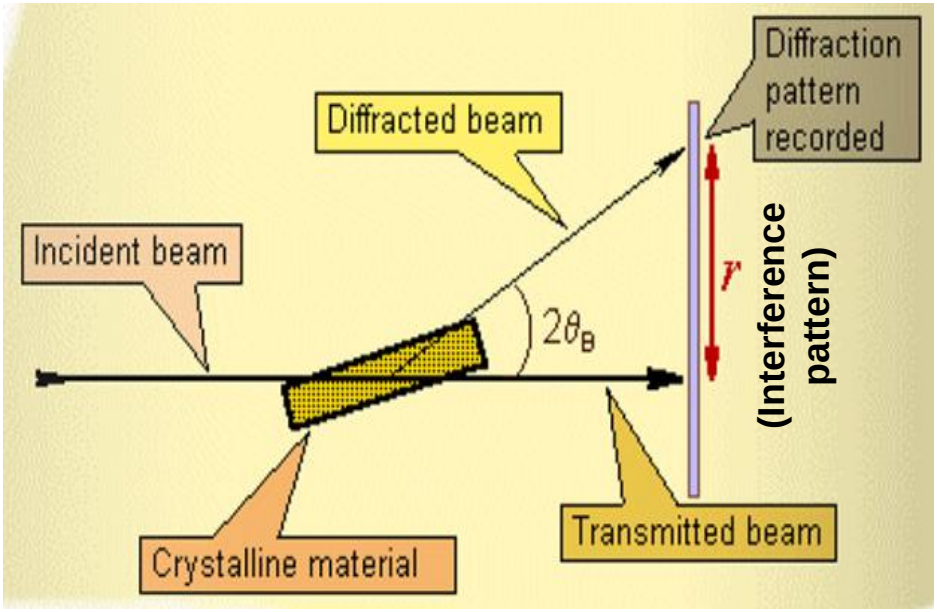
- XRD is a versatile, non-destructive characterization technique widely used in materials science and engineering for identifying unknown crystalline materials.
- XRD works by irradiating a material with incident X-rays, and then measuring the intensities and scattering angles of the X-rays that leave 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 and to determine atomic arrangement.
- XRD also yields information on how the actual structure deviates from the ideal one, owing to internal stresses and defects.

Selected Nobel Prize Winners involving X-ray crystallography

Year	Laureate(s)	Prize	Rationale
1914	Max von Laue	Physics	Discovery of diffraction of X-rays by crystals, an important step in the development of X-ray spectroscopy.
1915	William Henry Bragg	Physics	Analysis of crystal structure by means of X-rays.
1964	Dorothy Hodgkin	Chemistry	Determination of the structures of important biochemical substances.
2009	Ada E. Yonath, T.A. Steitz, R. Venkatraman	Chemistry	For studying the structure and function of the ribosome.
2012	Brian Kobilka	Chemistry	For studying G-protein-coupled receptors.



- **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.



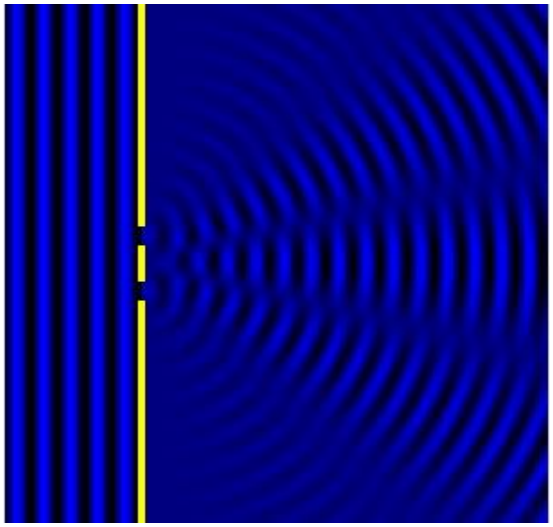
How XRD pattern produced?

- 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.

What is diffraction?

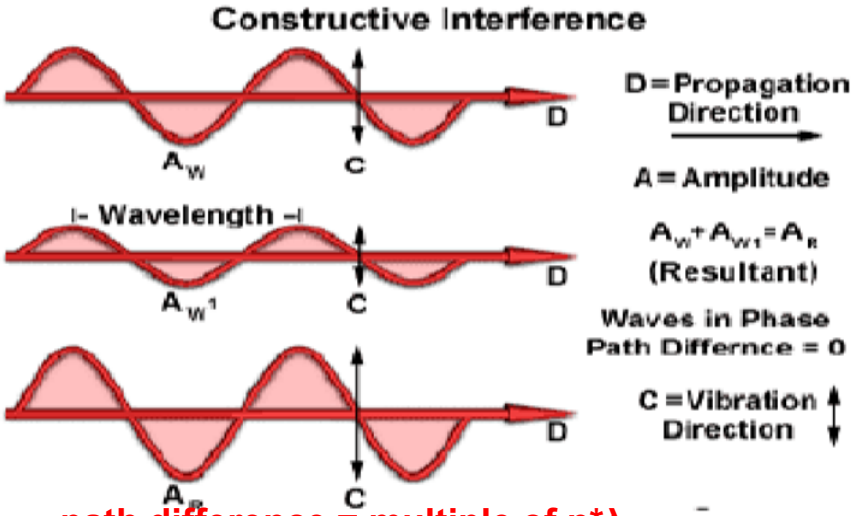
- Diffraction refers to different phenomena which occur when a wave encounters an obstacle.
- In classical physics, the diffraction phenomenon is described as the apparent bending of waves around small obstacles and the spreading out of waves past small openings.



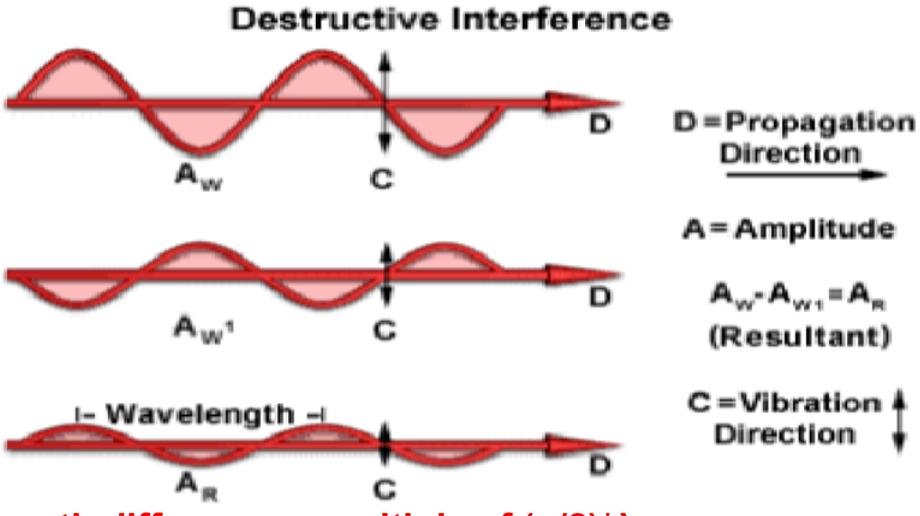
Interference of diffracted waves

- Interaction between diffracted waves is called interference.

- **Constructive Interference**: Waves are in-phase when each of their crests and troughs occur exactly at the same time. Those type of waves stack together to produce a resultant wave that has a **higher amplitude**. For constructive interference, path difference should be multiples of n*λ.
- **Destructive Interference**: If the waves are out of phase by multiples of (n/2)*λ, then destructive interference occurs and the amplitude of the resultant wave will be reduced.

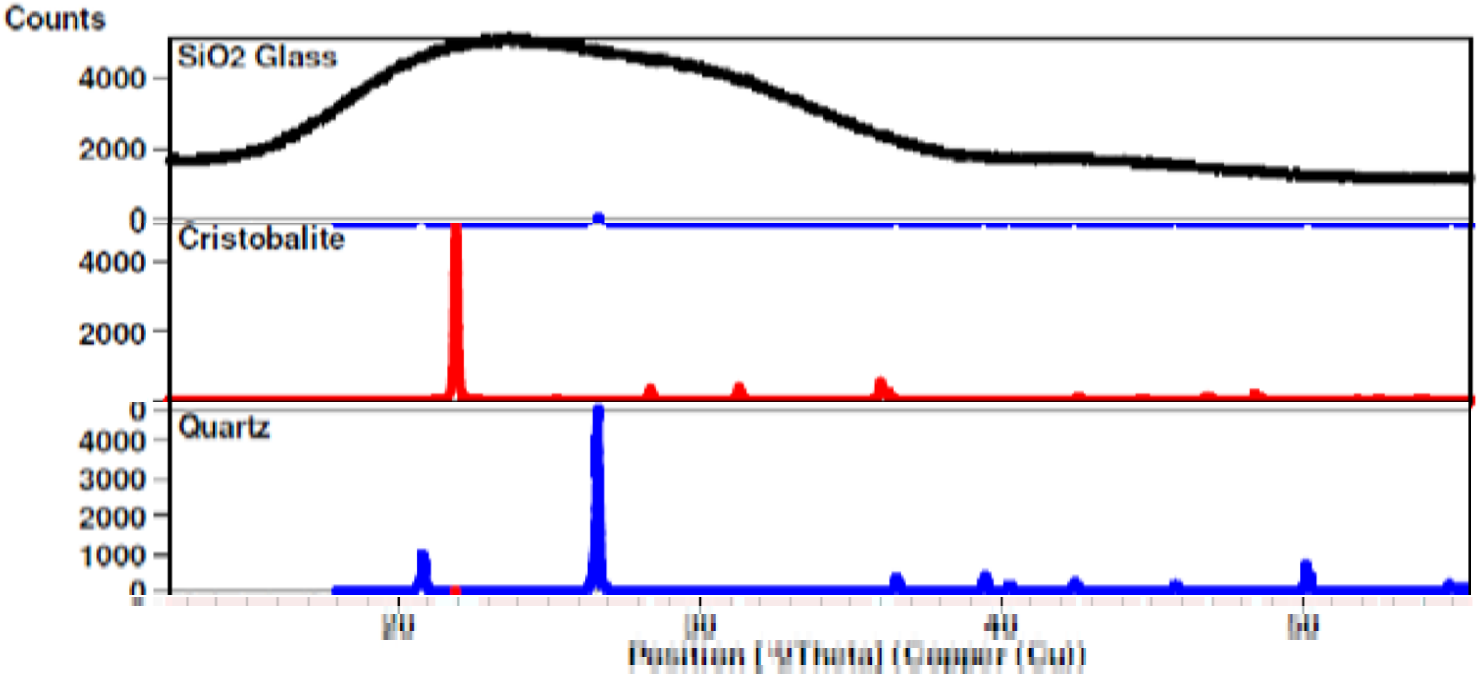


path difference = multiple of n*λ

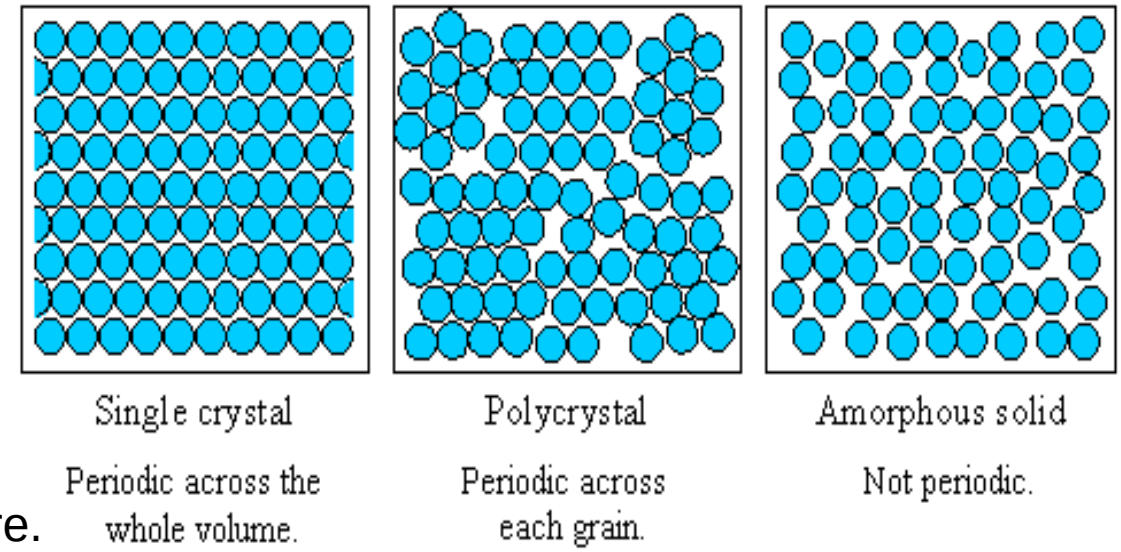


path difference = multiple of (n/2)*λ

XRD patterns for 3 different forms of SiO_2



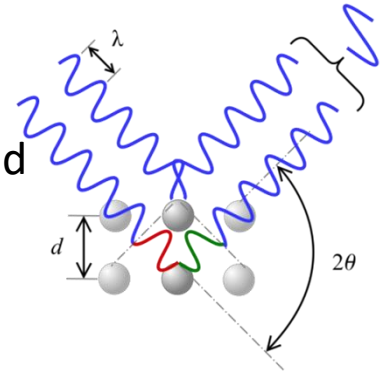
- These three phases of SiO_2 are chemically identical.
- The amorphous glass does not have long-range atomic order and therefore produces only broad pattern.
- Cristobalite form polycrystalline structure.
- Quartz form single crystal structure.



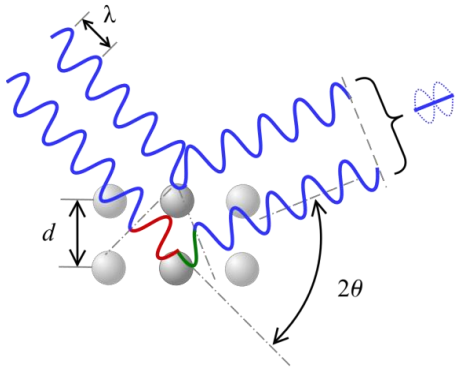
Bragg model of diffraction

- Crystals are regular arrays of atoms, whilst X-rays are waves of EM radiation. Crystal atoms scatter incident X-rays, primarily through interaction with the atom's electrons. This phenomenon is known as elastic scattering; the electron is known as the scatterer.
- A regular array of scatterers produces a regular array of spherical waves. In the majority of directions, these waves cancel each other out through destructive interference, however, they add constructively in a few specific directions, as determined by Bragg's law:
- $n\lambda = 2d\sin\theta$, where "n" is an integer, and "λ" is the beam wavelength, "d" is the spacing between diffracting planes and "θ" is the incident angle.

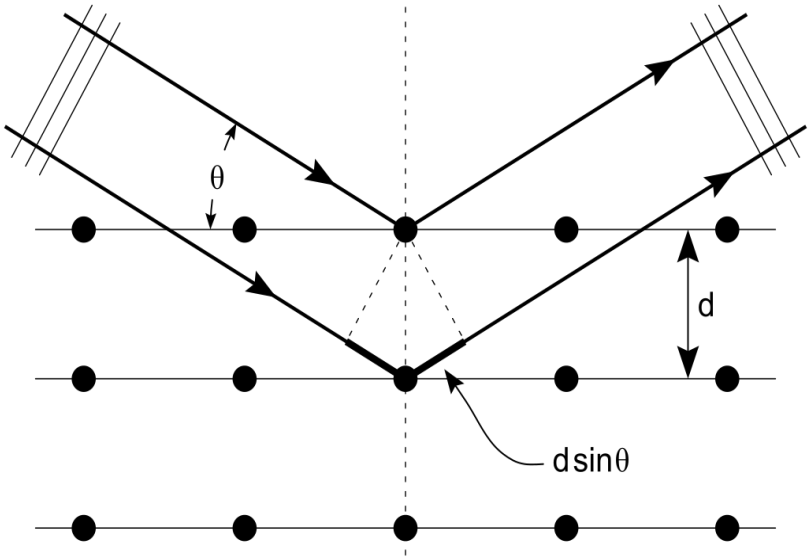
- X-rays scattered from adjacent planes will combine constructively (constructive interference) when angle θ between plane and X-ray results in path-length difference that is integer multiple "n" of X-ray wavelength "λ".

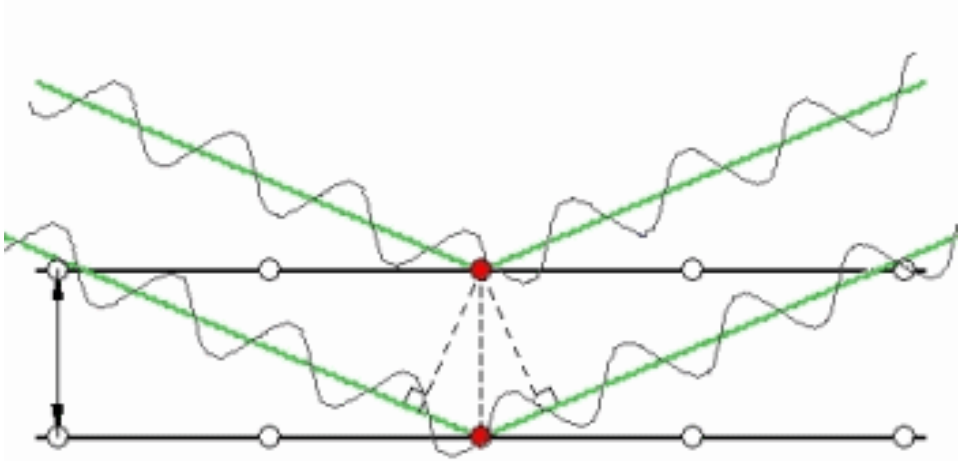


Constructive interference

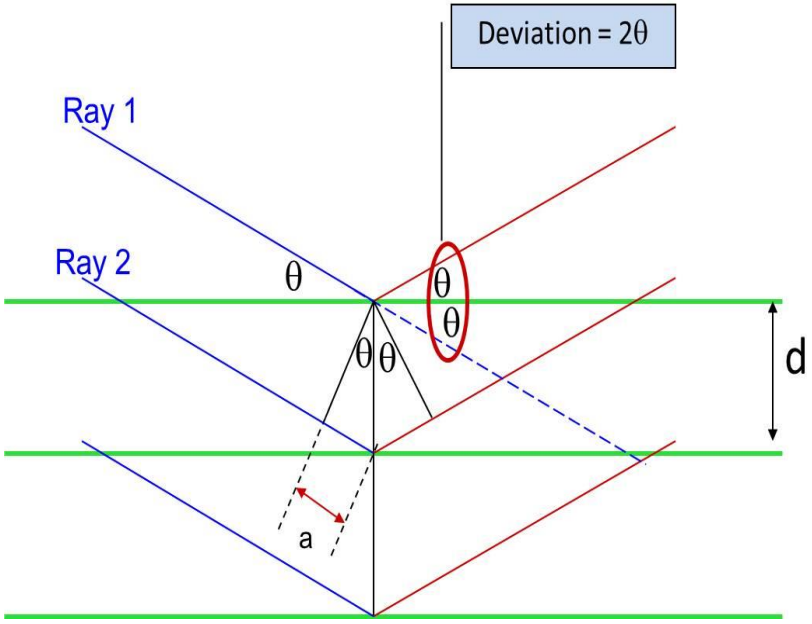


Destructive interference

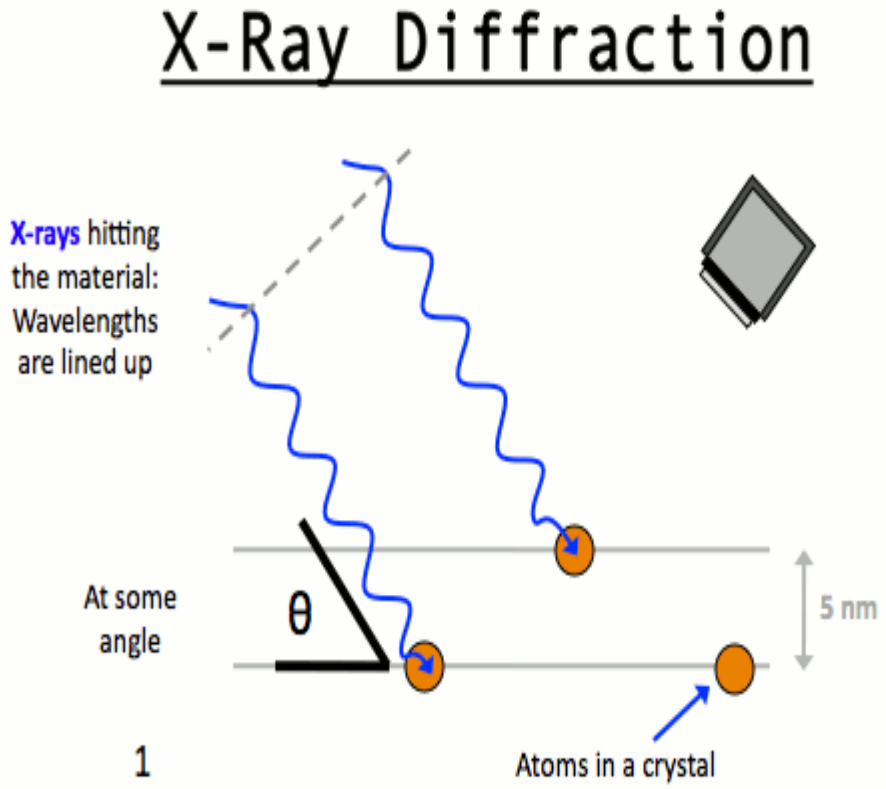




- In this model, a given reflection is associated with a set of evenly spaced sheets running through the crystal, usually passing through the centres of the atoms of the crystal lattice.
- Orientation of a particular set of sheets is identified by its three Miller indices (h, k, l), and let their spacing be noted by "d".
- Specific directions appear as spots on the diffraction pattern called reflections. Consequently, XRD patterns result from EM waves impinging on a regular array of scatterers.
- X-rays are used to produce the diffraction pattern because their wavelength, λ, is often the same order of magnitude as the spacing "d" between the crystal planes (1-100 Å).

