UV Spectroscopy

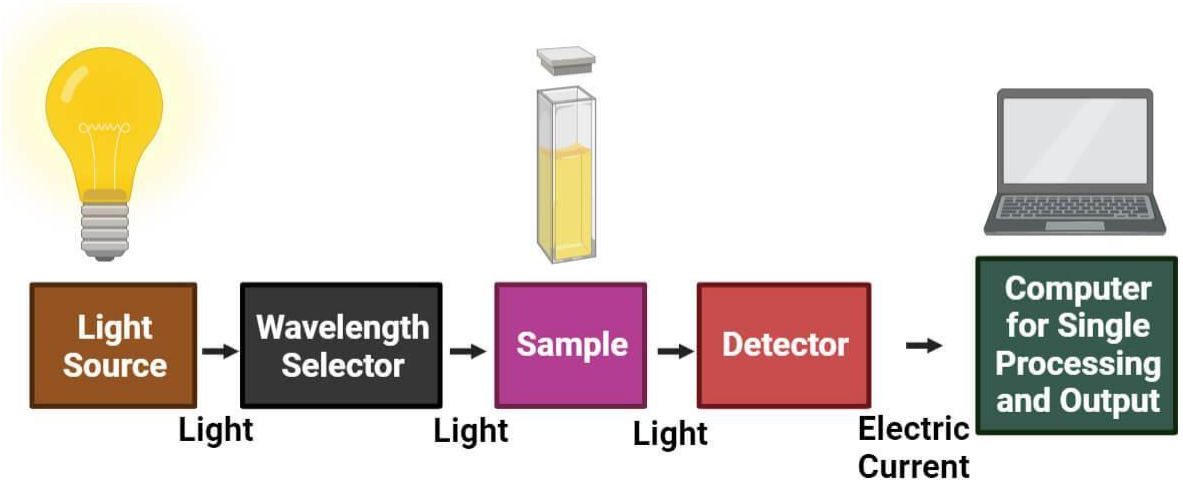
Introduction:

Ultraviolet (UV) Spectroscopy is an analytical technique used to study how a substance absorbs ultraviolet and visible light. The UV region ranges from **200 to 400 nm**, and the visible region spans **400 to 800 nm**. It is widely used in **qualitative** and **quantitative** analysis of organic and inorganic compounds. UV Spectroscopy is based on the electronic transitions within molecules. commonly used in pharmaceuticals, food, environmental testing, and research

UV-Vis Spectroscopy Principle

When a particular wavelength of light incidents on a molecule, that molecule gets excited. Once the electron excites, it excites from the ground (lower) energy state to the higher energy state. When an electron excites , it absorbs light energy because electrons in the orbital at a lower energy state utilize energy to move to a higher energy level. Energy is neither created nor destroyed but can transform energy from one form to another. On passing electromagnetic radiation, (UV- Vis range 200- 800 nm), only light which is possessing suitable energy can produce cause transitions of electron from one energy level to another. If the energy is utilized, the intensity of light received is lost. At this time, the energy absorbed by the electrons will equal the energy difference between the two energy levels. During this stage, electron transition occurs. So, after the interaction of electromagnet radiation, the spectra received are called absorption spectra.

Experimental Set-Up



Instrumentation of UV-Vis Spectroscopy

The main components of UV- Vis spectrophotometer are:

1. Light Source
2. Wavelength selector
3. Sample container
4. Detectors

1. Light Source

It is essential for emitting light in a wide range of wavelengths to work in a UV-Vis spectrometer. Commonly, a high-intensity light source used for both UV and Visible ranges is a **xenon lamp**. It is less stable and more costly. So, the two lamps for this instrument are a deuterium lamp for UV light and a halogen or tungsten lamp for visible light as a source of light. The two lamps provide good intensity.

2. Wavelength selector

In order to allow sample examination using the wavelengths that the light source emits; wavelength selection helps to ascertain which wavelength is appropriate for the type of analyte and sample. The commonly used wavelength selector in the UV-Vis spectrometer is the **monochromator.** It separates light into a narrow band of wavelength.

A monochromator contains a prism that separates all different wavelengths of light in a single beam. It bends the monochromatic light and produces non-linear dispersion. Only single radiation or colour of a specific wavelength will allow it to leave the monochromator and pass through exit slit.

3. Sample Container

In a single-beam spectrophotometer, all the radiation coming from the light source passes through the sample as one beam. Single-beam spectrophotometers can determine colour by comparing the light sources' intensities before and after a sample is inserted. The wavelength range measure is 190–750 nm; however, it may go up to 1100 nm.

In a double-beam spectrophotometer, all the radiation coming from the light source splits into two beams: one passes through the sample, and the other only passes through the reference.

For each wavelength, the light intensity passes through the beam separator to the reference chamber (Io) and sample chamber (**I**). The intensity of light symbolizes as **Io** measures the number of photons per second. When the light passes through the blank solution, it does not absorb light, referred to as (I). If sample I is less than Io, the sample has absorbed some light.

The absorbance (A) of the sample is related to **I and Io** according to the following equation:

Absorbance (A)= -log(T)= -log (I/Io)

This equation shows the relationships between absorbance and transmittance.

