LECTURE 4.3: Microscopy Techniques

4.3.1. Introduction

Electron Microscopes are scientific instruments that use a beam of highly energetic electrons to examine objects on a very fine scale. This examination can yield information about the topography (surface features of an object), morphology (shape and size of the particles making up the object), composition (the elements and compounds that the object is composed of and the relative amounts of them) and crystallographic information (how the atoms are arranged in the object).

4.3.2. Why Electron Microscopes?

Electron Microscopes were developed due to the limitations of Light Microscopes which are limited by the physics of light to 500x or 1000x magnification and a resolution of 0.2 μm. In the early 1930's this theoretical limit had been reached and there was a scientific desire to see the fine details of the interior structures of organic cells. This required 10,000x plus magnification which was just not possible using Light Microscopes. Owing to the much smaller wavelengths electron microscopes can provide 2-3 orders of magnitude higher resolution than light microscopes. So we need electron microscopes because of their very high resolution. Expression

The Transmission Electron Microscope (TEM) was the first type of Electron Microscope to be developed and is patterned exactly on the Light Transmission Microscope except that a focused beam of electrons is used instead of light to "see through" the specimen. It was developed by Max Knoll and Ernst Ruska in Germany in 1931.The first Scanning Electron Microscope (SEM) debuted in 1942 with the first commercial instruments around 1965. Its late development was due to the electronics involved in "scanning" the beam of electrons across the sample. Electron Microscopes (EMs) function exactly as their optical counterparts except that they use a focused beam of electrons instead of light to "image" the specimen and gain information as to its structure and composition.

4.3.3. Scanning Electron Microscope (SEM)

SEM gives information about

Topography**:** The surface features of an object on "how it looks", its texture, direct relation between these features and materials properties.

Morphology**:** The shape and size of the particles making up the object direct relation between these structures and materials properties.

Composition**:** The elements and compounds and the relative amounts of them; direct relationship between composition and materials properties.

Crystallographic Information**:** How the atoms are arranged in the object" direct relation between these arrangements and material projects.

Working principle of Electron microscope

The basis of electron microscopy is the interactions of electrons with matters which brings about changes in electrons or generate new electrons/photons with different energies. When an electron beam interacts with the atoms in a sample, they undergo in general two types of scattering: elastic and inelastic (Fig.4.3.1).

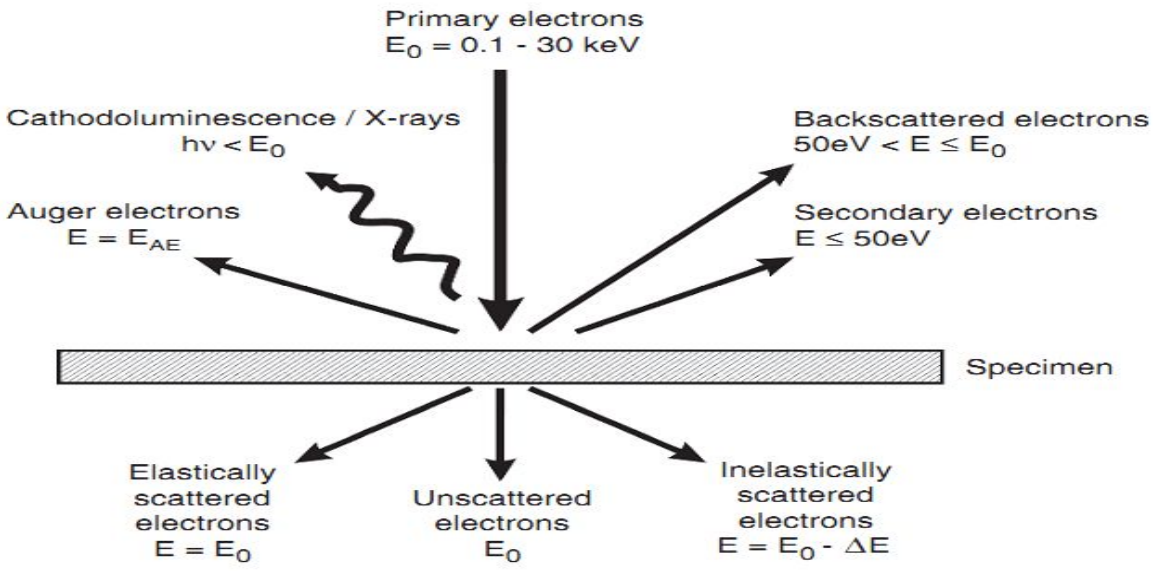


FIG.:4.3.1

In the former, they do not lose energy only the trajectory changes. In the case of inelastic scattering process there is loss of energy of primary electrons accompanied with secondary effects (secondary electrons, BSE, Cathodoluminescence, X-rays etc.). This interaction places the atom in an excited (unstable) state. Specimen interaction is what makes Electron Microscopy possible. The interactions (inelastic) sketched on the upper side of the specimen are utilized when examining thick or bulk specimens (Scanning Electron Microscopy, **SEM**) while on the lower side are those examined in thin or foil specimens (Transmission Electron Microscopy, **TEM**).