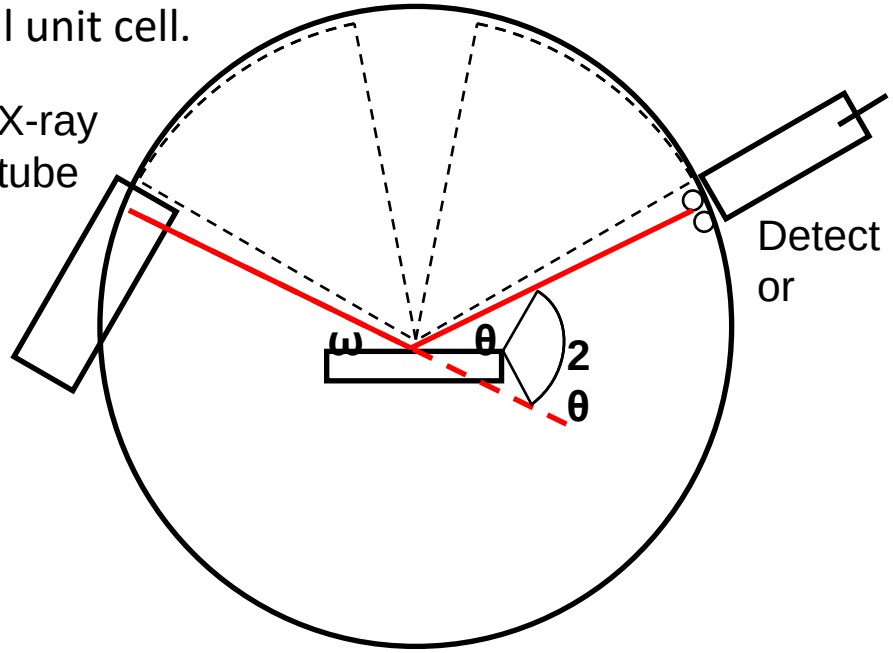


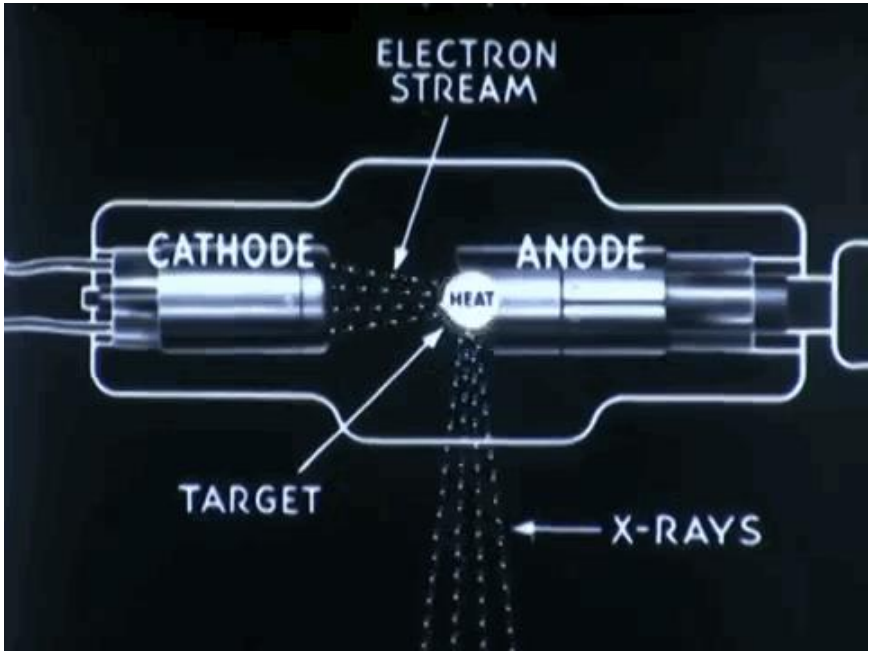
- The path difference between Ray 1 and 2: **2a = 2d sinθ**
- For constructive interference **2a = nλ = 2d sinθ**

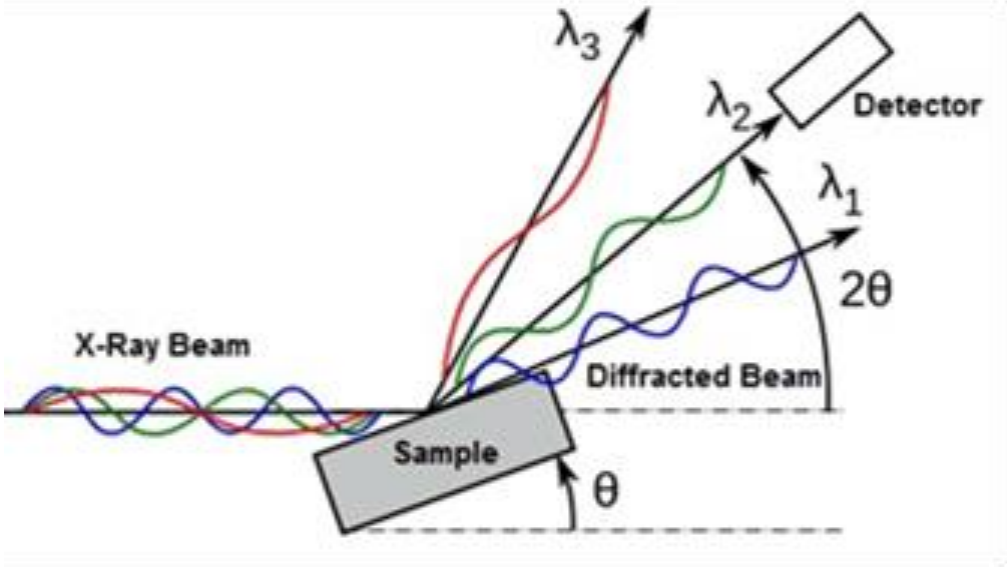


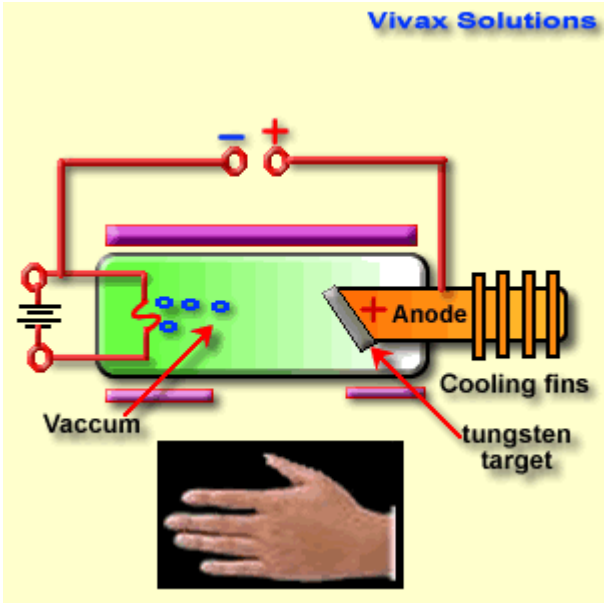
- Because crystal structures contain planes of atoms, each plane will reflect incident X-rays differently. For ex. let two monochromatic X-ray beams (of a specific wavelength) strike a crystal structure at an incoming angle of θ.
- Ray 1 will reflect off of the top atomic plane while Ray 2 will reflect from the second atomic plane. However, because Ray 2 has to cross deeper into the atomic plane, it travels a distance "2a" farther than Ray 1.
- If the distance "2a" is equal to the integral number (n*λ) of wavelength of 2 waves, then Ray 1 and 2 will be in-phase (constructively interfere) when they both exit the crystal.
- If we know wavelength λ of X-rays going into crystal and also measure angle θ of diffracted X-rays coming out of crystal, then we can determine *d*-spacing between atomic planes.
- If we now reorient the crystal to a different atomic plane, we can measure *d*-spacing in other planes. By doing multiple x-ray diffractions at different crystal orientations, we can determined crystal structure and size of crystal unit cell.

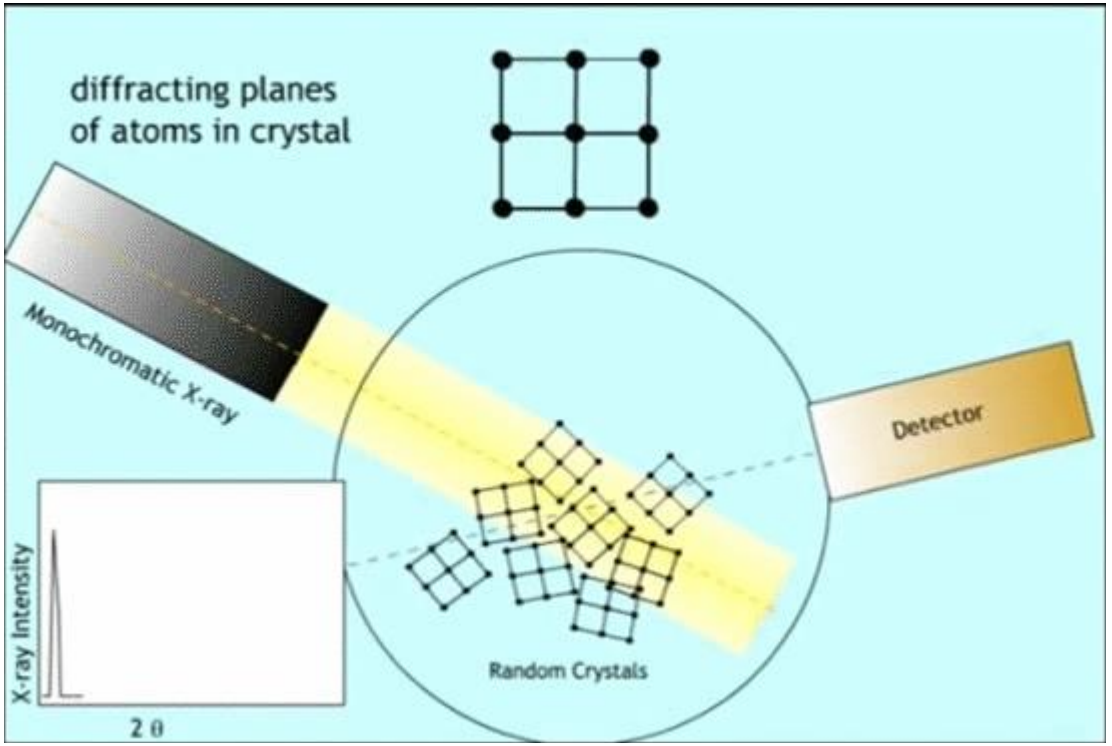
- Incident angle (ω) is defined between the X-ray source and sample.
- Diffracted angle (2θ) is defined between the incident beam and the detector angle.
- Incident angle (ω) is always ½ of the detector angle 2θ i.e. θ.
- In a typical XRD instrument, the X-ray tube is fixed, the sample rotates at θ°/min and detector rotates at 2θ°/min.

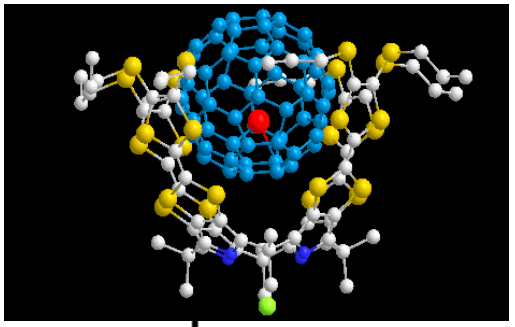


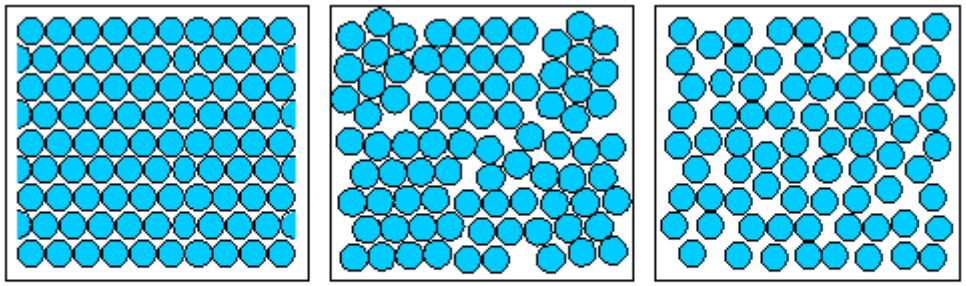

ELECTRON
STREAM
CATHODE
ANODE
HEAT
TARGET
X-RAYS


λ3
λ2
Detector
λ1
2θ
X-Ray Beam
Diffracted Beam
Sample
θ


Vivax Solutions
Anode
Cooling fins
Vaccum
tungsten
target


diffracting planes
of atoms in crystal
Monochromatic X-ray
Detector
X-ray Intensity
2 θ
Random Crystals





Single crystal

Periodic across the whole volume.

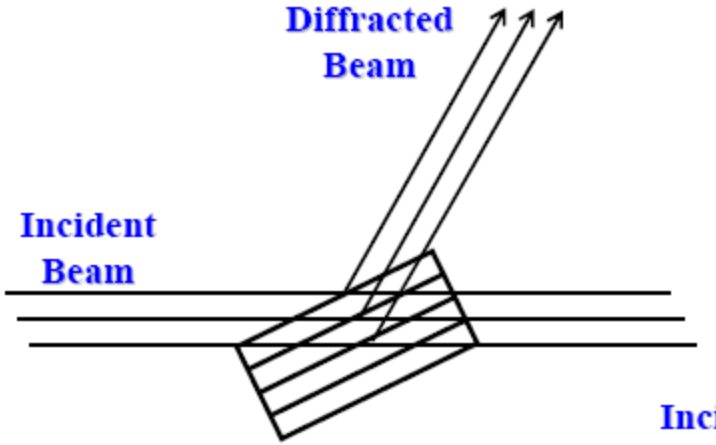
Polycrystal

Periodic across each grain.

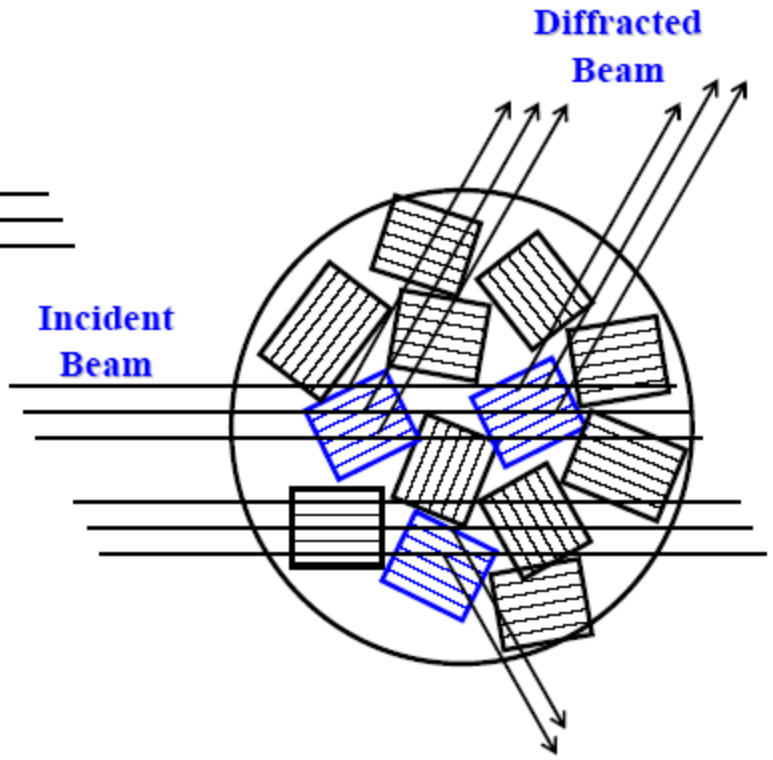
Amorphous solid

Not periodic.