T= I/Io = e–kbc

Where: – I o is the incident intensity

– I is the transmitted intensity

– e is the base of natural logarithms

– k is a constant

– b is the path length (usually in cms).

The lighter the refracted, the more transmittance occurs. The lower the absorbance, the higher the transmittance.

In UV and visible regions, fused silica or quartz cuvettes are commonly used.

4. Detector

Detectors rely on photoelectric coatings or semiconductors. It converts the incoming light from the sample into an electric signal or current. The higher the current, the greater the intensity. It has the properties of low noise and high sensitivity, so it gives a linear response. Each detector has a variety of wavelength ranges and different sensitivity. Finally, the data recorder usually plots the absorbance against wavelength (nm) in the UV and visible section of the electromagnetic spectrum.

Advantages of UV-Vis Spectroscopy

1. It is non-destructive and reusable.
2. It is easy to operate and the fastest method to interpret data because it gives accurate readings.

3. It is an inexpensive technique.
4. It is more convenient.

Disadvantages of UV-Vis Spectroscopy

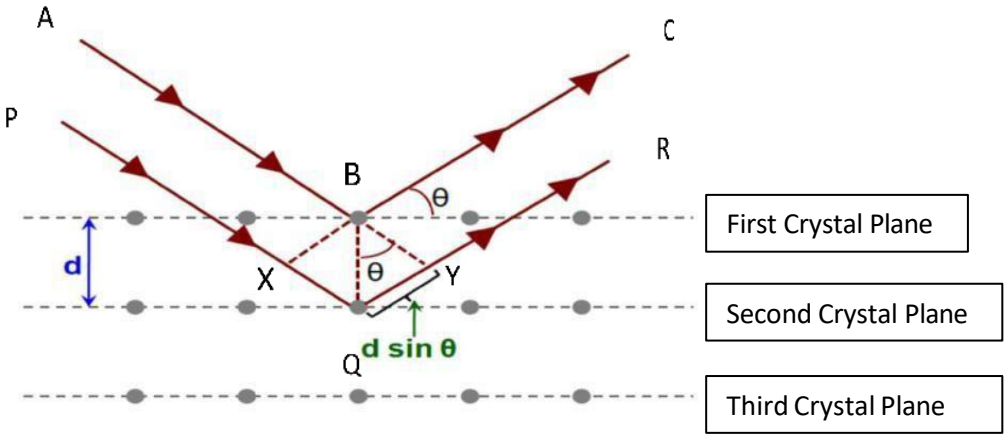
1. It may take time to prepare using the machine.
2. Spectrometer reading might be affected if it keeps with any electronic noise, outside light, and other contaminants.

X-Ray Diffraction

Braggs law

Braggs studied the diffraction pattern in crystals by using X-rays.

Consider the set of crystal planes with inter planar distance 'd' and when the X-rays are incident on the crystal planes then X-rays are scattered in different directions and forms maximum and minimum intensities.



Let the X-ray AB is incident on the atom of the first crystal plane then it is reflected in the form of BC. Let another X-ray PQ is incident on the atom of the second crystal plane then it is reflected in the form of QR. These two reflected X-rays **interfere with each other to form Interference** pattern. Depending on the path difference they form maximum or minimum intensity.

To find maximum or minimum intensity draw two normal lines BX and BY as shown in the figure.

the figure.

From the Triangle BXQ, $\sin\Theta = \frac{BX}{BQ} = \frac{BX}{d}$

$$BX = d \sin\Theta \quad \text{--- (1)}$$

From the Triangle BYQ, $\sin\Theta = \frac{BY}{BQ} = \frac{BY}{d}$

$$BY = d \sin\Theta \text{ ------ (2)}$$

$\therefore$ Path Difference $(\Delta) = BX + BY = 2d \sin\Theta$ -----(3)

To find Maximum intensity, the path difference should equal to = $n\lambda$

i.e $\Delta = n\lambda$ ----(4)

on equating 3 and 4 we get $2d \sin\Theta = n\lambda$.

From λ, Θ values we can calculate the inter planar distance as $d_{hkl} = \frac{a}{\sqrt{h^2+k^2+l^2}}$

Depending on the value of h2+k2+l2 we can determine the crystals as SCC, BCC and FCC.

Determination of Crystal Structures-

Crystal structures can be determined by using two methods 1. Laue method 2. Powder method.

1.Laue Method:-

One of the important method to determine the crystal structure is Laue method by using X ray Diffraction technique.

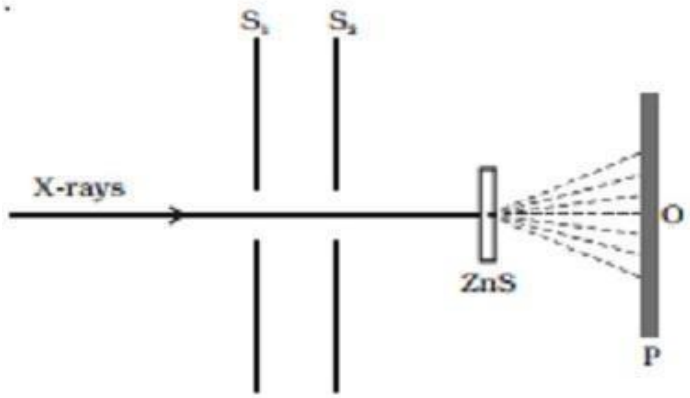


Fig (a) Laue experimental set up

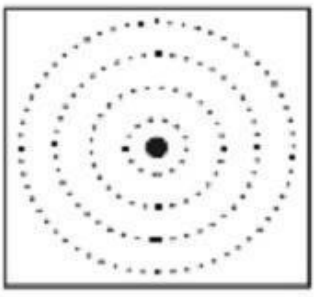


Fig (b) Laue spot

The Experimental arrangement consists of X-ray source which is passed through the two slits S1 and S2 , to obtain fine beam of X-rays. This fine beam of X-rays when incident on the crystal, the atoms inside the crystal diffract the X-rays in different directions and allowed to fall on a photographic plate.

This diffraction pattern on the screen i.e Photographic plate consists of bright spots. These bright spots are called Laue spots as shown in the figure. The bright

spots are formed on the screen due to maximum intensity satisfying Bragg's equation

$2d\sin\Theta = n\lambda$, for a particular wavelength of incident beam.

Merits & De-merits-

1. This method is used to determine the crystal orientation & symmetry & to study defects in crystal.
2. This method consists of several wavelengths of X- rays , which is not convenient to study actual crystal structures.

Powder Method

This Powder method was designed by Debye & Scherrer.

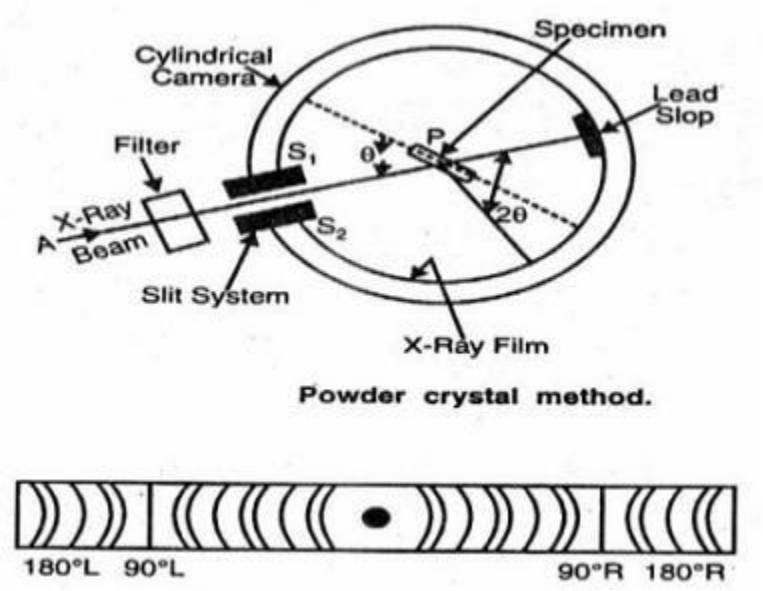


Fig- Powder Method

The experimental arrangement of powder method consists of Crystal which is in the form of powder. That powder is kept inside a thin-capillary tube made of non diffracting material. rays from source are passed through the filter & Pin holes to produce a fine beam of monochromatic X-rays. The fine beam after passing through small hole falls on capillary tube which is kept inside a cylindrical cone shape drum. As the powder consists of crystallites (atoms) which are arranged in an random manner, all possible Θ & d values are available for diffraction of X-rays.

The X-rays come out through the cylindrical drum & makes diffraction pattern on the film and forming as arcs. Arcs are formed on the screen, as we move away from the centre, the arcs are changed to straight lines as shown in figure.

Fourier Transform Infrared (FTIR) spectrometry

For almost seventy years, infrared spectroscopy has been an effective technique for materials analysis in the laboratory. An infrared spectrum is a sample's fingerprint, with absorption peaks corresponding to the frequency of vibrations between the bonds of the atoms that make up the material.

Principle:

- FT-IR stands for Fourier Transform Infrared, the preferred method for infrared spectroscopy. In Infrared Spectroscopy, IR radiation is passed through a sample.
- Some of the infrared radiation is absorbed by the sample and some of the radiation is passed (transmitted). The resulting spectrum represents the molecular absorption and transmission , creating a molecular finger print of the sample.
- Like a finger print no two unique molecular structures produce

This Fourier Transform is a Mathematical process for converting Amplitude Time signal to Amplitude- Frequency spectrum or vice versa and it is preferred because of the limitations with the dispersive instruments.