Single Crystal Diffraction

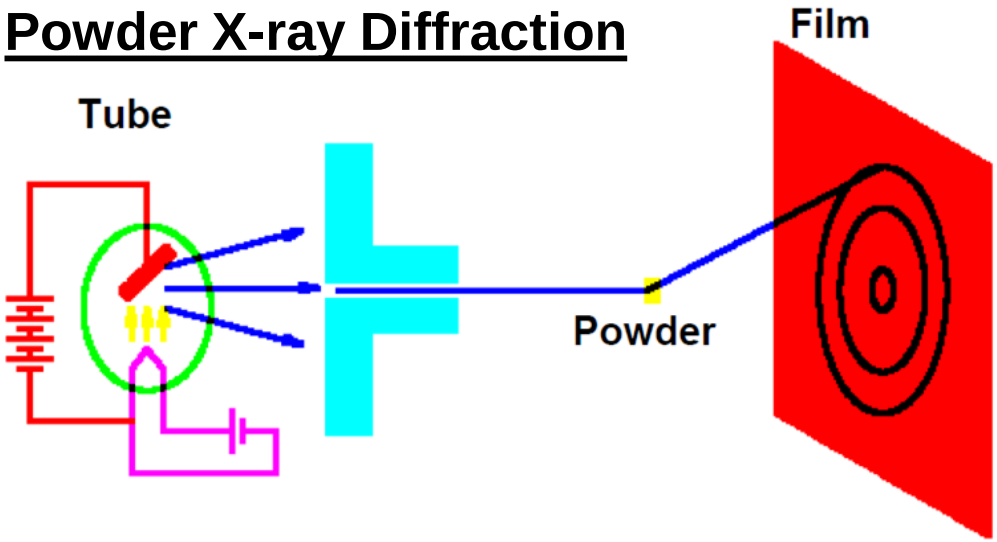


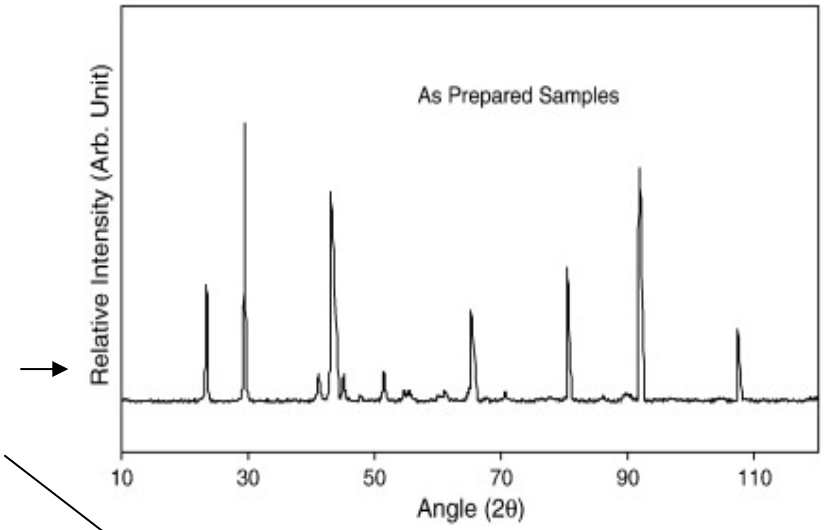
Powder Diffraction



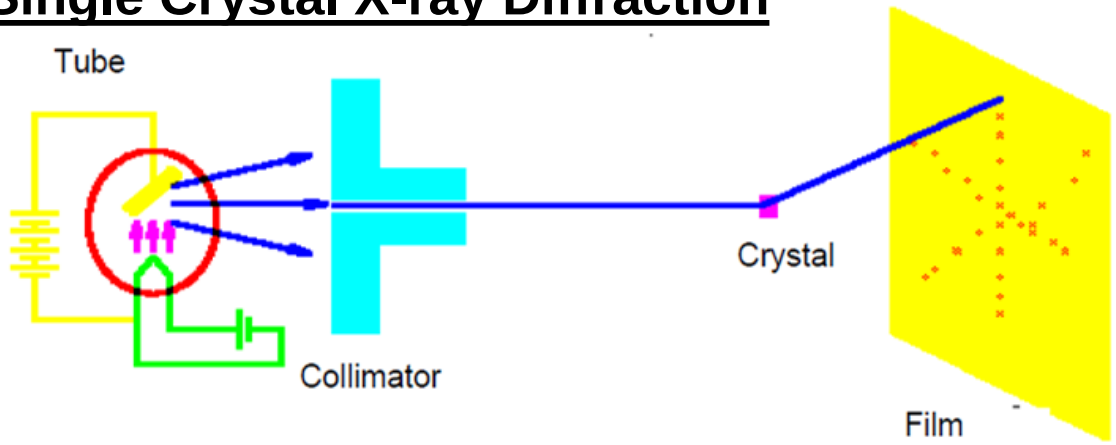
In powder diffraction only a small fraction of the crystals (shown in blue) are correctly oriented to diffract.

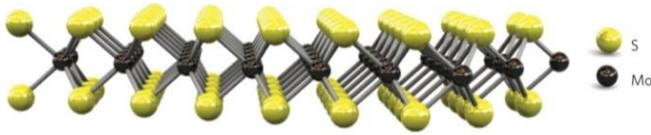
Powder X-ray Diffraction





Single Crystal X-ray Diffraction





- Powder 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.
- **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 $\Delta(2\vartheta)$

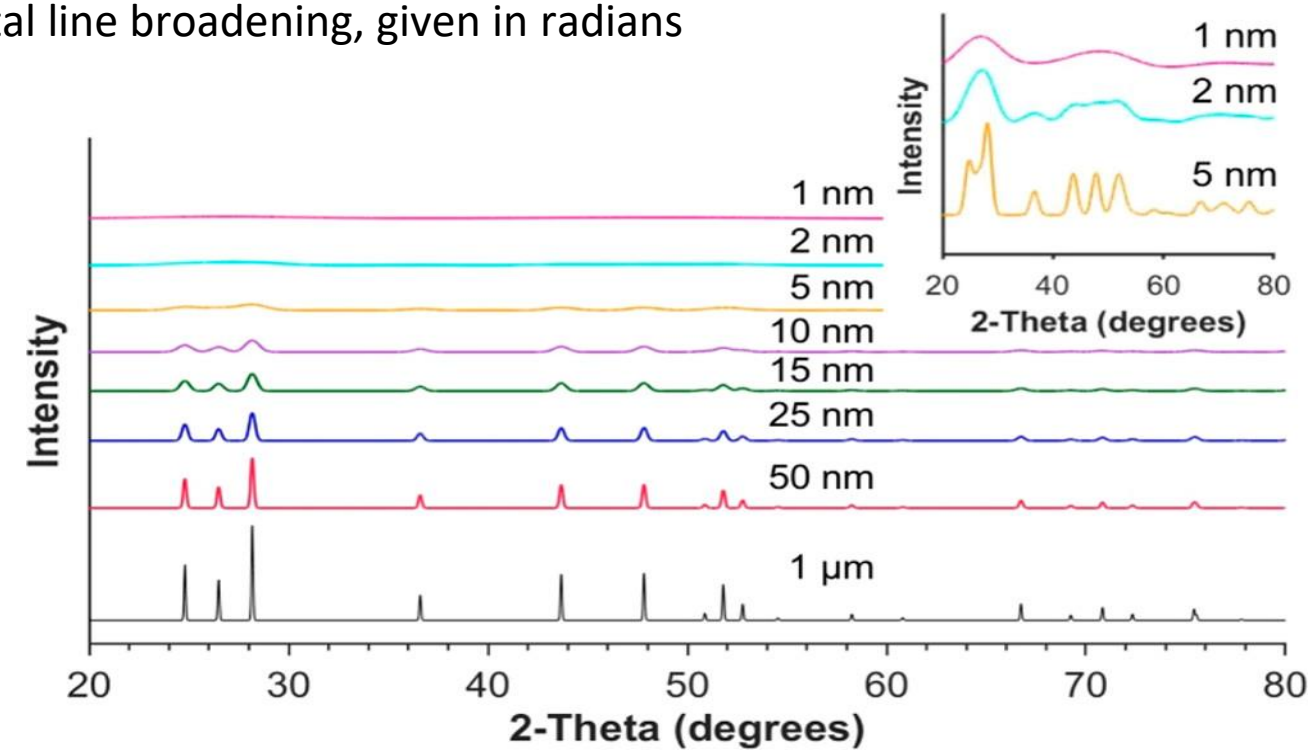


Fig. Simulated p-XRD patterns for wurtzite CdS spherical particles of different sizes from 1 μm to 1 nm. The inset shows the 1, 2, and 5 nm XRD patterns on an expanded y-axis scale for clarity.

XRD Numericals

Q. 1. In a NaCl crystal, there is a family of planes 0.252 nm apart. If the first-order maximum is observed at an incidence angle of 18.1°, what is the wavelength of the X-ray scattering from this crystal?

Ans.: Use the Bragg equation $n\lambda = 2d\sin\theta$ to solve for θ.

For the first-order, n=1. Then $\lambda = 2d\sin\theta/n = 2d\sin\theta/1$

$\lambda = 2(0.252\times10^{-9}\ m)\sin(18.1°)/1$

$= 0.504*0.31068 = 1.57\times10^{-10}$m or **0.157 nm**.

Q. 2. Estimate the crystallite size of the given nanomaterial using p-XRD data: Peak position $2\theta = 21.61^o$, FWHM of sample = 2.51^o, k = 0.9 and λ = 1.5406 Å (degree to radian = Degree × π/180).

Ans.: $2\theta = 21.61^o$ ($\theta = 10.805^o$) and FWHM = 2.51^o (0.043825 radian)

Crystalline grain size calculation by Scherrer's equation: k*λ/β*cosθ

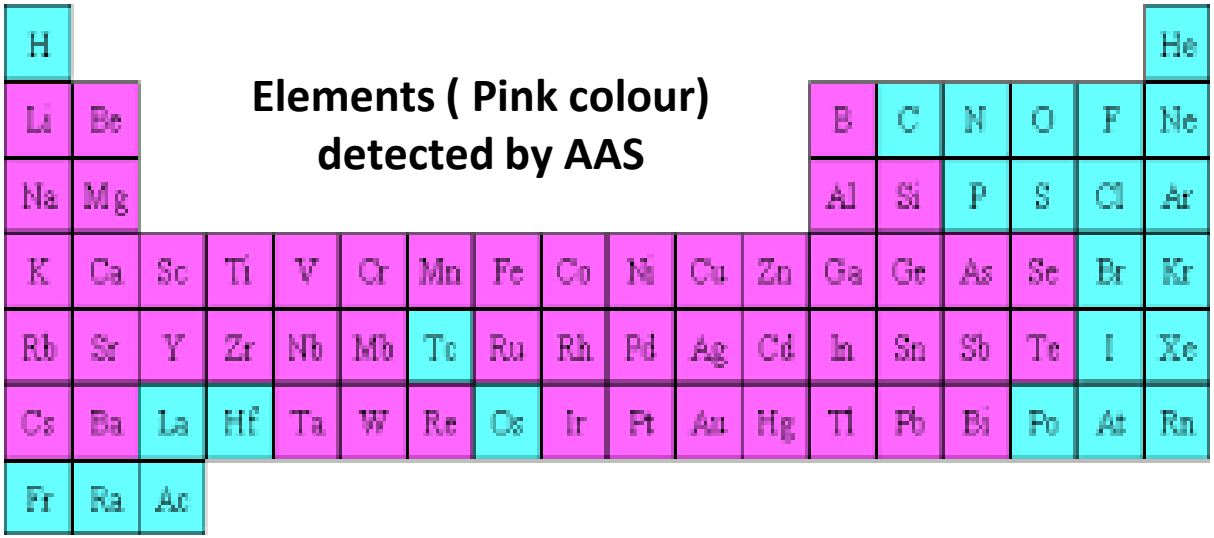
k = 0.9, **λ** = 1.5406 Å (0.15406 nm), **β** = FWHM in radian and **2θ** = Bragg's angle in ^{o} obtained from p-XRD data.

Crystallite size = (0.9*0.15406)/(0.043825*0.982257) nm = **3.22 nm**

(d)-(i): Overview of Atomic Absorption Spectroscopy (AAS)

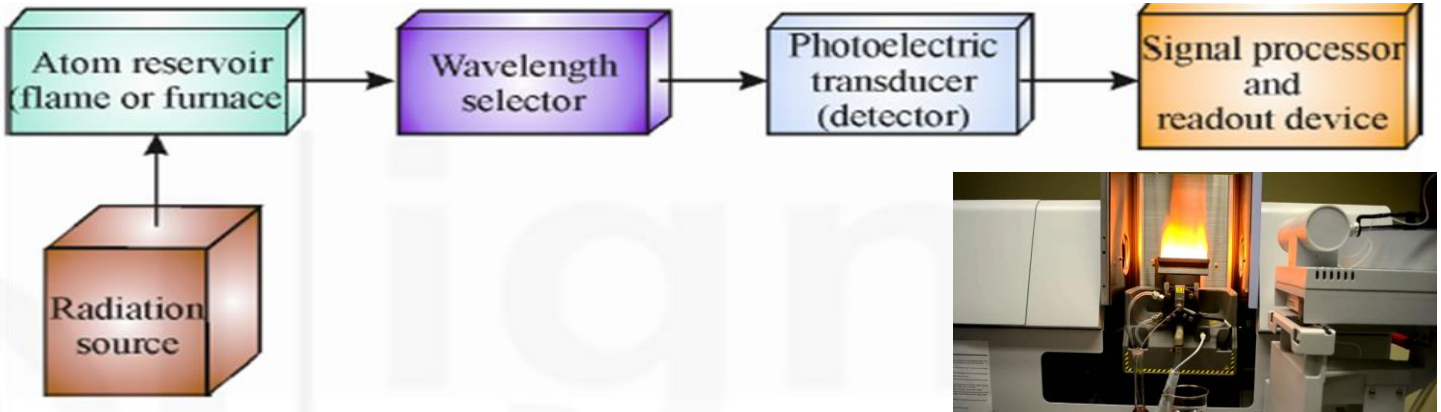
Basic Principle: In atomic spectrometry, the elements present in a sample are converted into gaseous atoms by a process called atomization and their interaction with the specific radiation is measured.

- The atomization is achieved by the thermal energy of the flame.
- Wavelength of the radiation absorbed and the extent of the absorption form the basis of the qualitative and quantitative determinations.



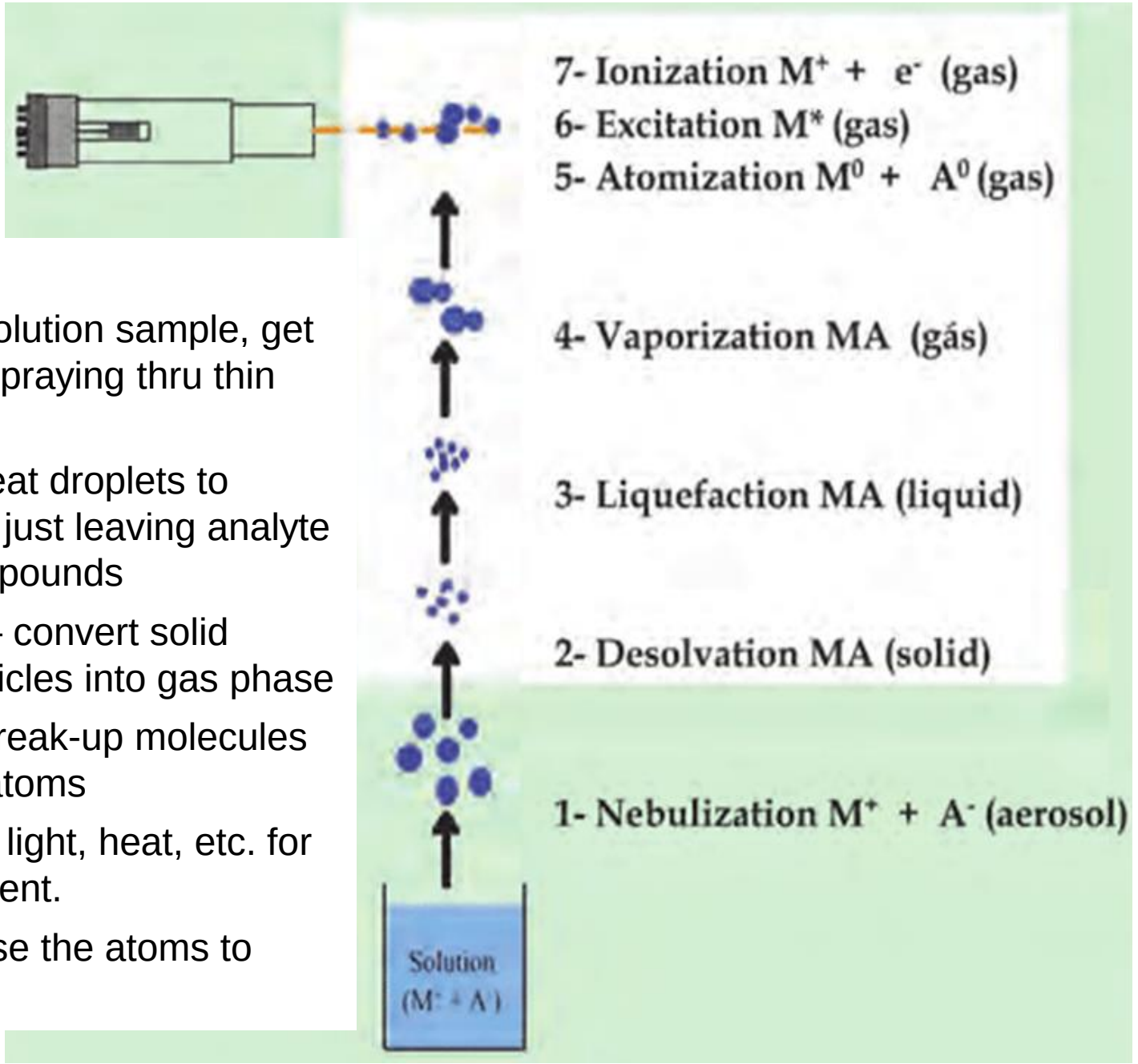
AAS components:

- Radiation source
- Atom reservoir
- Monochromator
- Detector
- Readout device



Sample Atomization process

Need to break sample into atoms to observe atomic spectra



Basic steps:

(1). nebulization – solution sample, get into fine droplets by spraying thru thin nozzle

(2). desolvation - heat droplets to evaporate off solvent just leaving analyte and other matrix compounds

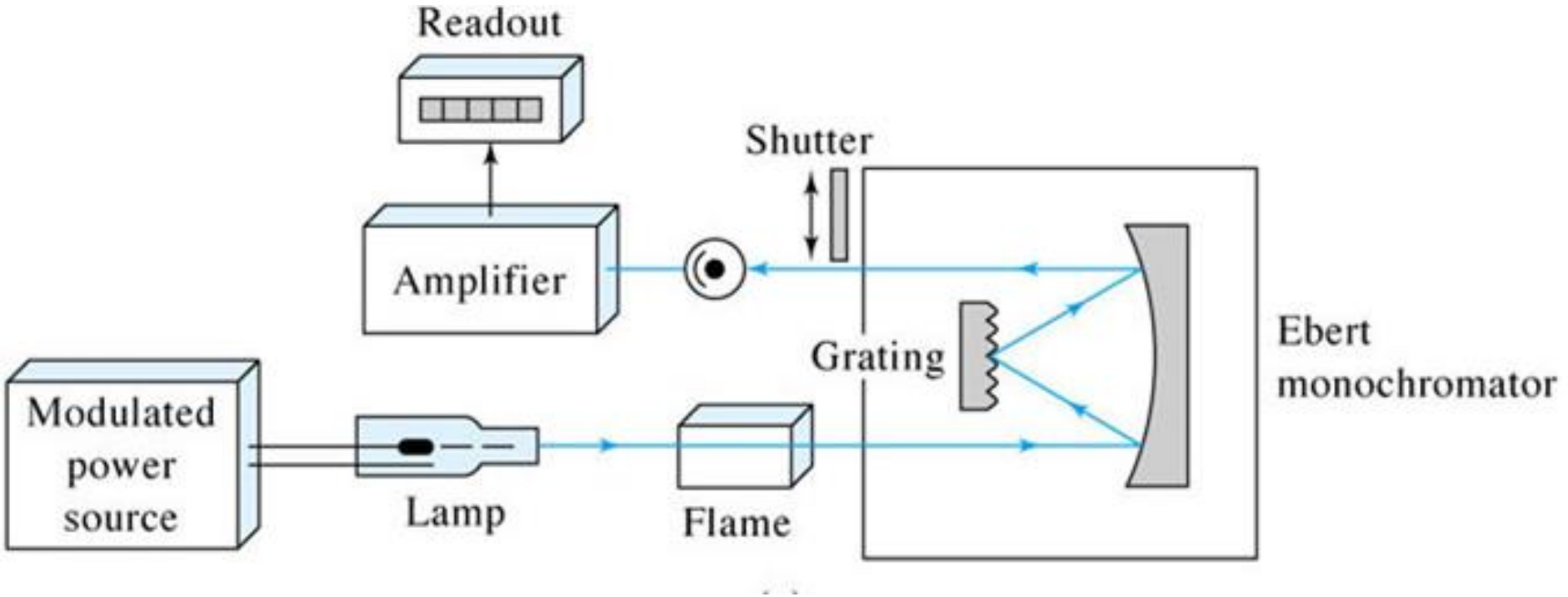
(3,4). volatilization – convert solid analyte/matrix particles into gas phase

(5). dissociation – break-up molecules in gas phase into atoms

(6). excitation – with light, heat, etc. for spectra measurement.