Essential Equations

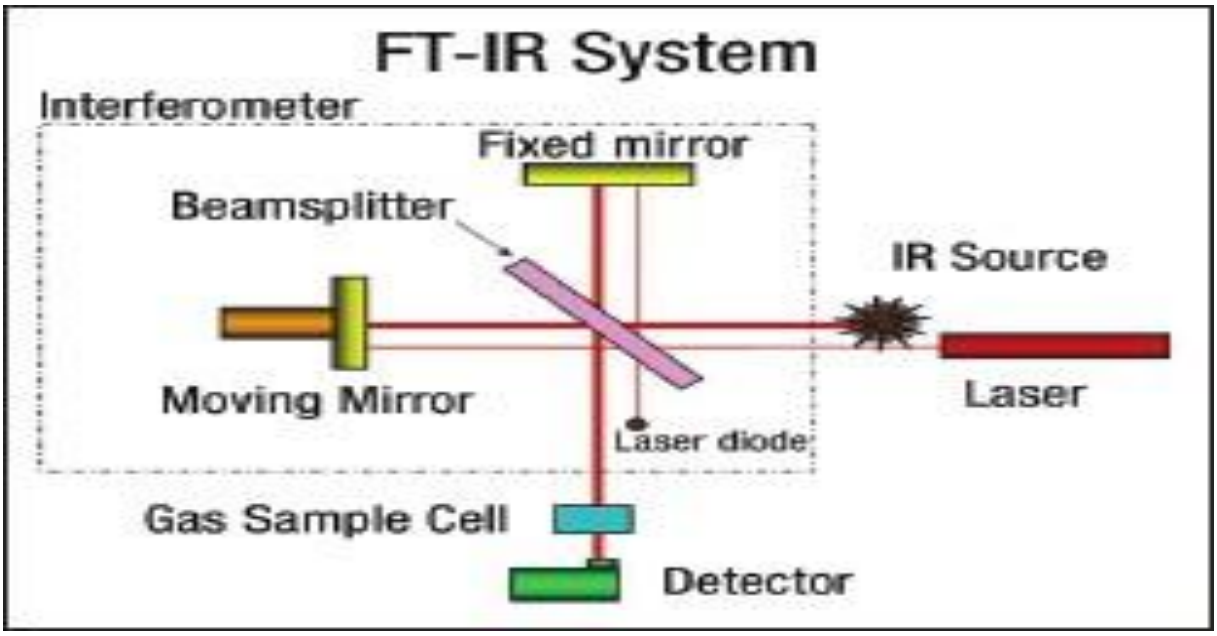
$$\mathbf{I(x) = \int B(\nu) \cos 2\pi\nu\ x\ d\nu}$$

Which is one half a cosine Fourier-Transform pair with the other one being.

$$\mathbf{B(\nu) = \int I(x) \cos 2\pi\nu\ x\ dx.}$$

This Fourier-transform spectrophotometer is based on the Michelson-Morely experiment used to measure the influence of the earth's rotation on the speed of the light. The Fourier transform spectrophotometer is considerably more accurate than the monochromator system.

FT-spectrometers include a small computer directly interfaced to the spectrometer in order to control the scan system and carry out the mathematical transformation. The most critical part of Fourier transform system is the mirror scan mechanism.



An FT-IR instrument uses an interferometer consists of a source, beam splitter, two mirrors, a laser and a detector. The energy goes from the source to the beam splitter which splits the beam into two parts. One part is transmitted to a moving mirror, the other is reflected to a fixed mirror. The moving mirror moves back and forth at a constant velocity, controlled by the calibrating laser's response. The two beams are reflected from the mirrors and recombined back at the beam splitter, generating an interference pattern, which is transmitted though the sample compartment (and if present the sample where absorbance occurs) to the detector. This signal is then subjected to the FT function to generate a spectrum.

Analysis using an FTIR proceeds as follows.

The source:

IR energy is emitted from a glowing black-body source and the beam passes through an aperture, which controls the amount of energy.

The interferometer:

The IR beam enters the interferometer where "spectral encoding" takes place as already described. The interferometer uses a reference laser for precise wavelength calibration.

The sample:

The IR beam enters the sample compartment where it is transmitted through or reflected off the surface of the sample; specific frequencies of energy that are uniquely characteristic of the sample are absorbed by the sample.

The detector:

The beam finally passes to the detector for final measurement.

The computer:

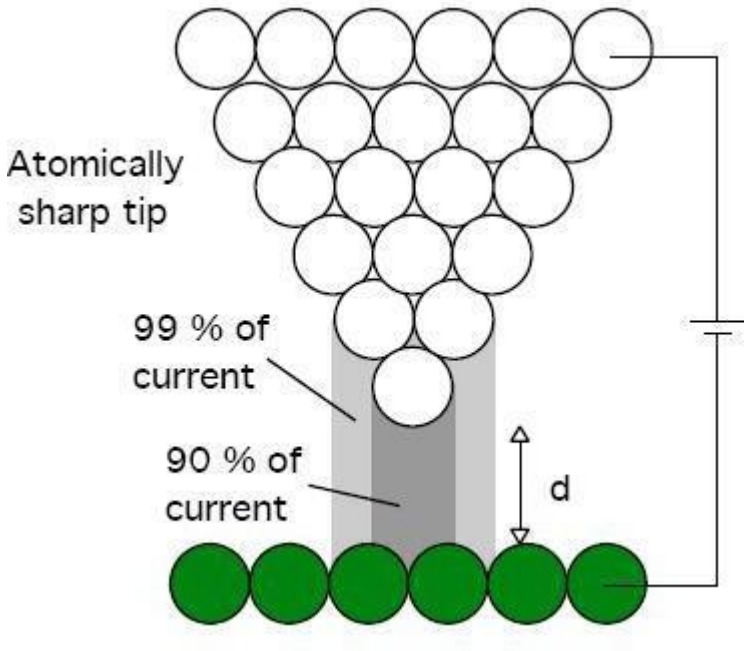
The signal is digitized, the FFT calculation takes place and final infrared spectrum is presented to the user.