(7). ionization – cause the atoms to become charged

Working procedure



AAS consists of

(i). source delivering the characteristic radiation of the analyte

(ii). an atom reservoir into which the analyte is introduced and atomized

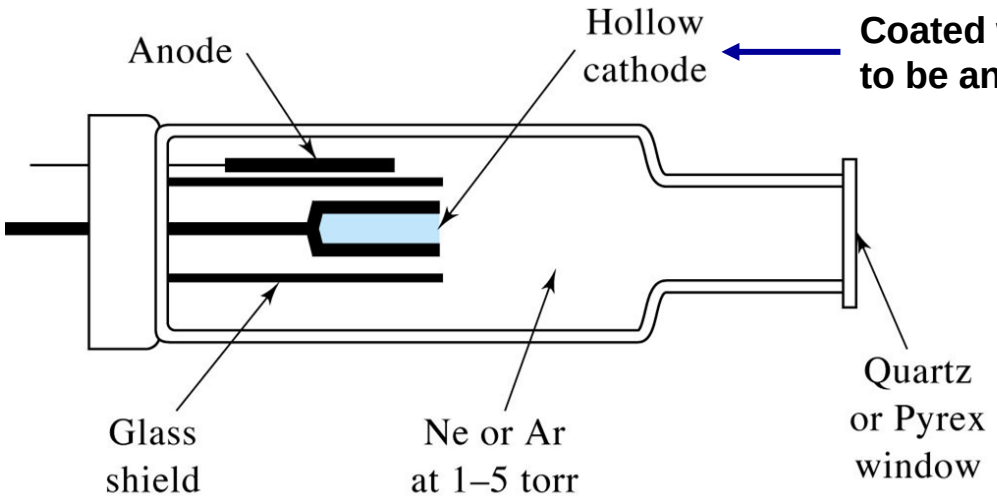
(iii). a monochromator

(iv). detector and

(v). readout device.

The radiation from a hollow cathode lamp is made to fall on the sample of the analyte aspirated into the flame where a part of it is absorbed. The transmitted radiation is then dispersed by a monochromator and sent to the detector.

Hollow Cathode Lamp



Process: *use element to detect element*

(1). ionizes inert gas to high potential (300V): Ar □ $Ar^+ + e^-$

(2). Ar^+ go to "-ve" cathode and hit surfaces

(3). As Ar^+ ions hit cathode, some of deposited element is excited and dislodged into gas phase (sputtering)

(4). excited element relaxes to ground state and emits characteristic radiation

Advantages: sharp lines specific for element of interest

Disadvantages: can be expensive, need to use different lamp for each element tested.

LIMITATIONS: Need for trace metal analyses at mg/L and even sub mg/L levels calls for a more sensitive technique. To meet this trace analysis, one has to use **GRAPHITE FURNACE ATOMIC ABSORPTION.**

(d)-(ii): Overview of Infrared (IR) Spectroscopy

- An IR spectrometer is an optical instrument used to measure properties of light over a specific portion of the EM spectrum in the range of 5 to 20 μ.
- **Fourier Transform Infrared (FTIR) spectrometer** obtains IR spectra by first collecting an interferogram of a sample signal using an interferometer and then performs a **Fourier Transform** on the interferogram to obtain the spectrum.
- An **interferometer** is an instrument that uses the technique of superimposing (interfering) two or more waves, to detect differences between them. FTIR spectrometer uses a Michelson interferometer.
- Fourier transform defines a relationship between a signal in time domain and its representation in frequency domain.
- Being a transform, no information is created or lost in the process, so the original signal can be recovered from the Fourier transform and vice versa.

What is Finger Print region and Functional Group region in IR spectroscopy?

- It is convenient to split an IR spectrum into two approximate regions: **4000-1000 cm^{-1} known** as the **functional group region**, and <1000 cm^{-1} known as the **fingerprint region**.
- It usually contains a large number of peaks, making it difficult to identify individual peaks.

Wavelength = 1/Wavenumber
For the IR, wavelength is in μ.
Wavenumber is typically in 1/cm, or cm^{-1}.

5 μ corresponds to 2000 cm^{-1}.
20 μ corresponds to 500 cm^{-1}.
15 μ corresponds to 667 cm^{-1}.

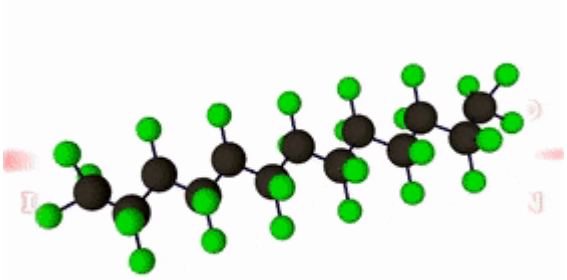
- The absorption of light, as it passes through a medium, varies linearly with the distance the light travels and with concentration of the absorbing medium.
- Where, “a” is the absorbance, “**ε**” is a characteristic constant for each material at a given wavelength (known as the extinction coefficient or absorption coefficient), “c” is concentration, and “l” is the length of the light path, the absorption of light may be expressed by the simple equation a= **ε**cl
- Infrared spectroscopy is the measurement of the wavelength and intensity of the absorption of mid-infrared light by a sample. Mid-infrared is energetic enough to excite molecular vibrations to higher energy levels.
- The wavelength of infrared absorption bands is characteristic of specific types of chemical bonds, and infrared spectroscopy finds its greatest utility for identification of organic and organometallic molecules.

Theory of Infrared Absorption Spectroscopy

- For a molecule to absorb IR, the vibrations or rotations within a molecule must cause a net change in the dipole moment of the molecule. The alternating electrical field of the radiation (remember that EM radiation consists of an oscillating electrical field and an oscillating magnetic field, perpendicular to each other) interacts with fluctuations in the dipole moment of the molecule.
- If the frequency of the radiation matches the vibrational frequency of the molecule then radiation will be absorbed, causing a change in the amplitude of molecular vibration.

Beer-Lambert Law

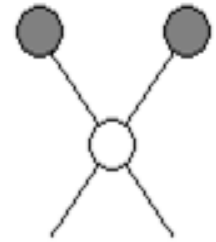
- When IR light passes through a molecular material, absorption can occur.
- The extent of absorption is given by the Beer-Lambert Law (also known as Beer's Law), where *A* is the absorption, *T* is the transmittance, I_0 is the incoming intensity of light, and *I* is the light transmitted through the sample. On the right side, ε is the absorptivity, *L* is the path length, and *c* is the concentration of the specific analyte.
- The absorptivity characterizes how much light is absorbed by a specific molecule at a specific wavelength. The product *Lc* effectively represents how many molecules are in the beam.



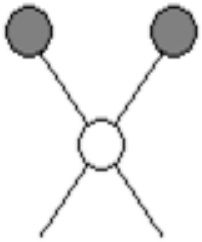
$$A = -log_{10}\left(\frac{I}{I_0}\right) = -log_{10}T = \varepsilon Lc$$

- Rotational levels are quantized, and **absorption of IR by gases yields line spectra**.
- However, **in liquids or solids, these lines broaden** into a continuum due to molecular collisions and other interactions.
- In general, a molecule which is an excited vibrational state will have rotational energy and can lose energy in a transition which alters both the vibrational and rotational energy content of the molecule.
- The total energy content of the molecule is given by the **sum** of the vibrational and rotational energies.
- For a molecule in a specific vibrational and rotational state, denoted by the pair of quantum numbers (***v***, ***J***), we can write its energy as:

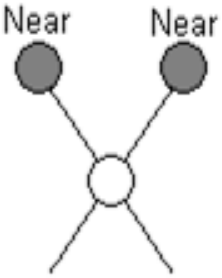
$$E(v, J) = E_{vib}(v) + E_{rot}(J)$$



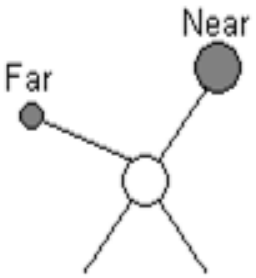
In-plane rocking



In-plane scissoring

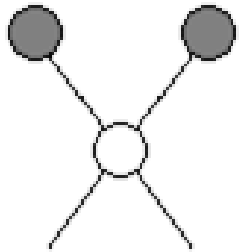


Out-of-plane wagging

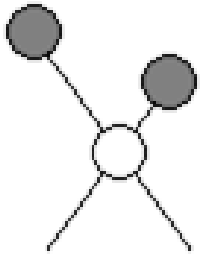


Out-of-plane twisting

Bending Vibrations

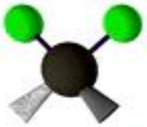


Symmetric

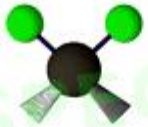


Asymmetric

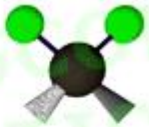
Stretching Vibrations



SYMMETRIC STRECHING



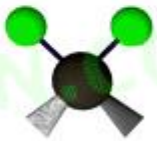
ANTISYMMETRIC STRECHING



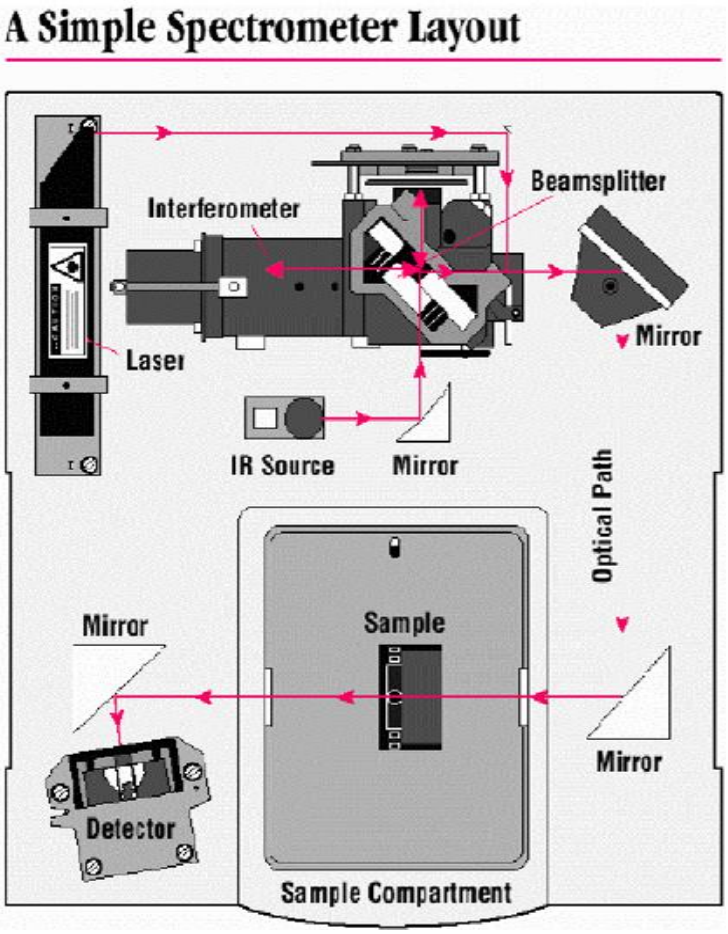
ROCKING

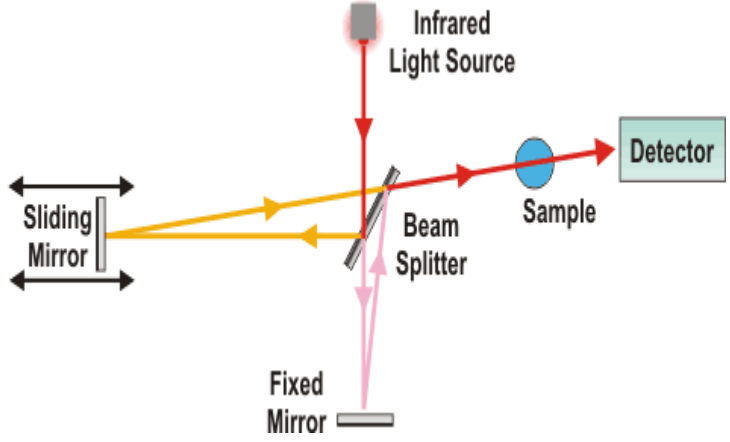
WAGGING

TWISTING



SCISSORING



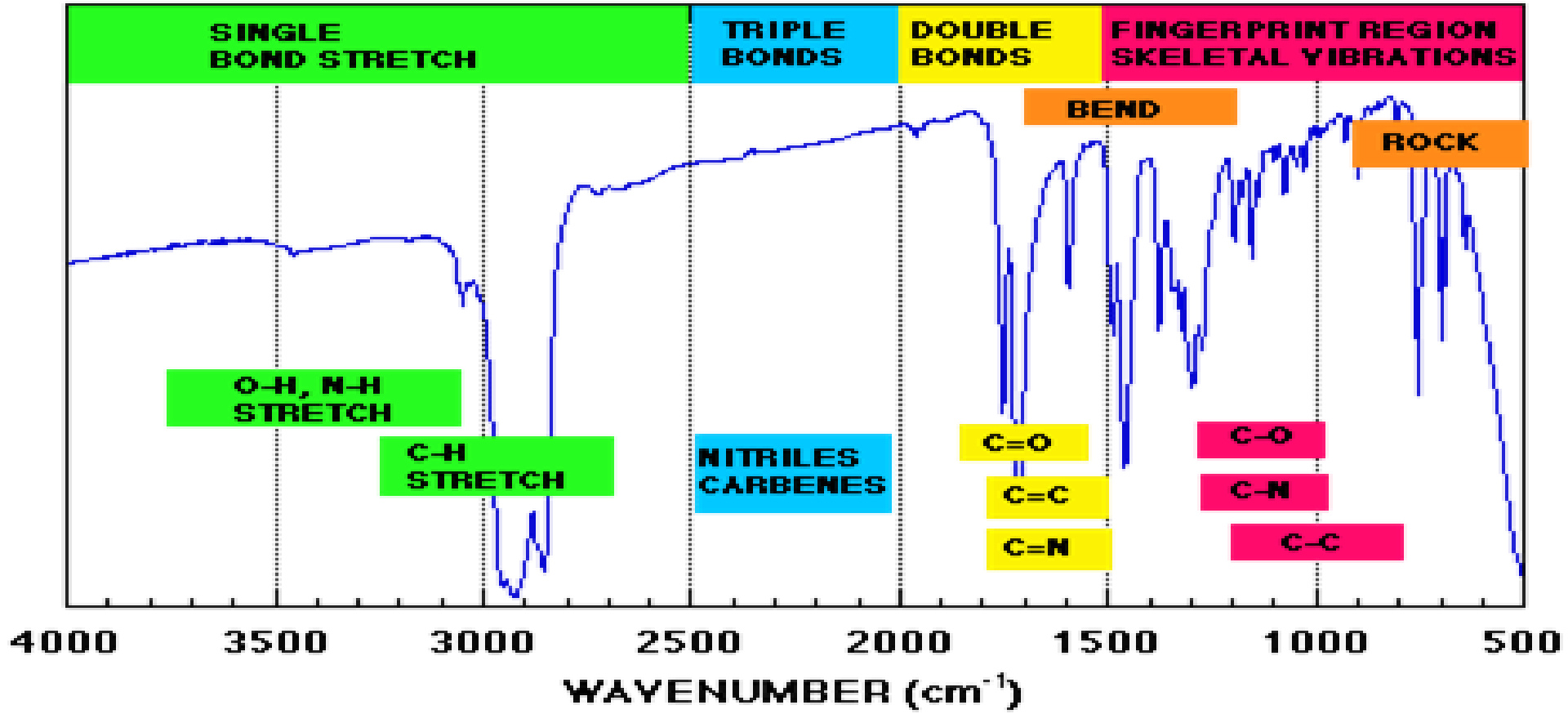


- An IR spectrophotometer is an instrument that passes IR light through an organic molecule and produces a spectrum that contains a plot of the amount of light transmitted on the vertical axis against the wavelength of infrared radiation on the horizontal axis.

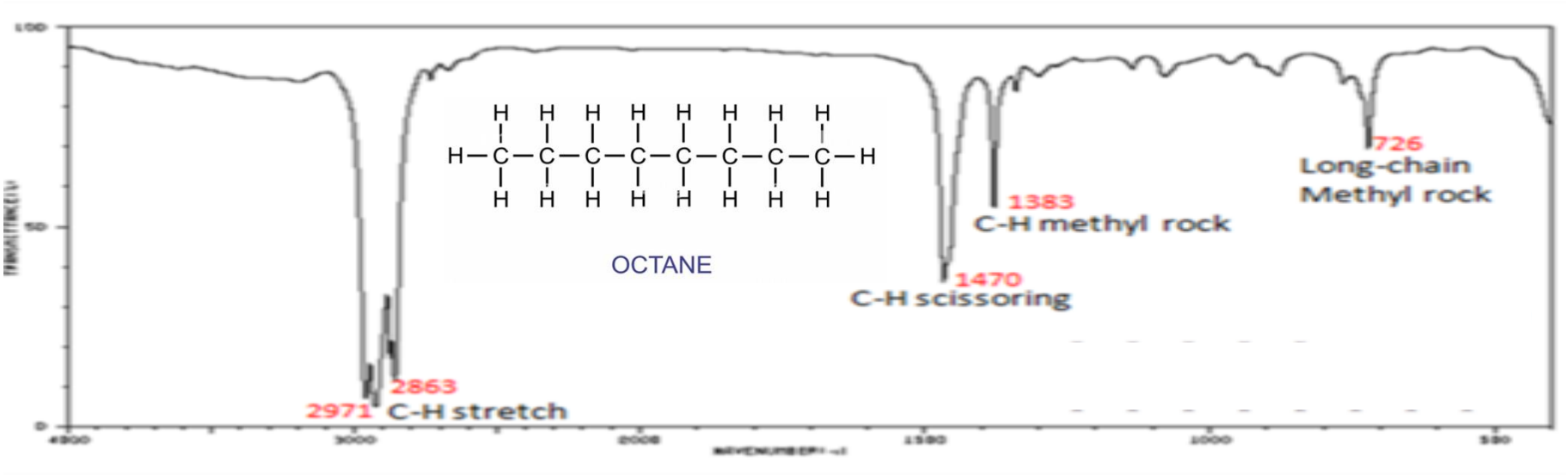
- The mirror moves at a fixed rate. Its position is determined accurately by counting the interference fringes of a collocated Helium-Neon laser.
- The Michelson interferometer splits a beam of radiation into two paths having different lengths, and then recombines them.
- Detector measures the intensity variations of the exit beam as a function of path difference.
- Monochromatic source would show a simple sine wave of intensity at detector due to constructive and destructive interference as the path length changes.

- In IR spectra, the absorption peaks point downward because the vertical axis is the % transmittance of the radiation through the sample.
- Absorption of radiation lowers the % transmittance value. Since all bonds in an organic molecule interact with IR radiation, IR spectra provide a considerable amount of structural data.

Absorbance of organic functional groups and bonds in the IR region

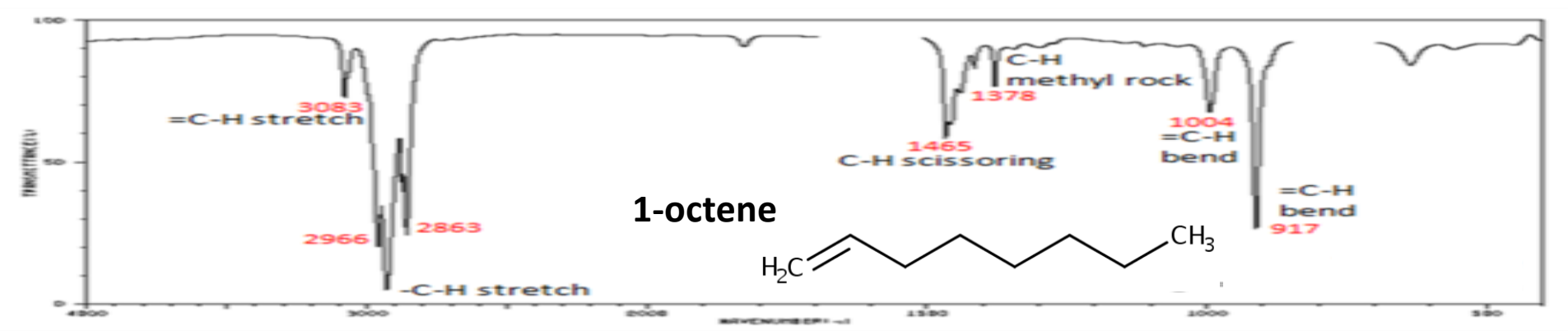


Examples:



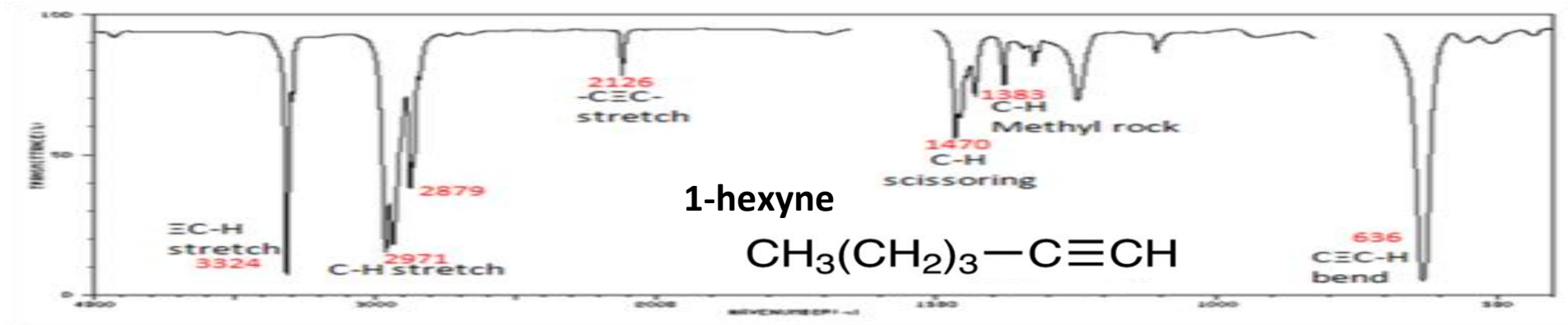
Hydrocarbons

- Hydrocarbons compounds contain only C-H and C-C bonds, and more information can be obtained from IR spectra arising from C-H stretching and C-H bending.
- In alkanes, which have very few bands, each band in the spectrum can be assigned:
- C–H stretch from 3000–2850 cm^{-1}
- C–H bend or scissoring from 1470-1450 cm^{-1}
- C–H rock, methyl from 1370-1350 cm^{-1}
- C–H rock, methyl, seen only in long chain alkanes, from 725-720 cm^{-1}
- Above IR spectrum is for Octane. Note the change in dipole moment with respect to distance for the C-H stretching is greater than that for others shown, which is why the C-H stretch band is the more intense.



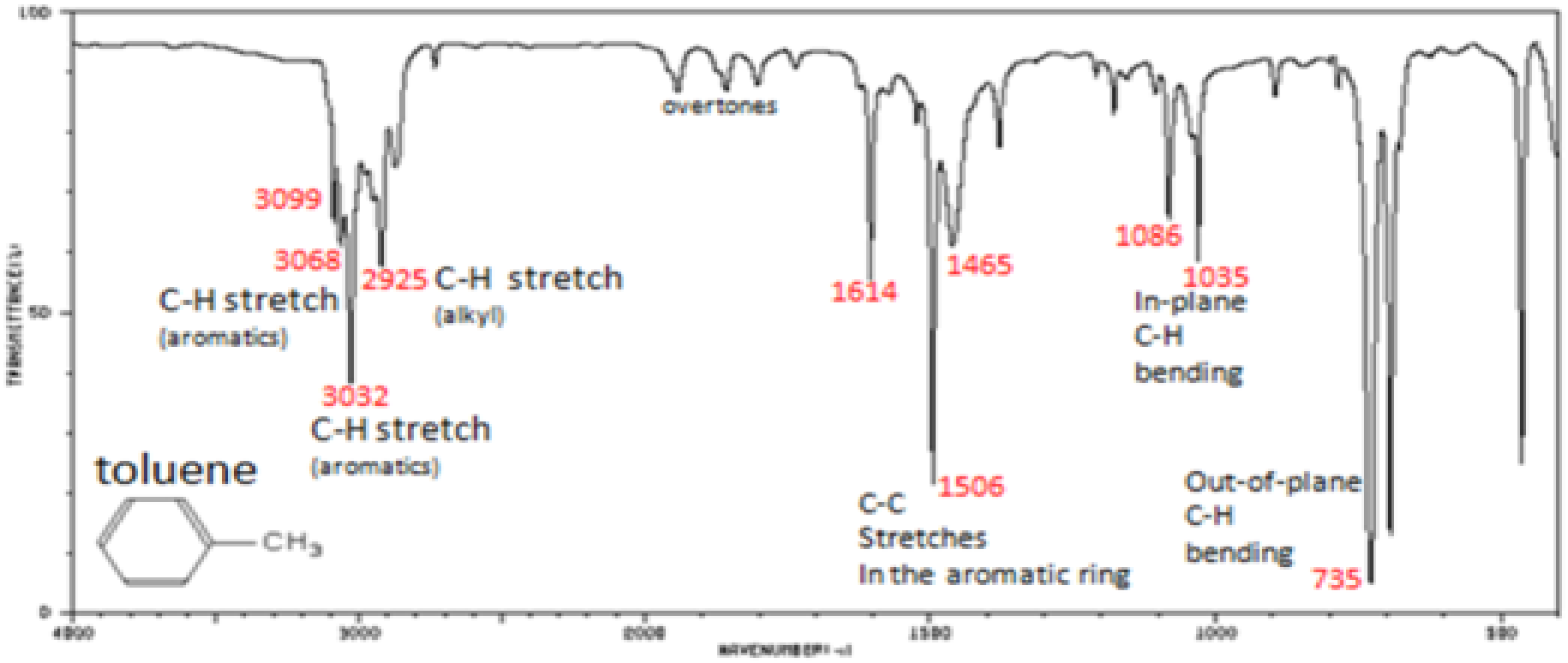
Above figure shows the IR spectrum of 1-octene. In alkenes compounds, each band in the spectrum can be assigned:

- C=C stretch from 1680-1640 cm^{-1}
- =C–H stretch from 3100-3000 cm^{-1}
- =C–H bend from 1000-650 cm^{-1}
- As alkanes compounds, these bands are not specific and are generally not noted because they are present in almost all organic molecules.



Spectrum of 1-hexyne, a terminal alkyne, is shown above. In alkynes, each band in the spectrum is assigned:

- –C≡C– stretch from 2260-2100 cm^{-1}
- –C≡C–H: C–H stretch from 3330-3270 cm^{-1}
- –C≡C–H: C–H bend from 700-610 cm^{-1}



Above is the spectrum of toluene. In aromatic compounds, each band in spectrum is assigned :

- C–H stretch from 3100-3000 cm^{-1}
- overtones, weak, from 2000-1665 cm^{-1}
- C–C stretch (in-ring) from 1600-1585 cm^{-1}
- C–C stretch (in-ring) from 1500-1400 cm^{-1}
- C–H “oop” from 900-675 cm^{-1}
- Note that this is at slightly higher frequency than is the –C–H stretch in alkanes. This is a very useful tool for interpreting IR spectra.
- Only alkenes and aromatics show a C–H stretch slightly higher than 3000 cm^{-1}.

What advantages does *in-situ* FTIR Spectroscopy bring to Reaction Analysis?

- The mid-IR energy region yields detailed “fingerprint” spectra of starting materials, intermediates, products and by-products allowing continually tracking of these key species as a function of time.
- Real-time measurement, performed every minute or less.
- In-situ, no extractive sampling required; measure chemistry without disturbing the reaction
- Non-destructive; preserving the integrity of the chemical reaction
- Measures reactions in batch, semi-batch or continuous flow operation
- Measures reactions run under pressure or at elevated or low temperature
- Measures reactions in aqueous or non-aqueous media and over a broad range of pH
- Spectral data can be converted into real-time concentration data, enabling the development of key kinetic parameters and precise endpoint determination
- Provides a primary means of obtaining important kinetic data and factual evidence supporting proposed mechanisms
- In-situ FTIR helps to identify and track transient intermediates that might affect product yield and quality and is key to mechanistic understanding
- Reaction trends are followed in real time, allowing for the monitoring key reaction events such as initiation, steady-state, endpoint and decomposition.

Where FTIR Spectroscopy used for?

Academic Research	Pharmaceutical Industry	Chemical Industry
Organocatalysis	Chemical Synthesis	Intermediates
Metal-Mediated Chemistry	Hydrogenation Reactions	Surfactants
Chemo- and Biocatalysis	Metal Catalyzed Reactions	Flavors and Fragrances
C-H Activation	Biocatalysis/Enzymatic Catalysis	Coatings/Pigments
Mechanistic Studies	Crystallization and Recrystallization (Supersaturation)	Agrochemicals
Reaction Kinetics/Reaction Progress Kinetics Analysis	Halogenations/Lithiations/Fluorine and Fluorination Chemistry	Initiators
Catalyst Cycles	Suzuki and Other Cross-Coupling Reactions	Bulk Chemicals
Polymerization Kinetics	Organometallic Chemistry	Isocyanate Reactions
	Low Temperature Chemistry	Ethylene Oxide and Propylene Oxide (EO/PO)
	Quality by Design and Process Analytical Technology	Highly Oxidizing Reactions
		Hydroformylation
		Catalytic Reactions
		Phosgenations
		Esterifications
		Halogenations

(d)-(iii). Overview of Nuclear Magnetic Resonance (NMR) Spectroscopy

- NMR spectroscopy or magnetic resonance spectroscopy (MRS) is a spectroscopic technique used to observe local magnetic fields around atomic nuclei.
- The sample is placed in a magnetic field and the NMR signal is produced by excitation of the nuclei sample with radio waves into nuclear magnetic resonance, which is detected with sensitive radio receivers.
- The intramolecular magnetic field around an atom in a molecule changes the resonance frequency, thus giving access to details of the electronic structure of a molecule and its individual functional groups.
- As the fields are unique or highly characteristic to individual compounds, in modern organic chemistry practice, NMR spectroscopy is the definitive method to identify monomolecular organic compounds.

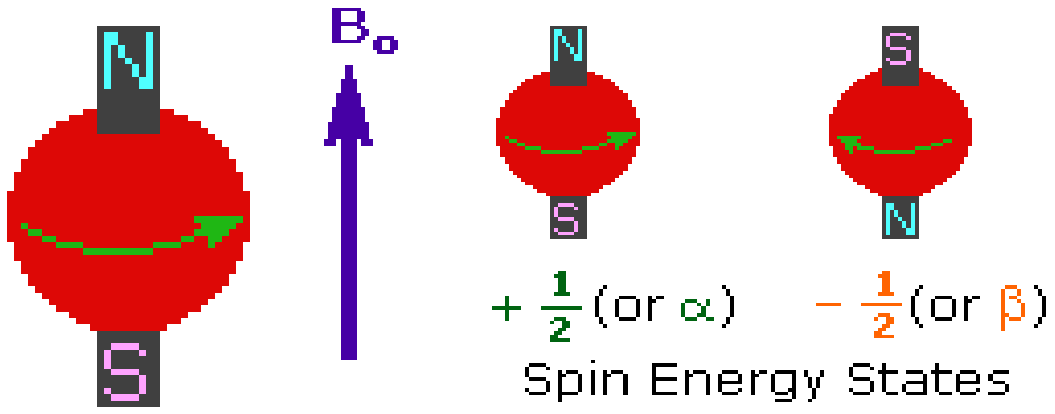
- Similarly, biochemists use NMR to identify proteins and other complex molecules. Besides identification, NMR spectroscopy provides detailed information about the structure, dynamics, reaction state, and chemical environment of molecules. The most common types of NMR are proton and ^{13}C-NMR spectroscopy, but it is applicable to any kind of sample that contains nuclei possessing spin.

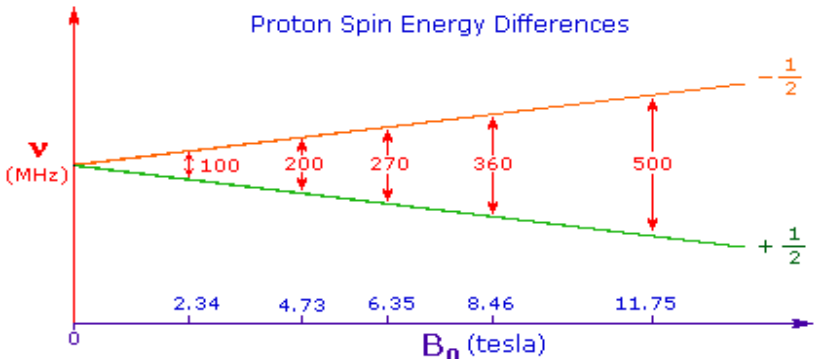
Principle of NMR: usually involves 3 sequential steps:

- Alignment (polarization) of magnetic nuclear spins in an applied, constant magnetic field B_0.
- Perturbation of this alignment of the nuclear spins by a weak oscillating magnetic field; usually referred to as a radio-frequency (RF) pulse.
- Detection and analysis of EM waves emitted by nuclei of the sample as a result of this perturbation.

The following features lead to the NMR phenomenon:

- A spinning charge generates a magnetic field. The resulting spin-magnet has a magnetic moment (**μ**) proportional to the spin.
- In the presence of an external magnetic field (**B_0**), two spin states exist [**+1/2** and **-1/2**].
- Magnetic moment of the lower energy +1/2 state is aligned with the external field, but that of the higher energy -1/2 spin state is opposed to the external field. Note that the arrow representing the external field points North.
- Difference in energy between the two spin states is dependent on the external magnetic field strength, and is always very small. The following diagram illustrates that the two spin states have the same energy when the external field is zero, but diverge as the field increases. At a field equal to B_x a formula for the energy difference is given (remember I = 1/2 and μ is the magnetic moment of the nucleus in the field).





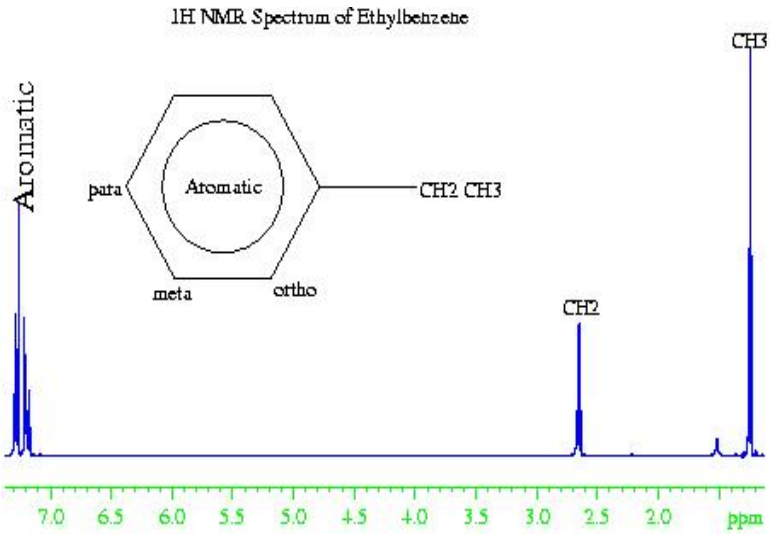
Chemical shift

- The precise resonant frequency of the energy transition is dependent on the effective magnetic field at the nucleus. This field is affected by electron shielding which is in turn dependent on the chemical environment. As a result, information about the nucleus' chemical environment can be derived from its resonant frequency.
- In general, the more electronegative the nucleus is, the higher the resonant frequency. Other factors such as ring currents (anisotropy) and bond strain affect the frequency shift.
- It is customary to adopt tetramethylsilane (TMS) as the proton reference frequency. This is because the precise resonant frequency shift of each nucleus depends on magnetic field used. Frequency is not easy to remember (for example, the frequency of benzene might be 400.132869 MHz) so it was decided to define chemical shift as follows to yield a more convenient number such as 7.17 ppm.

$$\delta = (\nu - \nu_0)/\nu_0$$

- The chemical shift, using this equation, is not dependent on the magnetic field and it is convenient to express it in ppm where (for proton) TMS is set to ν_0 thereby giving it a chemical shift of zero. For other nuclei, ν_0 is defined as $\Xi\ \nu_{TMS}$ where Ξ (Greek letter Xi, uppercase Ξ, lowercase **ξ**) is the frequency ratio of the nucleus (*e. g.*, 25.145020% for ^{13}C).

- In the case of ^{1}H-NMR spectrum of ethyl benzene, the methyl (CH_3) group is the most electron withdrawing (electronegative) and therefore resonates at the lowest chemical shift.
- The aromatic phenyl group is the most electron donating (electropositive) so has the highest chemical shift.