Scanning Tunnelling Microscope

The principle of STM is based on tunneling of electrons between this conductive sharp probe and sample.

Tunneling is a phenomenon that describes how electrons flow (or tunnel) across two objects of differing electric potentials when they are brought into close proximity to each other. When a voltage is applied between probe and surface electrons will flow across the gap (probe-sample distance) generating a measurable current.

The tunneling phenomena can be explained by quantum mechanics. Tunneling originates from the wavelike properties of electrons. When two conductors are close enough there is an overlapping of the electron wavefunctions. Electrons can then diffuse across the barrier between the probe (tip-terminating ideally in a single atom) and the sample when a small voltage is applied. The resulting diffusion of electrons is called tunneling.



An important characteristic of tunneling is that the amplitude of the current exhibits an exponential decay with the distance, d. One way to describe this relationship is by the equation:

$$I \sim Ve–cdI \sim Ve–cd$$

- I = Tunneling current
- V = voltage between probe and sample
- c = constant
- d = probe-sample separation distance

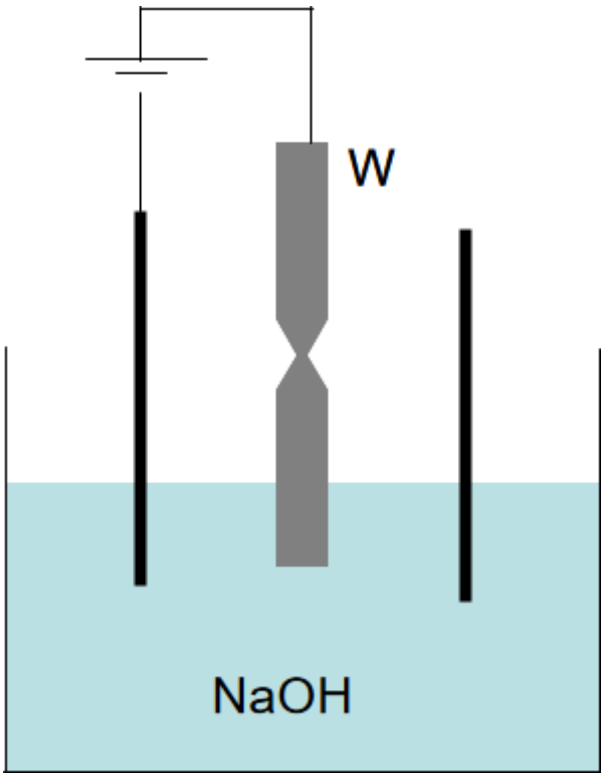
Key factor for STM: Very small changes in the probe-sample separation induce large changes in the tunneling current! (i.e. at a separation of a few Å the current rapidly decreases)

This dependency on tunneling current and probe-sample distance allows for precise control of probe-sample separation, resulting in high vertical resolution(<1 Å) . Furthermore, tunneling is only carried out by the outermost single atom of the probe. This allows for high lateral resolution (<1 Å).

STM probes

Commercial probes are available but often users make their own probes. A common method is to electrochemically etch tungsten, W, wire in NaOH to create a sharp probe. A problem with W probes is that they oxidize over time. Platinum iridium (Pt-Ir) is preferred for use in air because platinum does not

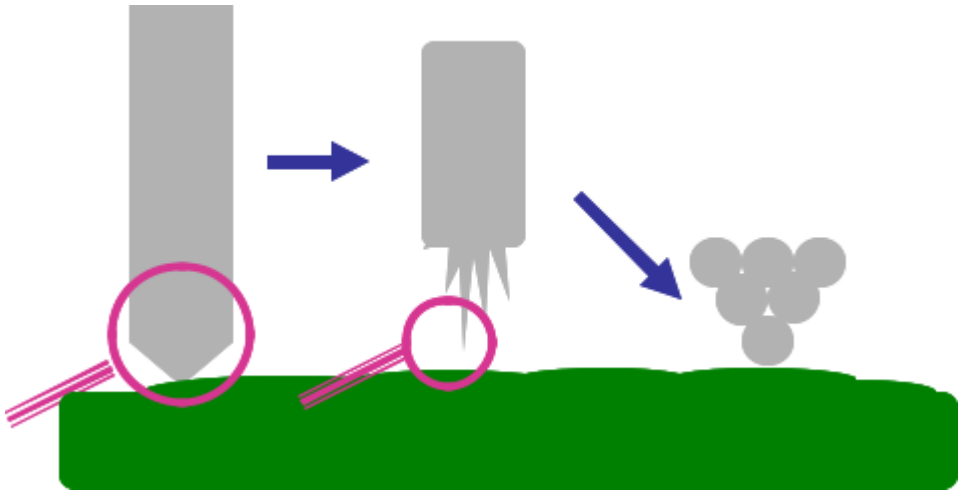
easily oxidize. The tiny fraction of Iridium in the alloy makes it much harder. The Pt-Ir tips are usually shaped by cutting Pt-Ir wire with a wire cutter.



Set up for etching W probe for STM.

It should be noted that a tip does not necessarily have to be one perfect point.

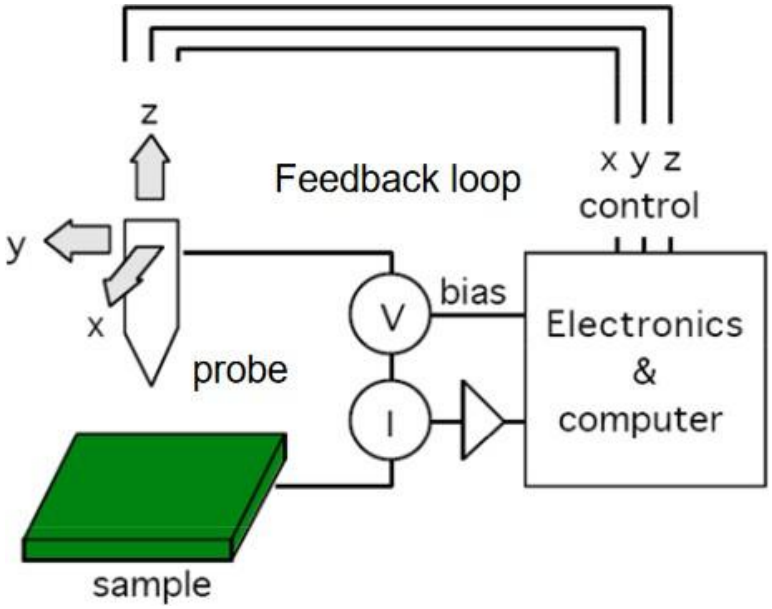
STM Probe



STM probe magnified to an atomic scale.

In STM a voltage is applied between the metallic probe and the sample, typically (0-3 V). When the probe is close to the surface (2-4 Å) the voltage

will result in a current, due to tunneling between the probe and sample. When the probe is far away from the surface, the current is zero. The tunneling current produced is low (pA-nA) but can be monitored using amplifiers. A 3D scanner with an electronic feedback loop is used to raster the probe across the sample to obtain a topographical image and monitor the tunneling current.

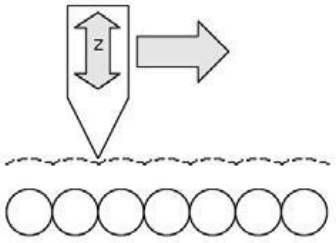


Imaging Methods

There are two methods of imaging in STM:

1) Constant Current

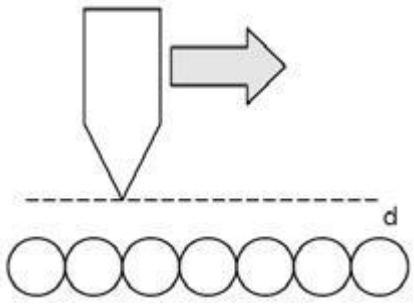
A constant tunneling current is maintained during scanning (typically 1 nA). This is done by vertically (z) moving the probe at each (x,y) data point until a "setpoint" current is reached. The vertical position of the probe at each (x,y) data point is stored by the computer to form the topographic image of the sample surface. This method is most common in STM.



Constant current imaging with STM.

2) Constant Height

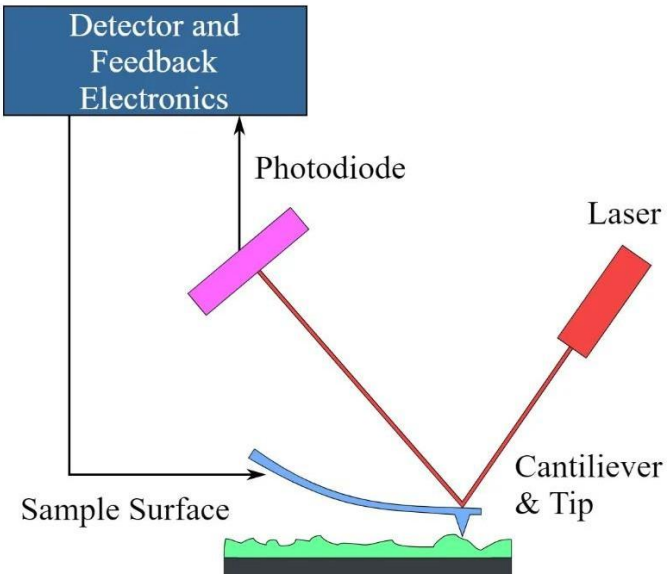
In this approach the probe-sample distance is fixed. A variation in tunneling current forms the image. This approaching allows for faster imaging, but only works for flat samples.