- The methylene (CH_2) falls somewhere in the middle. However, if the chemical shift of the aromatics were due to electro positivity alone, then they would resonate between 4 and 5 ppm. The increased chemical shift is due to the delocalized ring current of the phenyl group.

Uses of NMR spectroscopy

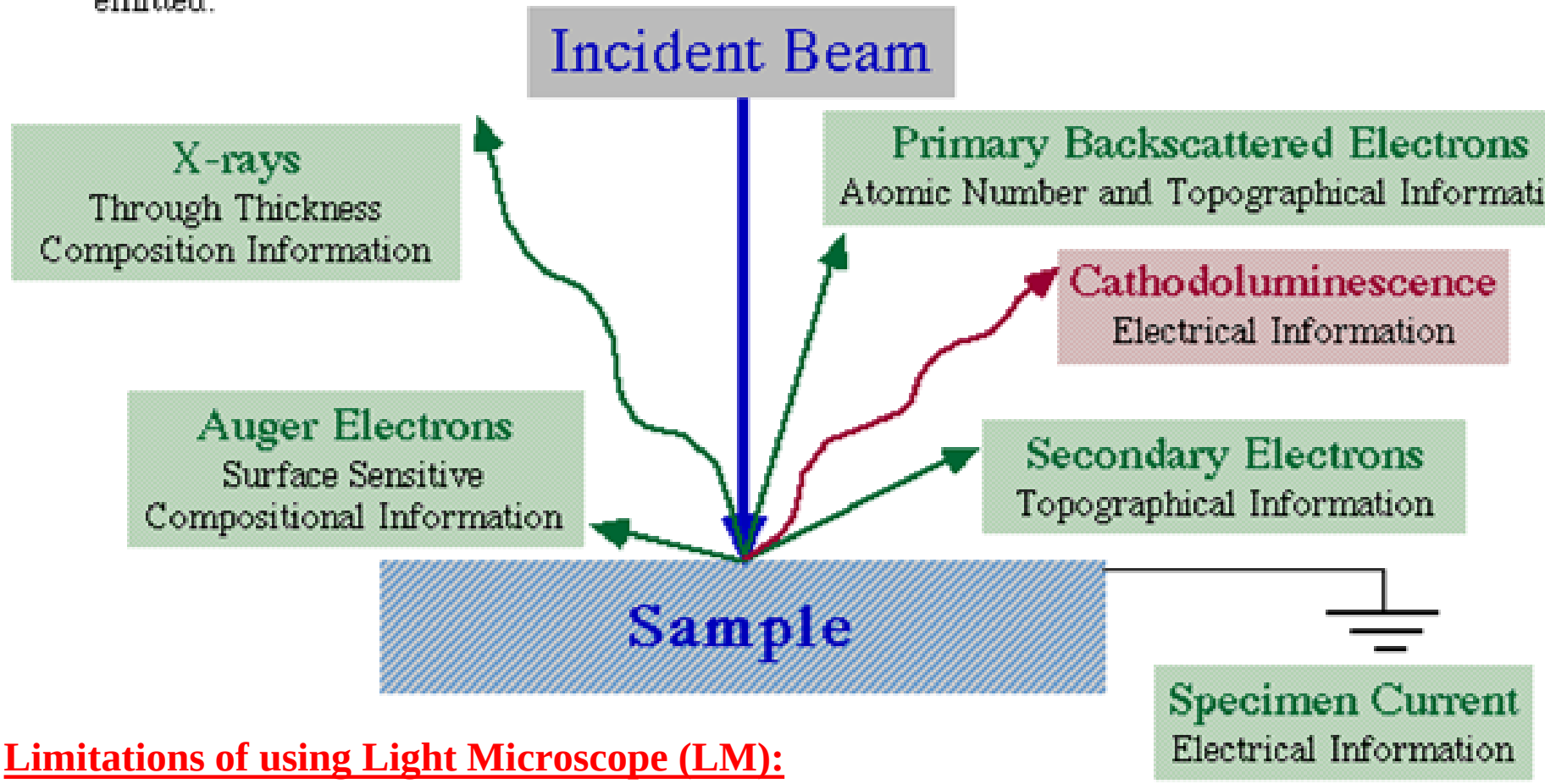
- NMR spectroscopy is used in quality control and research for determining the content and purity of a sample as well as its molecular structure.
- For ex. NMR can quantitatively analyse mixtures containing known compounds. For unknown compounds, NMR can either be used to match against spectral libraries or to infer the basic structure directly. Once the basic structure is known, NMR can be used to determine molecular conformation in solution as well as studying physical properties at the molecular level such as conformational exchange, phase changes, solubility and diffusion.

(d)-(iv). Overview of Scanning Electron Microscopy (SEM) & (d)-(v). Transmission Electron Microscopy (TEM)

Scanning Electron Microscopy (SEM)	Transmission Electron Microscopy (TEM)
	

Electron/Specimen Interactions

When the electron beam strikes the sample, both **photon** and **electron** signals are emitted.



Limitations of using Light Microscope (LM):

- LM has a magnification of 1000x with a resolution of 200 nm.
- Resolving power of LM is limited by the number and quality of the lenses but also by the wavelength of the light used for illumination.
- Using light with a short wavelength (blue or ultraviolet) gave a small improvement.
- Immersing the specimen and the front of the objective lens in a medium with a high refractive index (oil) gave another small improvement leading upto 100 nm.

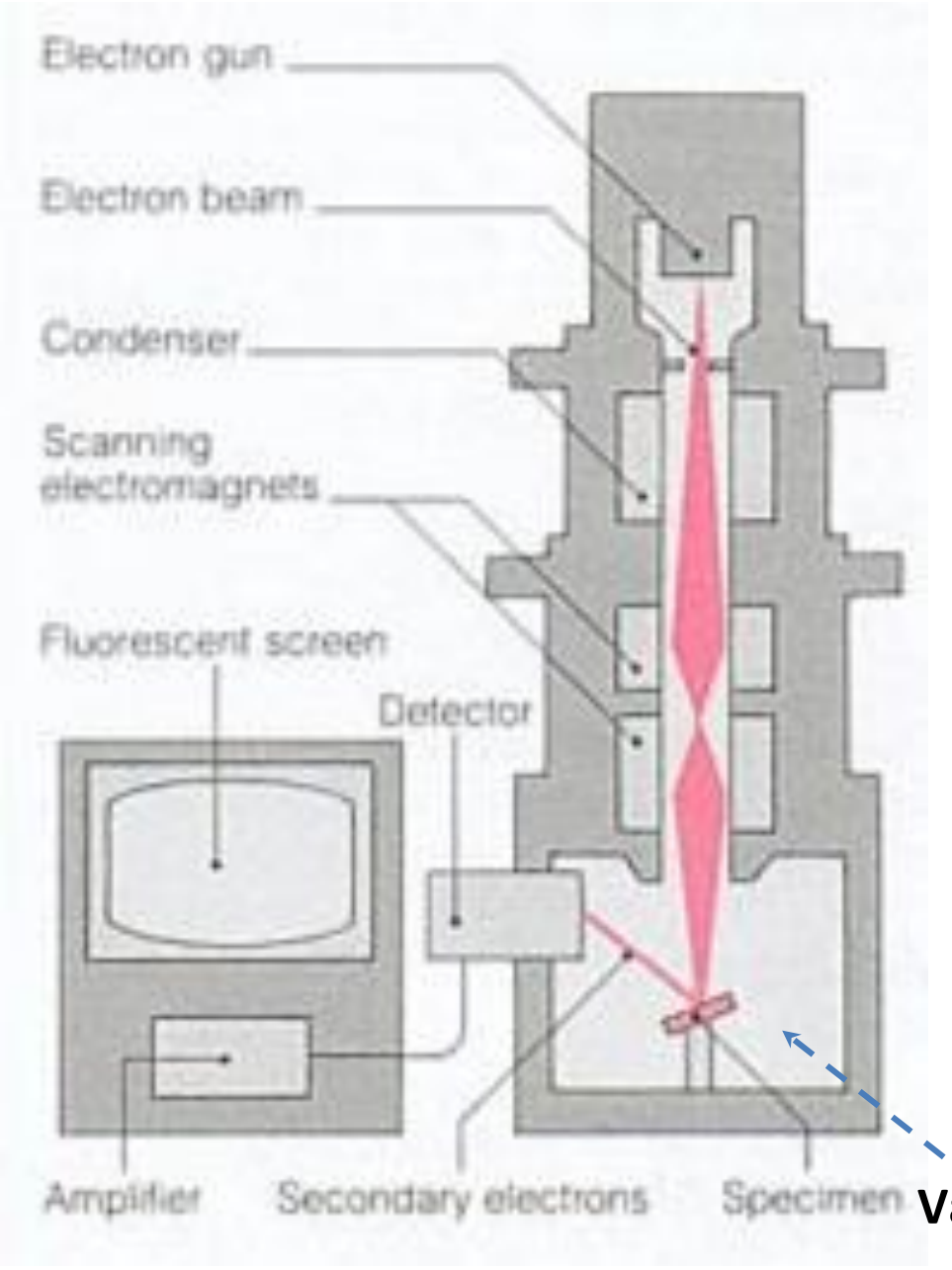
SEM basics

- Imagine yourself alone in an unknown darkened room with only a fine beam torch. You might start exploring the room by scanning the torch beam systematically from side to side gradually moving down so that you could build up a picture of the objects in the room in your memory.
- **SEM** uses an **electron beam** instead of a **torch**, an **electron detector** instead of **eyes** and a **fluorescent screen and camera** as **memory**.

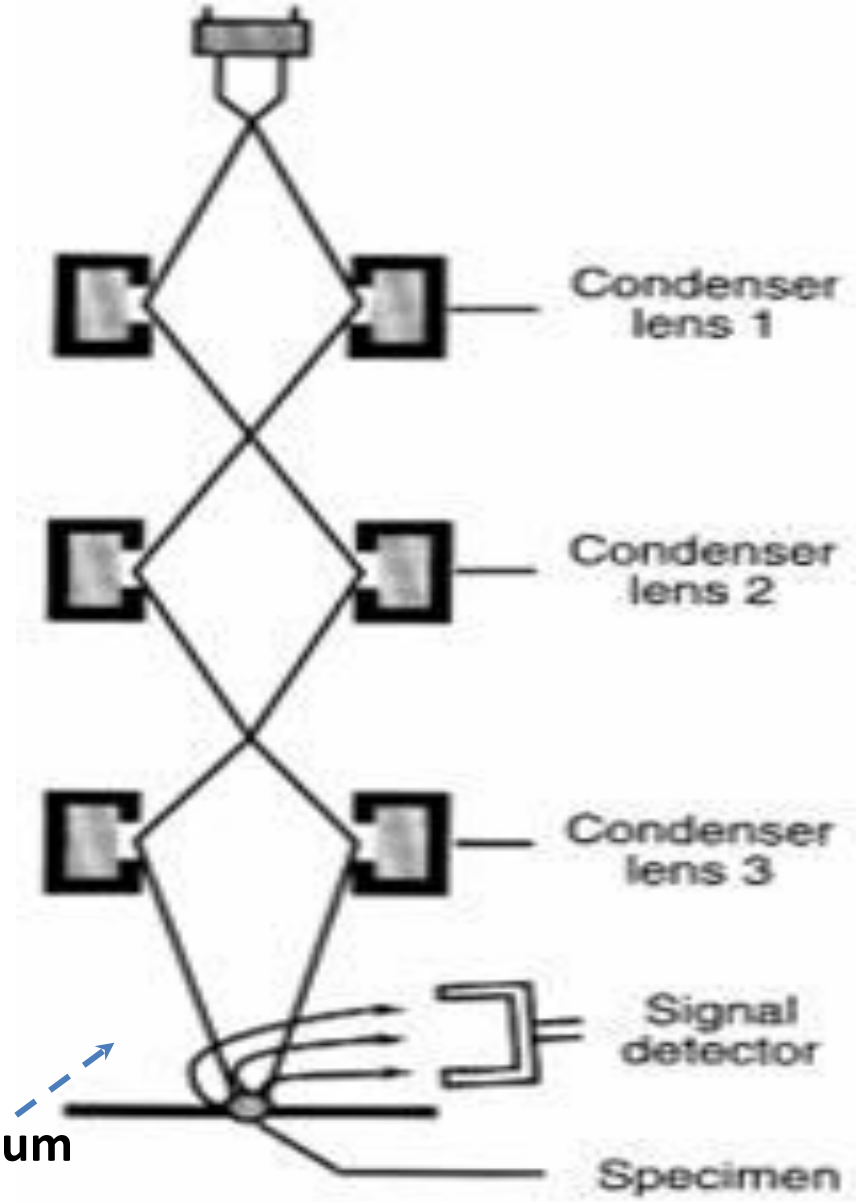
- Accelerated electrons behave in vacuum just like light. They travel in straight lines and have a wavelength which is about 100 000 times smaller than that of light.
- Furthermore, electric and magnetic fields have the same effect on electrons as glass lenses and mirrors have on visible light.
- The first electron microscope used two magnetic lenses and later added a third lens to achieve a resolution of 100 nm, twice as good as that of the light microscope.
- Now, the electron microscope uses five magnetic lenses in the imaging system, a resolving power of 0.1 nm at magnifications of over 1 million times.

Essential components of SEM

1. Electron gun as Source
2. Electron Lenses
3. Sample Stage
4. Detectors for all signals of interest
5. Display/Data output devices
6. Infrastructure Requirements: (a). Power Supply, (b). Vacuum System, (c). Cooling system, (d). Vibration-free floor and (e). Room free of ambient magnetic and electric fields.


Electron gun
Electron beam
Condenser
Scanning electromagnets
Fluorescent screen
Detector
Amplifier
Secondary electrons
Specimen

Vacuum


Condenser lens 1
Condenser lens 2
Condenser lens 3
Signal detector
Specimen

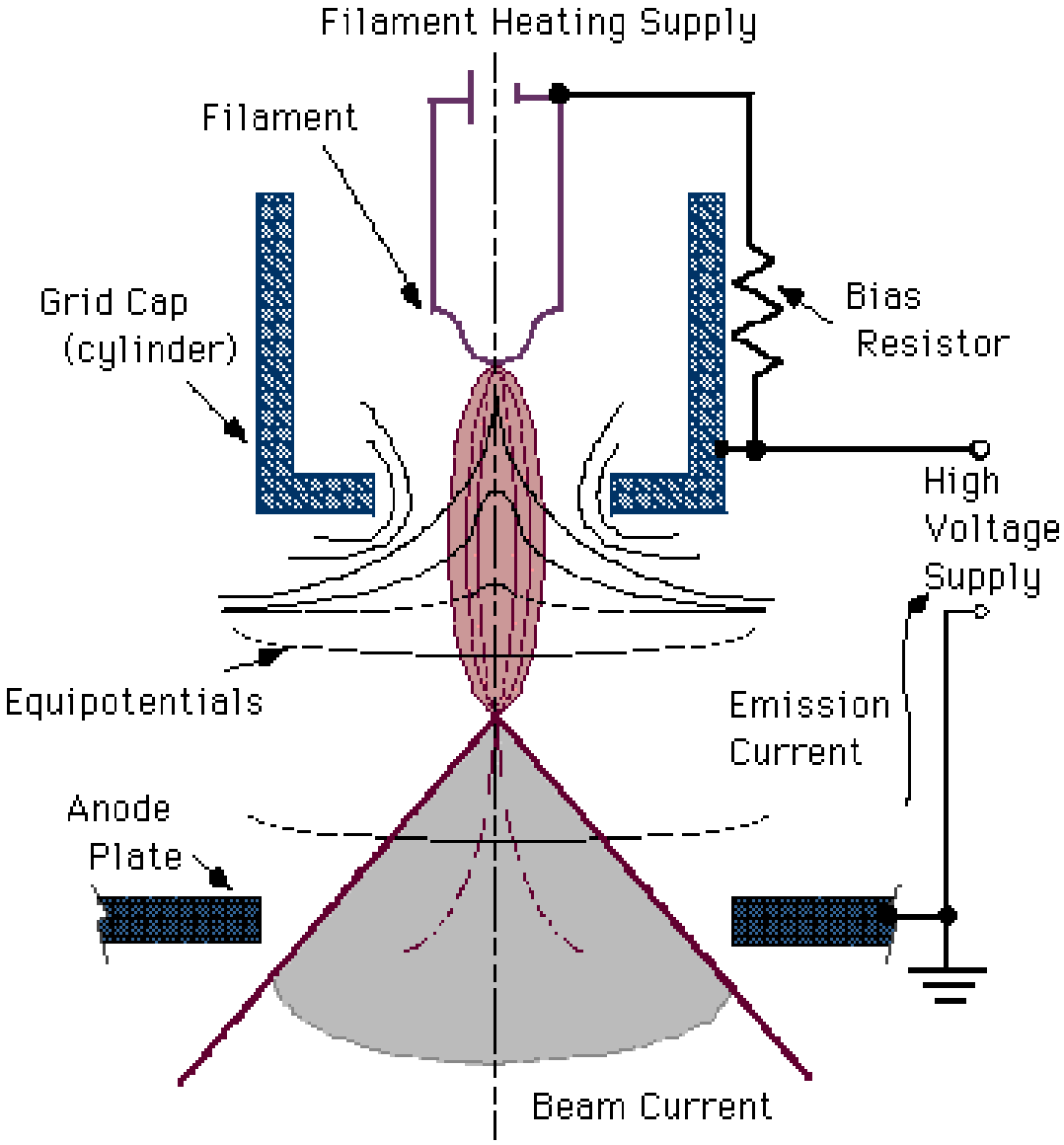
1. Electron gun as Source:

Electron gun consists of 3 important components:

(i). Filament made from hair-pin shaped Tungsten (or Lanthanum Hexaboride) functioning as Cathode. Voltage applied to the filament heats it up to 2700 °C;

(ii). Wehnelt cylinder (also known as grid cap or cylinder) made from platinum or tantalum foil. It is used for focusing and controlling the electron beam and has the shape of a hollow cylinder. The bottom side of the cylinder has an opening at its centre with 200 to 1200 µm diameter; and

(iii). Anode, which is positive with respect to the filament, forms powerful attractive forces for electrons. This causes electrons to accelerate (several hundred thousand km/sec) toward the anode. Some accelerate right by the anode and down by the column onto the sample.



These 3 components together form a **triode electron gun** which is a very stable source of electrons.

2. Electron lenses - Magnification and resolution

- Magnification is entirely determined by electronic circuitry that scans the beam over the specimen (and simultaneously over fluorescent screen of monitor where the image appears).
- Magnification can be as high as 3,00,000x. Increasing the magnification is achieved by reducing the size of the area scanned on the specimen.
- Resolution of 1 nm can be attained depending on:
  a) Diameter of electron beam on the specimen surface
  b) Specimen properties
  c) Specimen preparation technique
  d) Instrumental parameters such as
     i. beam intensity
     ii. accelerating voltage
     iii. scanning speed
     iv. distance from the last lens to specimen (referred to as the working distance) and
     v. angle of the specimen surface with respect to the detector.

3. Sample stage: What happens to the specimen during electron bombardment?

Electron beam scan the specimen in a rectangular spot less than 4 nm in diameter.

a) Specimen itself emits secondary electrons.
b) Some of the primary electrons are reflected (back-scattered electrons).
c) Electrons are absorbed by the specimen.
d) Specimen emits X-rays, and sometimes emits photons (light).