Constant height imaging with STM. (d= fixed probe-sample distance).

Atomic Force Microscope

The atomic force **microscope** (AFM) is a type of scanning probe microscope whose primary roles include measuring properties such as magnetism, height, friction. The resolution is measured in a nanometer, which is much more accurate and effective than the optical diffraction limit. It uses a probe for measuring and collection of data involves touching the surface that has the probe.



Principle of Atomic Force Microscope

The Atomic Force Microscope works on the principle measuring intermolecular forces and sees atoms by using probed surfaces of the specimen in nanoscale. Its functioning is enabled by three of its major working principles that include Surface sensing, Detection, and Imaging.

The Atomic Force Microscope (AFM) performs surface sensing by using a cantilever (an element that is made of a rigid block like a beam or plate, that attaches to the end of support, from which it protrudes making a perpendicularly flat connection that is vertical like a wall). The cantilever has a sharp tip that scans over the sample surface, by forming an attractive force between the surface and the tip when it draws closer to the sample surface. When it draws very close making contact with the surface of the sample, a repulsive force gradually takes control making the cantilever avert from the surface.

During the deflection of the cantilever away from the sample surface, there is a change in direction of reflection of the beam, and a laser beam detects the aversion, by reflecting off a beam from the flat surface of the cantilever. Using a positive-sensitive photo-diode (PSPD- a component that is based on silicon PIN diode technology and is used to measure the position of the integral focus of an incoming light signal), it tracks these changes of deflection and change in direction of the reflected beam and records them.

The Atomic Force Microscope (AFM) takes the image of the surface topography of the sample by force by scanning the cantilever over a section of interest. Depending on how raised or how low the surface of the sample is, it determines the deflection of the beam, which is monitored by the Positive-sensitive photo-diode (PSDP). The microscope has a feedback loop that controls the length of the cantilever tip just above the sample surface, therefore, it will maintain the laser position thus generating an accurate imaging map of the surface of the image.

Parts of Atomic Force Microscope

Atomic Force Microscopes have several techniques for measuring force interactions such as van der Waals, thermal, electrical and magnetic force interactions for these interactions done by the AFM, it has the following parts that assist in controlling its functions.

Modified tips which are used to detect the sample surface and undergo deflections Software adjustments used to image the samples.

Feedback loop control – they control the force interactions and the tip positions using a laser deflector. the laser reflects from the back of the cantilever and the tip and while the tip interacts with the surface of the sample, the laser's position on the photodetector is used in the feedback loop for tracking the surface of the sample and measurement.

Deflection – The Atomic Force Microscope is constructed with a laser beam deflection system. The laser is reflected from the back of the AFM lever to the sensitive detector. They are made from silicon compounds with a tip radius of about 10nm.

Force measurement – the AFM works and depends highly on the force interactions; they contribute to the image produced. The forces are measured by calculation of the deflection lever when the stiffness of the cantilever is known. This calculation is defined by Hooke's law, defined as follows:

F= -kz, where F is the force, k is the stiffness of the lever, and z is the distance the lever is bent.

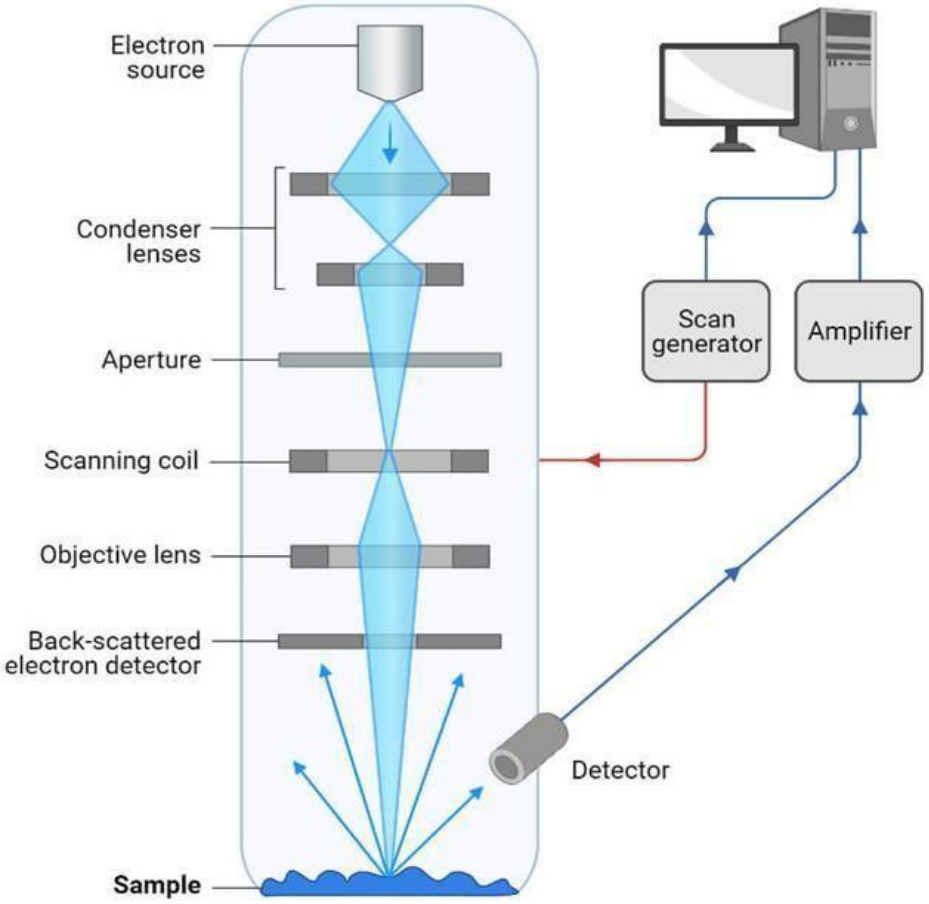
Scanning Electron Microscope (SEM)

Scanning Electron Microscope (SEM) is a type of electron microscope that scans surfaces of microorganisms that uses a beam of electrons moving at low energy to focus and scan specimens. Principle of Scanning Electron Microscope (SEM)

The Scanning electron microscope works on the principle of applying kinetic energy to produce

signals on the interaction of the electrons. These electrons are secondary electrons, backscattered electrons, and diffracted backscattered electrons which are used to view crystallized elements and photons. Secondary and backscattered electrons are used to produce an image. The secondary

electrons are emitted from the specimen play the primary role of detecting the morphology and topography of the specimen while the backscattered electrons show contrast in the composition of the elements of the specimen.



Parts of a Scanning Electron Microscope (SEM)

The major components of the Scanning Electron Microscope include;

Electron Source – This is where electrons are produced under thermal heat at a voltage of 1-40kV.

the electrons condense into a beam that is used for the creation of an image and analysis. There are

three types of electron sources that can be used i. e Tungsten filament, Lanthanum hexaboride, and Field emission gun (FEG)

Lenses – it has several condenser lenses that focus the beam of electrons from the source through the column forming a narrow beam of electrons that form a spot called a spot size.

Scanning Coil – they are used to deflect the beam over the specimen surface.

Detector – It's made up of several detectors that are able to differentiate the secondary electrons, backscattered electrons, and diffracted backscattered electrons. The functioning of the detectors highly depends on the voltage speed, the density of the specimen.

The display device (data output devices) Power supply Vacuum system

Working

When the primary electron beam from the electron gun interacts with the sample, the electrons lose energy by repeated random scattering and absorption within the specimen.

The energy exchange between the electron beam and the sample results in the reflection of high-energy electrons by elastic scattering, emission of secondary electrons by inelastic scattering and the emission of electromagnetic radiation, each of which can be detected by specialized detectors.

The beam current absorbed by the specimen can also be detected and used to create images of the distribution of specimen current.

TRANSMISSION ELECTRON MICROSCOPE:

Construction:

It consists of an electron gun to produce electrons. Magnetic condensing lens is used to condense the electrons and is also used to adjust the size of the electron that falls on to the specimen. The specimen is placed in between the condensing lens and the objective lens as shown.

The magnetic objective lens is used to block the high angle diffracted beam and

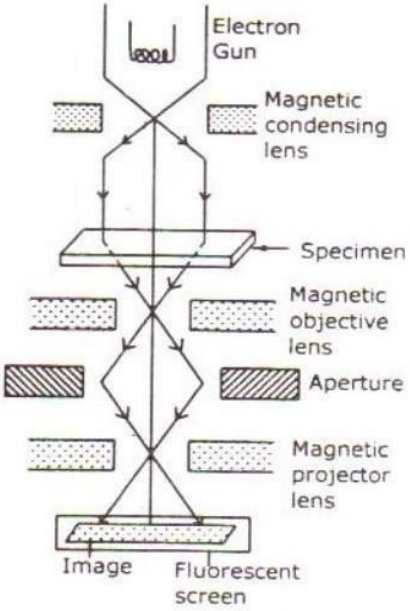
the aperture is sued to eliminate the diffracted beam (if any) and in turn increases the contrast of the image.
The magnetic projector lens is placed above the fluorescent screen in order to achieve higher magnification,. The image can be recorded by using a fluorescent (Phosphor) screen or (CCD – Charged Coupled device) also.

Working:

Stream of electrons are produced by the electron gun and is made to fall over the specimen using the magnetic condensing lens.

Based on the angle of incidence the beam is partially transmitted and partially diffracted. Both these beams are recombined at the E-wald sphere to form the image. The combined image is called the phase contrast image.

In order to increase the intensity and the contrast of the image, an amplitude contrast has to be obtained. This can be achieved only by using the transmitting beam and thus the diffracted beam can be eliminated.



Now in order to eliminate the diffracted beam, the resultant beam is passed through the magnetic objective lens and the aperture. The aperture is adjusted in such a way that the diffracted image is eliminated. Thus, the final image obtained due to transmitted beam alone is passed through the projector lens for further magnification. The magnified image is recorded in fluorescent screen or CCD. This high contrast image is called Bright Field Image. Also, it has to be noted that the bright field image obtained is purely due to the elastic scattering (no energy change) i.e., due to transmitted beam alone.