All these phenomena are interrelated and depends on the specimen topography, its chemical state and atomic number. The number of backscattered electrons, secondary electrons and absorbed electrons at each point of the specimen depends on the specimen's topography to a much greater extent than the other properties mentioned above.

Specimen orientation and manipulation

- SEM image quality depends on specimen orientation and distance from detectors and lens.
- Specimen stage allows the specimen to be moved in a horizontal plane (X and Y direction) and up and down (Z direction) and rotated and tilted as required. These movements are often motorized and controlled by the PC.
- SEM models differ in size of their specimen chambers allowing various sizes of specimens to be introduced and manipulated. Maximum specimen size also determines the price because larger the specimen chamber, larger the goniometer movement needed and larger the pumping system needed to maintain a good vacuum.
- The simplest model accepts specimens of a few cm in diameter and can move them 50 mm in the X and Y directions. Largest chamber accepts samples up to 200 mm in diameter and can move them 150 mm in each direction.
- All models allow samples to be tilted up to high angles and rotated through 360^{o}.
- There are special stages for heating, cooling and straining specimens.

4. Detectors for all signals of interest

- Detectors for backscattered electrons and secondary electrons are either a scintillation detector or a solid state detector.
- In the case of scintillation detector, electrons strike a fluorescent screen, which then emits light that is amplified and converted into an electrical signal by photomultiplier tube.
- In the case of solid state detector, it works by amplifying the minute signal produced by the incoming electrons in a semiconductor device.
- Amplified signal of the electron beam is also impacted on a cathode ray tube. Both the beam in the microscope and the one in the CRT are scanned at the same rate and the 1-to-1 relationship between each point on the CRT screen and the corresponding point on the

5. Display/Data output devices

- SEM is usually equipped with two image monitors, one for observation by operator and other a high resolution monitor, equipped with ordinary photo camera (or Polaroid)
- To facilitate the observation and correct choice of the parameters mentioned above, SEM have an image store in which the image is built up scan by scan and displayed at TV speed so that there is a steady, flicker-free image on the viewing monitor.
- Digital images are stored electronically for subsequent enhancement and analysis.
- Since the SEM image is electronically produced, it can be subjected to contrast enhancement, inversion (black becomes white), mixing of images from various detectors, subtraction of the image from one detector from that produced by a different detector, colour coding and image analysis.
- All these techniques may be applied if it suits the primary aim of extracting the best possible information from the specimen.

6. Importance of Vacuum in SEM column:

- In SEM, the column through which electron beam passes through must always be at vacuum.
- If the sample is in a gas filled environment, the electron beam cannot be generated or maintained because of a high instability in the beam. Also, gases could react with the electron source, causing it to burn out, or cause electrons in the beam to ionize, which produces random discharges and leads to instability in the beam.
- Transmission of beam through electron optic column would also be hindered by the presence of other molecules from the sample or the microscope itself, and could form compounds and condense on the sample. This would lower the contrast and obscure detail in the image.
- Sufficiently low vacuum in SEM column is produced by either an oil diffusion pump or a turbo-molecular pump backed by a rotary pre-vacuum pump.
- These combinations provide reasonable exchange times for specimen, filament and aperture (less than 2 minutes) without the need to use vacuum airlocks.
- SEM vacuum system is fully automatically controlled and protected against operating failures.

Strengths of SEM

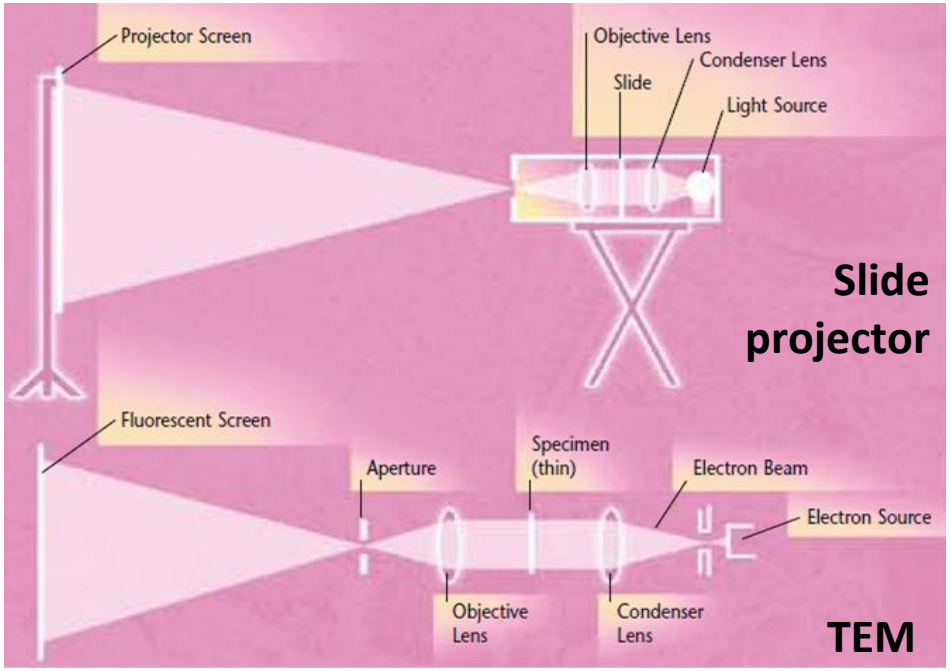
- **Gold as conductive coating:** A heavy element like gold is preferred for use as conductive coating as it gives a good yield of secondary electrons and thereby a good quality image. In addition, it gives a fine grain coating and is easily applied in a sputter coater. The layer required to ensure a conducting layer is quite thin (about 10 nm).
- **Operational features:** Most SEM models are easier to operate, with user-friendly interfaces, minimal sample preparation and rapid data acquisition (less than 5 min/image).
- SEM generate data in digital formats, which are highly portable.

Limitations of using SEM:

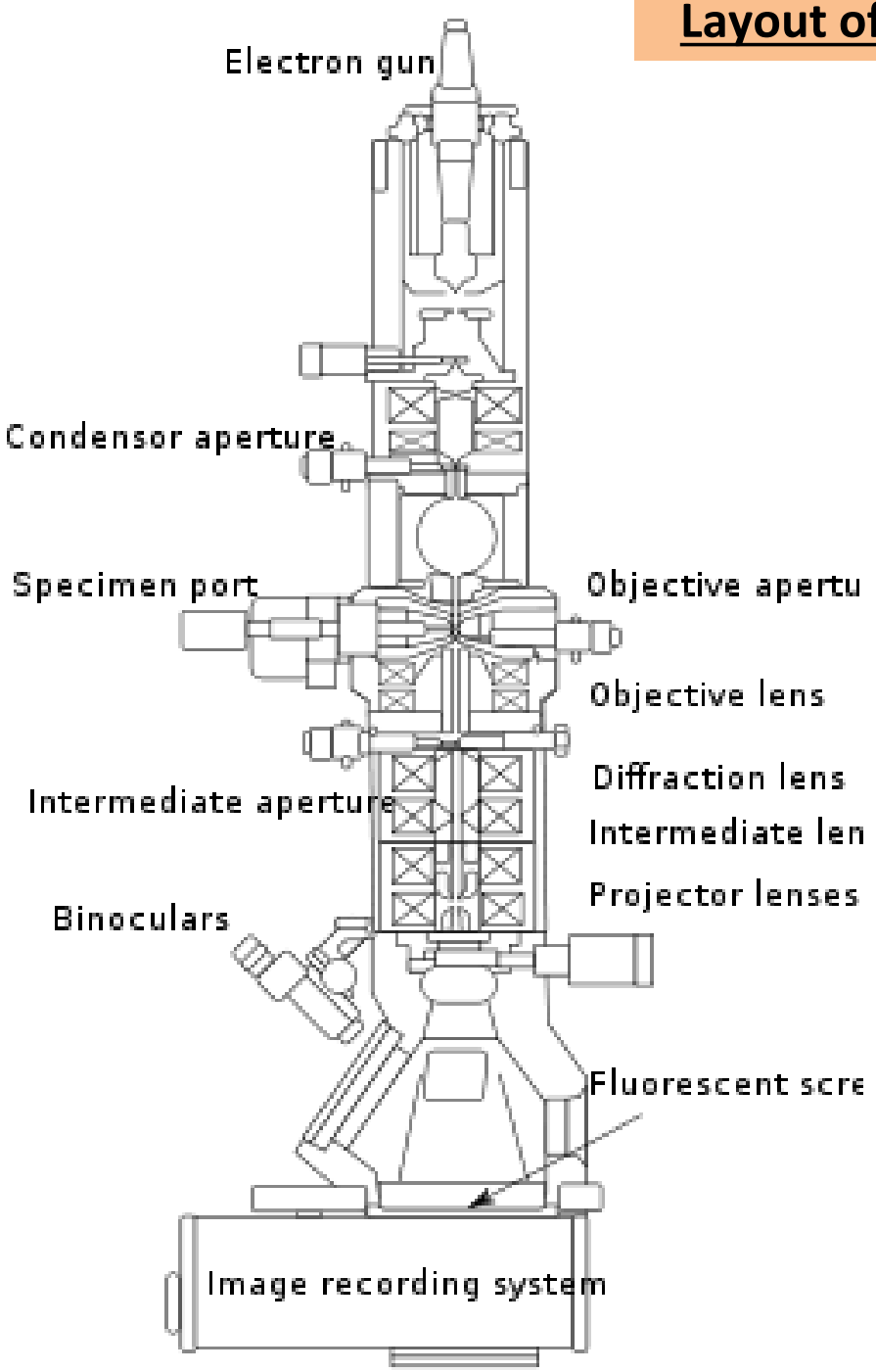
- **Sample condition:** Must be solid and stable in a vacuum to order of 10^{-5}~10^{-6} torr. Samples likely to outgas at low pressures (rock sample saturated with hydrocarbons, wet samples such as coal, organic materials or swelling clays) are unsuitable for examination in conventional SEM. However, they can be measured using "low vacuum" SEM.
- SEM cannot measure samples that fail to withstand the electron bombardment.
- If the specimen contains any volatile components such as water, it need to be removed using a drying process (or can be frozen solid).
- Non-conducting specimens will charge up under electron bombardment, and hence an electrically conductive coating must be applied to electrically insulating samples for study in conventional SEM, unless the instrument is capable of operation in a low-vacuum mode.
- **Detector type:** Solid state x-ray detector (EDS) cannot detect elements with A.N. less than 11 (Na). While EDS is very fast and easy to utilize, they have relatively poor energy resolution

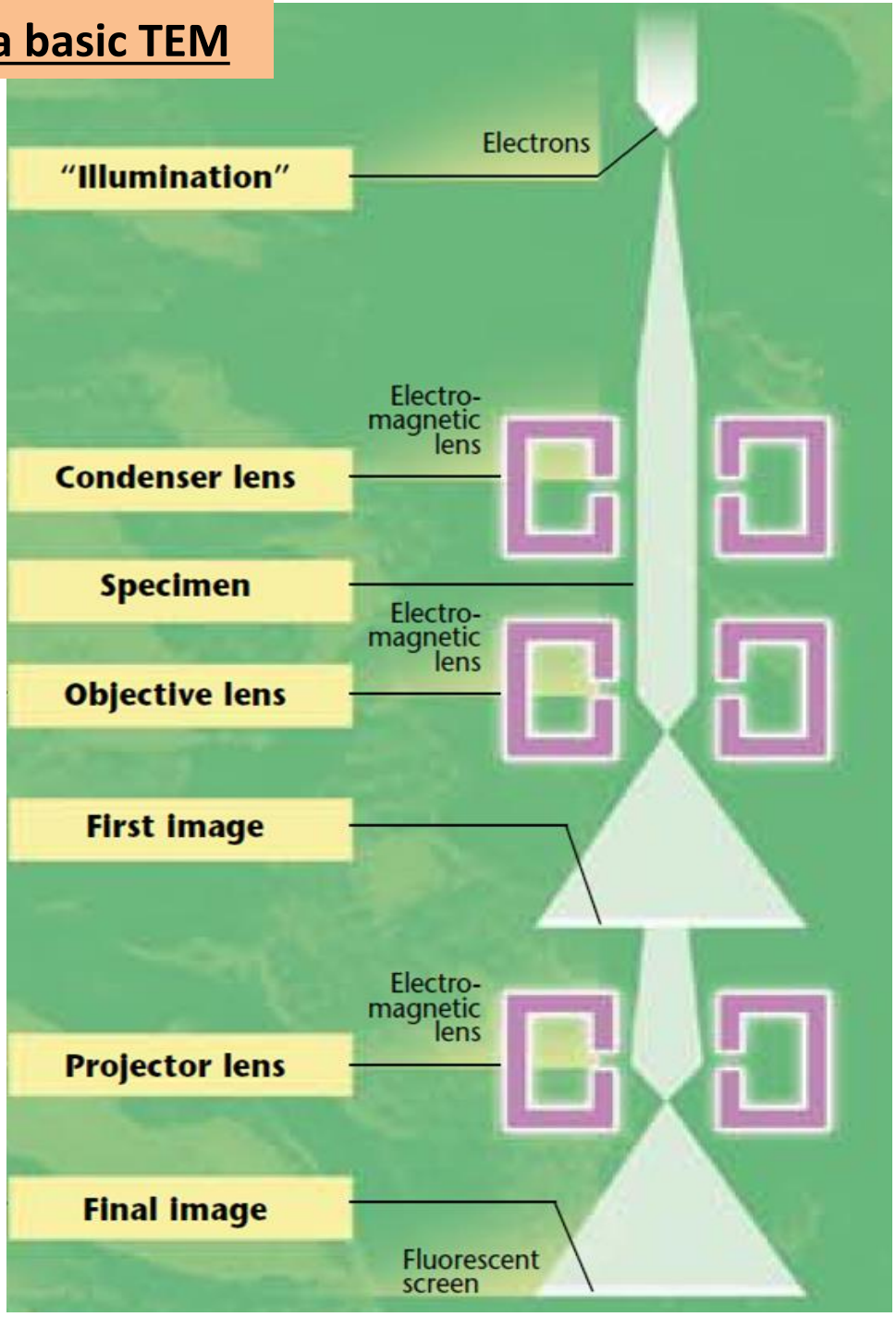
Transmission Electron Microscope (TEM)

- TEM can be compared with a slide projector. In projector case, light from a source is made into a parallel beam by condenser lens, and passed through the slide (object), which is further focused as an enlarged image onto the screen by the objective lens.
- In TEM case, the light source is replaced by an electron source (tungsten filament heated in vacuum), glass lenses are replaced by magnetic lenses and the projection screen is replaced by a fluorescent screen which emits light when struck by electrons.
- Electromagnetic lenses are variable, i.e. by varying the current through the lens coil, the focal length which determines the magnification can be varied.
- Entire electron path from gun to screen has to be under vacuum (otherwise the electrons would collide with air molecules and be absorbed). The final image is viewed through a window in the projection chamber.
- Specimen (object) has to be very thin to allow the electrons to pass through it.
- TEM specimens are usually **0.5 µm or less thick.** Higher the electrons speed, higher the accelerating voltage in the gun, and thicker the specimen that can be studied.



Layout of a basic TEM





What happens in the specimen during the electron bombardment in TEM?

When electrons impinge on the specimen, a number of things can happen:

a. Some of the electrons are **absorbed** as a function of thickness and composition of the specimen, which causes **amplitude contrast** in the image.
b. Other electrons are **scattered** over small angles, depending on the composition of the specimen, which causes **phase contrast** in the image.
c. In crystalline specimens, electrons are **scattered** in very distinct directions which are a function of the crystal structure, which causes **diffraction contrast** in the image.
d. Some of the impinging electrons are **reflected**, and are called **backscattered electrons**.
e. The impinging electrons can cause the **specimen itself to emit electrons** (these are called **secondary electrons**).
f. The impinging electrons cause the specimen to **emit X-rays** whose energy and wavelength are related to the specimen's **elemental composition**.
g. The impinging electrons cause the specimen to **emit photons or light (**called **cathodo-luminescence.**
h. Finally, electrons which have lost an amount of energy because of interaction with the specimen can be detected by **an Energy Loss Spectrometer** which is the equivalent of a **prism in light optics**.

Essential components of TEM

1. Electromagnetic lenses

- When an electrical current is passed through the coils (C), an electromagnetic field is created between the pole pieces (P) which form a gap in the magnetic circuit.
- By varying the current through the coils, the magnification of the lens can be varied. This is the essential difference between the magnetic lens and the glass lens.
- Otherwise they behave in the same way and have the same types of aberration: spherical aberration (the magnification in the centre of the lens differs from that at the edges), chromatic aberration (the magnification of the lens varies with the wavelength of the electrons in the beam) and astigmatism (a circle in the specimen becomes an ellipse in the image).
- Spherical aberration is a very important characteristic which is largely determined by the lens design and manufacture. Chromatic aberration is reduced by keeping the accelerating voltage as stable as possible and using very thin specimens. Astigmatism can be corrected by using variable electromagnetic compensation coils. The condenser lens system focuses the electron beam onto the specimen under investigation as much as necessary to suit the purpose.
- The **objective lens** produces an image of the specimen which is then magnified by the remaining imaging lenses and projected onto the fluorescent screen. If the specimen is crystalline there will be a diffraction pattern at a different point in the lens known as the back focal plane.

- By varying the strength of the lens immediately below the objective lens, it is possible to enlarge the diffraction pattern and project this onto the fluorescent screen. In the **Tecnai series of TEMs**, the objective lens is followed by four lenses: a diffraction lens, an intermediate lens and two projector lenses.
- To guarantee a high stability and to achieve the highest possible magnification, the lenses in a modern TEM are water cooled. On the way from the filament to the fluorescent screen, the electron beam passes through a series of apertures with different diameters. These apertures stop those electrons which are not required for image formation (e.g. scattered electrons).
- Using a special holder carrying four different apertures, the diameter of the apertures in the condenser lens, the objective lens and the diffraction lens can be selected from outside the column as dictated by circumstances.

2. Observation and recording of the image

- Image on fluorescent screen can be observed through a large window in the projection chamber. In order to examine fine detail or to assist correct focusing of image, a special fine-grain focusing screen is inserted into beam and observed through high-quality **12x binocular viewer.**
- Electrons have same influence on photographic material as light. It's only necessary **to replace the fluorescent screen with a photographic film** in order to record the image.
- In practice the fluorescent screen hinges up to allow the image to be projected on the film below. It is also possible to use a TV camera to record the image digitally, making it suitable for subsequent enhancement and analysis. It can also be used for recording dynamic phenomena using a video tape recorder or as the input signal for an image analysis system.

3. Diffraction

- When a wave passes through a periodic structure whose periodicity is of the same order of magnitude as the wavelength, the wave emerging is subject to interference which produces a pattern beyond the object.
- This phenomenon can be observed when ocean waves pass through a regular line of posts or when a street lamp is seen through an umbrella. The street lamp appears as a rectangular pattern of spots of light, bright in the centre and then getting fainter. This is caused by diffraction of light by the weave of the umbrella fabric and the size and form of the pattern provide information about the structure (closeness of weave and orientation).
- In exactly the same way, electrons are diffracted by a crystal and the pattern of spots on the screen of the microscope gives information about the crystal lattice (shape, orientation and spacing of the lattice planes).

4. Vacuum

- Electrons behave like light only when they are manipulated in vacuum. The whole column from gun to fluorescent screen and including the camera is evacuated. Various levels of vacuum are necessary: the highest vacuum is around the specimen and in the gun; a lower vacuum is found in the projection chamber and camera chamber.
- Different vacuum pumps are used to obtain and maintain these levels. The highest vacuum attained is of the order of a ten millionth of mm Hg.
- To avoid having to evacuate the whole column every time a specimen or photographic material or a filament is exchanged, a number of airlocks and separation valves are built in. In modern TEMs the vacuum system is completely automated and the vacuum level is continuously monitored and fully protected against faulty operation.

5. Electronics

- To obtain the very high resolution of which modern TEMs are capable, the accelerating voltage and the current through the lenses must be extremely stable.
- The power supply cabinet contains a number of power supplies whose output voltage or current does not deviate by more than one millionth of the value selected for a particular purpose. Such stabilities require very sophisticated electronic circuits.
- Digital electronic techniques in general and microprocessor-based techniques in particular play an important role in this respect. Modern electron microscopes employs a fast, powerful PC (personal computer) to control, monitor and record the operating conditions of the microscope.
- This results in a dramatic reduction in number of control knobs compared with earlier models and a microscope which is very easy to use. Furthermore, it allows special techniques to be embedded in the instrument so that the operator can carry them out using the same controls.
- PC can be attached to a network to allow automatic back-ups to be made and results to be downloaded to other workstations. The microscope can always be brought up to date by installing new software or by replacing the computer with the latest model.

6. Specimen orientation and manipulation

- With most specimens, it is not sufficient to move them only in the horizontal plane. Although the specimen is thin, there is nevertheless information in the image coming from various depths within the specimen.
- This can be seen by tilting the specimen and taking stereo photographs. In order to define the axis of tilt, it is necessary to be able to rotate the specimen.
- Crystalline specimens need to have a second tilt axis perpendicular to the first tilt axis in order to be able to orient a part of the specimen so as to obtain the required diffraction pattern. These requirements can be fulfilled in a device called a **goniometer.**
- The **goniometer** is a specimen stage designed to provide, in addition to X and Y translation of the specimen, tilt about one or two axes and rotation as well as Z movement (specimen height) parallel to the beam axis.
- It is usual also to provide for heating, cooling and straining of the specimen for specialised experiments in the microscope.
- The goniometer is mounted very close to the objective lens; the specimen is actually located in the objective lens field between the pole pieces because it is there that the lens aberrations are smallest and the resolution is highest.
- The goniometer itself provides motorised X, Y and Z movement and tilt about one axis. The specimen is mounted near the tip of a rod-shaped holder which in turn is introduced into the goniometer through an air lock.
- It is the specimen holder rod which provides the extra tilt axis or the rotation or heating, cooling or straining, a special holder being needed for each purpose.

Specimen preparation

- TEM can be used in any branch of science and technology where it is desired to study the internal structure of specimens down to the atomic level.
- It must be possible to make the specimen stable and small enough (~3 mm in dia.) to permit its introduction into the evacuated microscope column and thin enough (less than about 0.5 μm) to permit the passage of electrons.

Every branch of research has its own specific methods of preparing the specimen for electron microscopy.

1) In biology, tissues are treated as follows: at first, there is a chemical treatment to remove water and preserve the tissue as much as possible in its original state; it is then embedded in a hardening resin; after resin has hardened, slices (sections) with average thickness of 0.5 μm are cut with an instrument called **ultra-microtome equipped with a glass or diamond knife**.
2) The tiny sections thus obtained are placed on a specimen carrier – usually a 3 mm diameter copper specimen grid which has been coated with a structure-less carbon film 0.1 μm thick.
3) In metallurgy, a 3 mm diameter disc of material (thickness ~0.3 mm) is chemically treated in such a way that in the center of the disc the material is fully etched away. Around this hole, there will usually be areas that are sufficiently thin (approximately 0.1 μm) to permit electrons to pass through.
4) In a semiconductor, it is sometimes desired to cut out a section of material perpendicular to the surface in order to investigate a defect. This is done by ion-beam etching.

Limitations of TEM:

Not all specimens can be made thin enough for the TEM. Only a very narrow region of the specimen appears in focus in the image and there is considerable distortion. The technique has not found wide application in the study of surfaces.

The most important differences between TEM and SEM are:

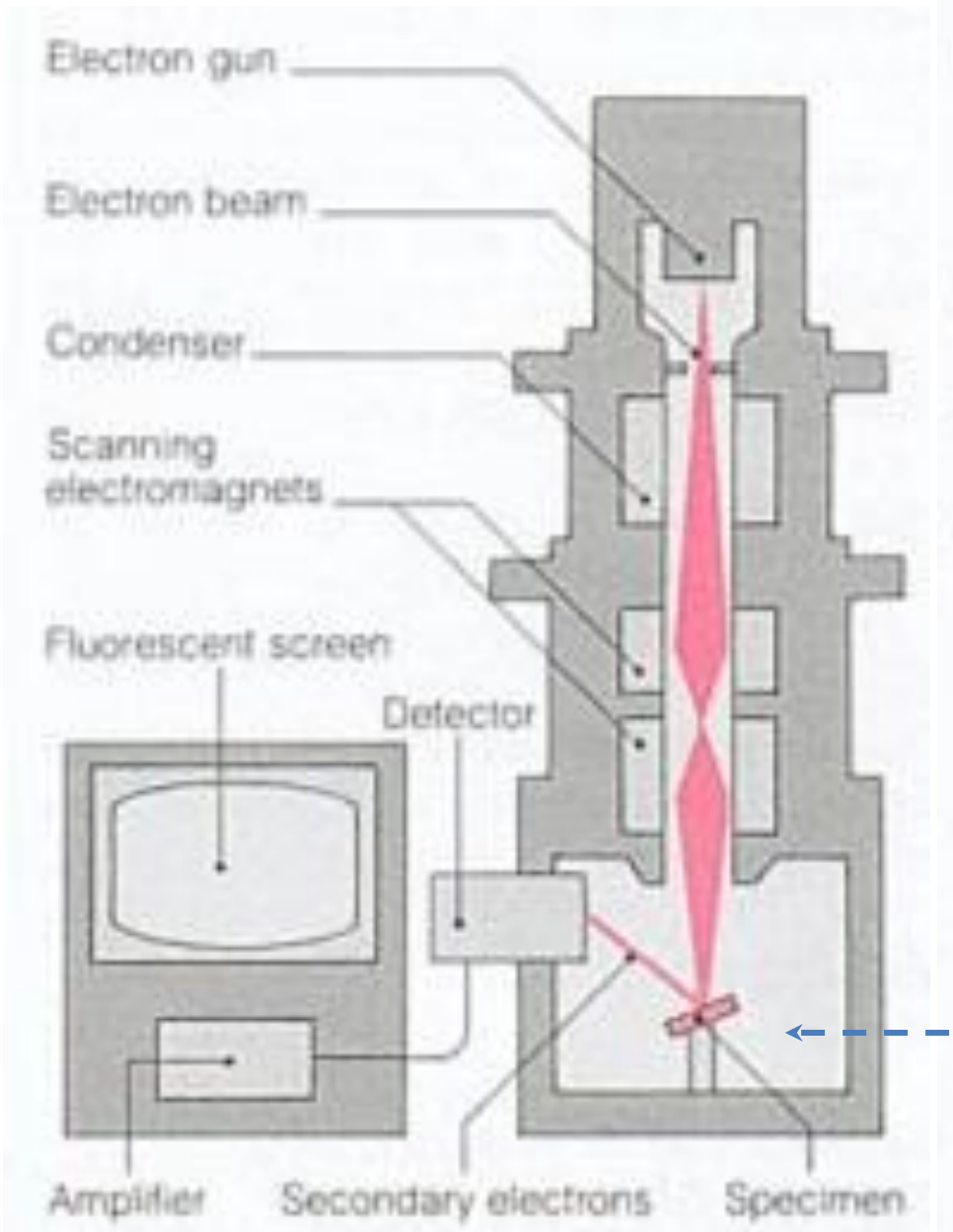
(1). the beam is not static as in the TEM: with the aid of an electromagnetic field, produced by the scanning coils, the beam is scanned line by line over an extremely small area of the specimen's surface

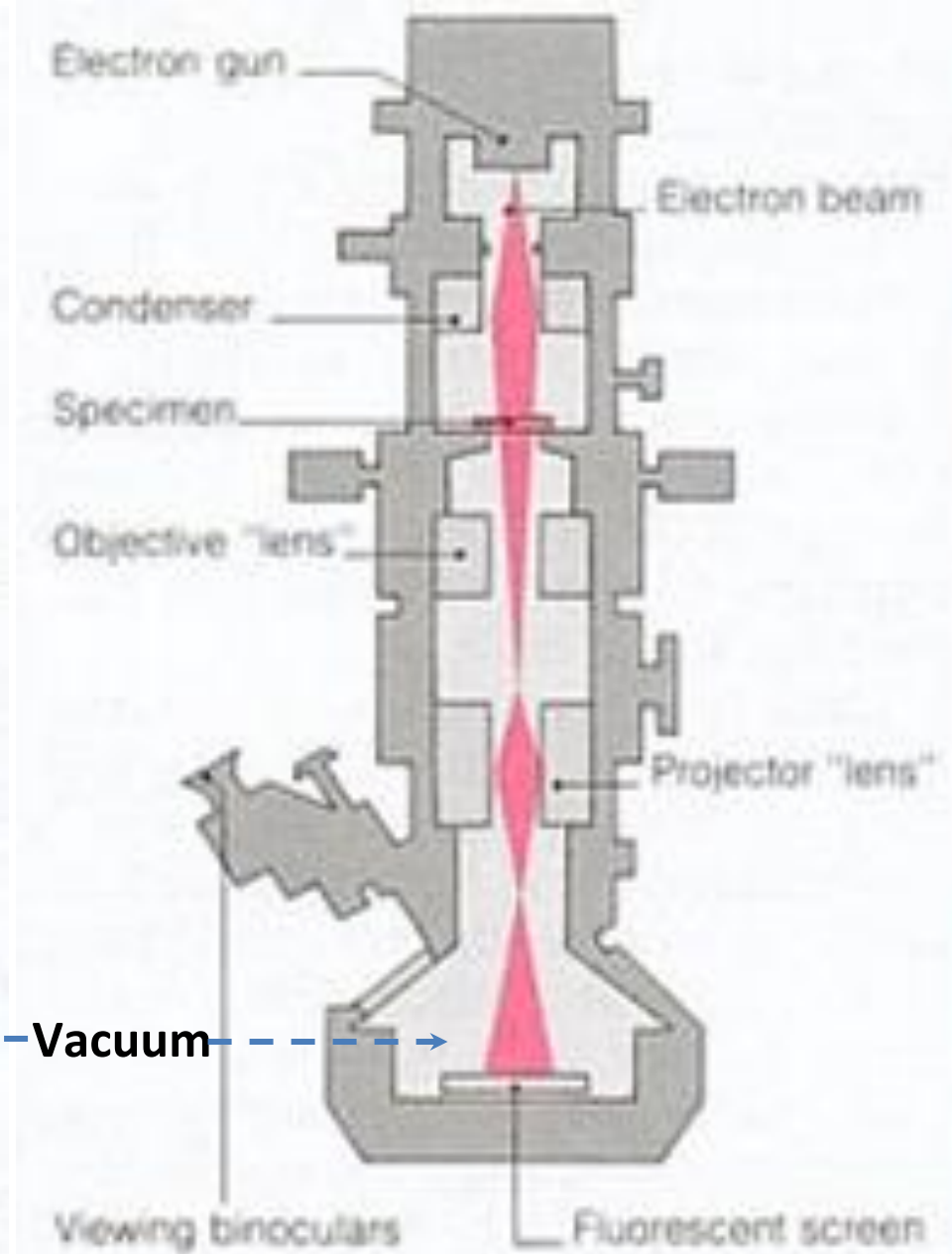
(b). the accelerating voltages are much lower than in TEM because it is no longer necessary to penetrate the specimen; in a SEM they range from 200 to 30.000 volts.

(c). specimens need no complex preparations.

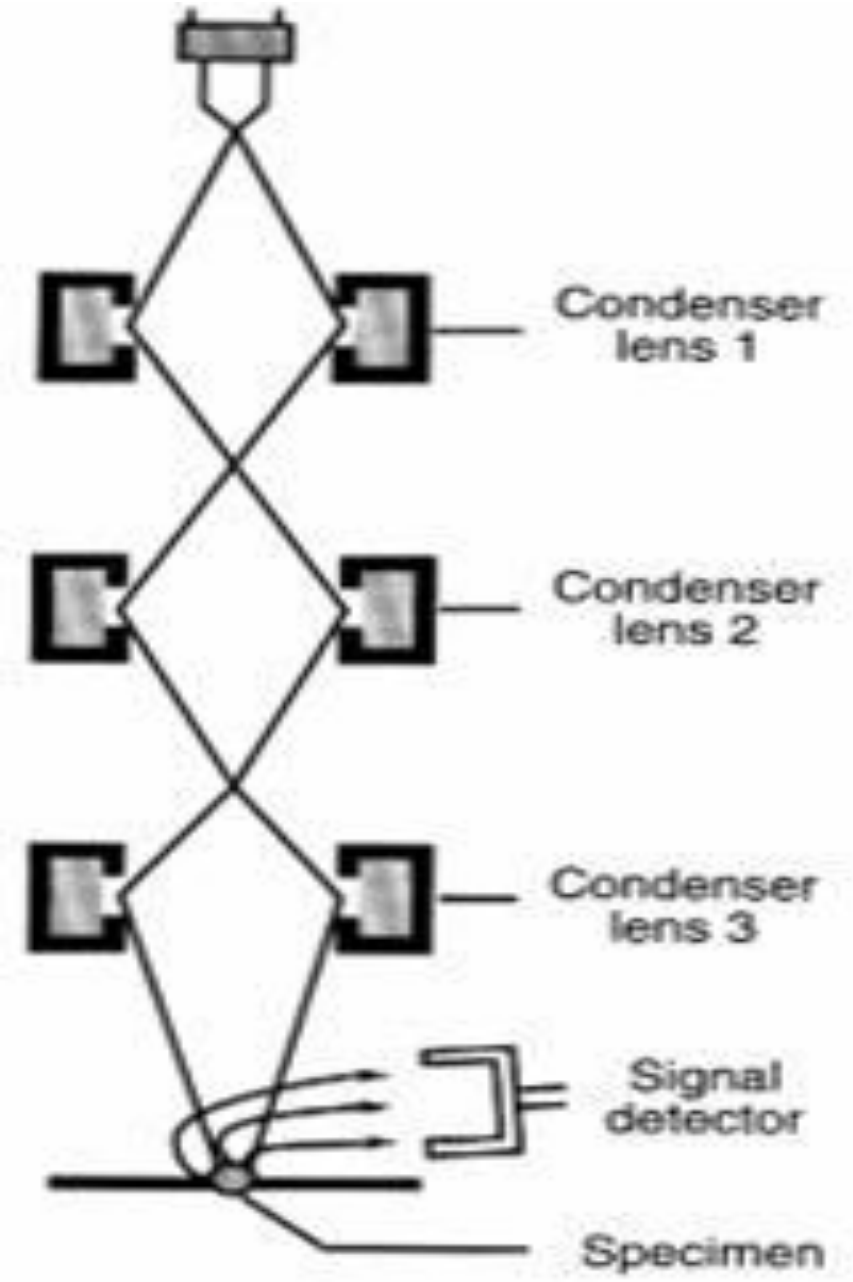
TEM specimens have to be very thin in order to be imaged with electrons. Typically, the specimen must be no thicker than a few hundred nm.

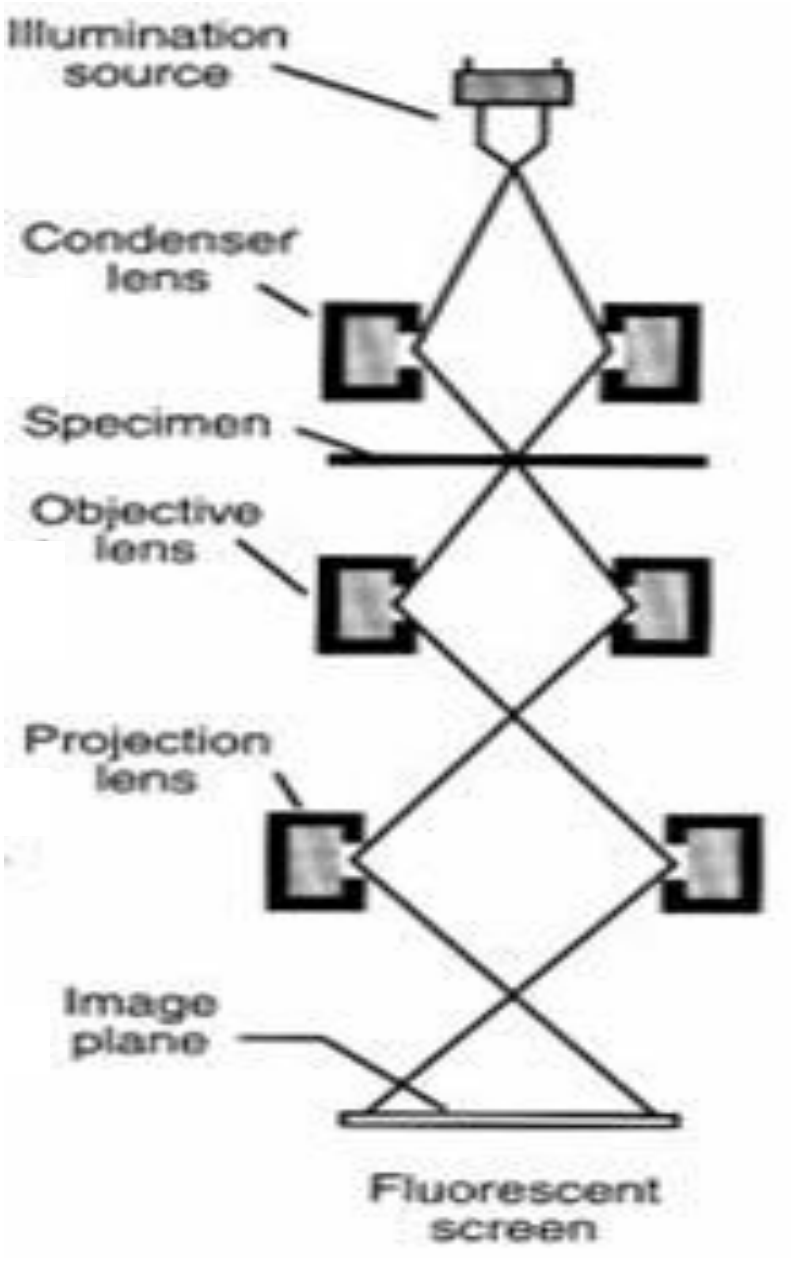
SEM	TEM


Electron gun
Electron beam
Condenser
Scanning electromagnets
Fluorescent screen
Detector
Amplifier
Secondary electrons
Specimen


Electron gun
Electron beam
Condenser
Specimen
Objective "lens"
Projector "lens"
Vacuum
Viewing binoculars
Fluorescent screen

SEM	TEM


Condenser lens 1
Condenser lens 2
Condenser lens 3
Signal detector
Specimen


Illumination source
Condenser lens
Specimen
Objective lens
Projection lens
Image plane
Fluorescent screen

End of Module 6

End of Syllabus