Principle of SEM

The scanning electron microscope (SEM) "sees" low kinetic energy secondary electrons from solid surfaces. When an electron beam interacts with a solid, various types of elastic and inelastic processes occur (as mentioned above), including electron scattering and excitation, which produces:

(1) Secondary electrons,
(2) Backscattered electrons,
(3) Auger electrons,
(4) Characteristic x-rays,
(5) Bremsstrahlung or continuous x-rays,
(6) Photons of various energies, including those in the infrared, visible, and ultraviolet.

The fraction of energy deposited by an electron beam in a sample associated with these different processes is dependent on the sample. Secondary and Auger electrons can only be observed when they come from the near-surface region of a solid (typically < 500 Å for insulators, such as silicate minerals, and < 100 Å for metals such as gold). Thus, measurements involving these types of electrons are "surface sensitive".

Basic signals utilized in SEM

1. Secondary Electrons:

Secondary electrons are generated by the primary electron beam as it enters a sample as well as by backscattered electrons as they exit a sample. Secondary electrons, which

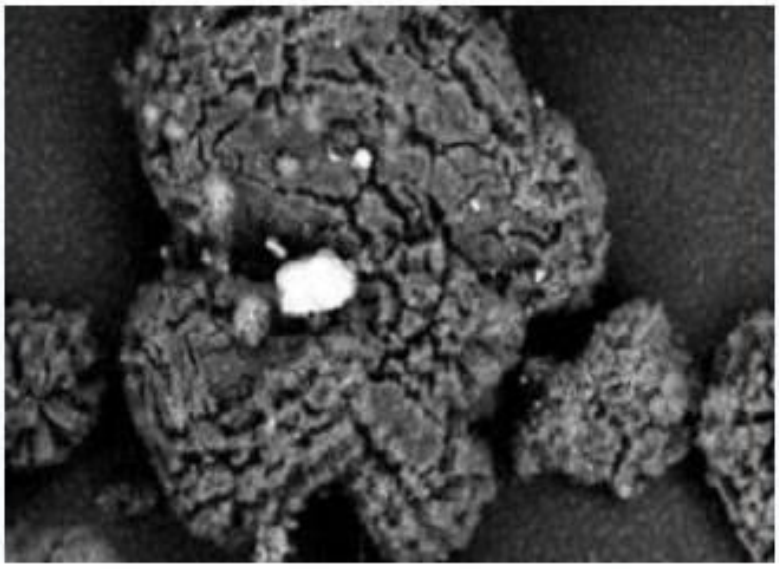
typically have kinetic energies < 50 eV, are sensitive enough to differences in surface topology that they can be readily observed from the surface of a sample. Such electrons form the basis of scanning electron microscopy.

In order to enhance the number of secondary electrons from an insulating sample, the sample is often coated with a thin layer of gold-palladium alloy or another electron-rich conducting material that produces abundant secondary electrons when struck by a focused electron beam. A thin metal coating will not mask surface features or the overall topology of the underlying sample. The gold-palladium coating also conducts electrons away, so that the sample does not develop a significant charge when it loses secondary electrons and other types of electrons. This type of coating is essential for insulator samples, which don't conduct charged particles. Such samples, if uncoated, would be difficult to image using an SEM because of the fact that they would develop a negative charge (due to build-up of electrons), which would cause the image to become defocused due to deflection of the exciting electron beam.

The secondary electron yield depends on many factors, and is generally higher for high atomic number targets, and at higher angles of incidence.

2. Backscattered Electrons

They are beam electrons that are reflected from the sample by elastic scattering. BSE are often used in analytical SEM along with the spectra made from the characteristic X-rays. Because the intensity of the BSE signal is strongly related to the atomic number (Z) of the specimen, so different production rates causes higher atomic number elements to appear brighter than lower atomic number elements. This interaction is utilized to differentiate parts of the specimen that have different average atomic number. Fig. 4.3.2, illustrates a backscattered electron image. Thus BSE images can provide information about the distribution of different elements in the sample. For the same reason, BSE imaging can image colloidal gold immune-labels of 5 or 10mm diameter which would otherwise be difficult or impossible to detect in secondary electron images in biological specimens



Bacscattered electron image (SEM)

Fig.:4.3.2

3. **Relaxation effects:** There are several types of signals that are generated from a specimen viz. X-Rays, cathodoluminescence and Auger electrons when the atoms get de-excited after undergoing inelastic scattering under an electron beam. But x-ray signal is typically the only other signal that is used for scanning electron microscopy. The x-ray signal is a result of recombination interactions between free electrons and positive electron holes that are generated within the material. The x-ray signal can originate from further down into the surface of the specimen surface and allows for determination of elemental composition through EDS (energy dispersive x-ray spectroscopy) analysis of characteristic x-ray signals.

Instrumentation in electron microscopy

Electron Column: The electron column is where the electron beam is generated under vacuum, focused to a small diameter, and scanned across the surface of a specimen by electromagnetic deflection coils see Fig.4.3.3. The lower portion of the column is called the specimen chamber. The secondary electron detector is located above the sample stage inside the specimen chamber. Specimens are mounted and secured onto the stage which is controlled by a goniometer. The manual stage controls are found on the front side of the specimen chamber and allow for x-y-z movement; 360^{o} rotation and 90^{o} tilt

however only the tilt cannot be controlled through the computer system thus there is no need to use all of the manual controls manipulates the orientation of the sample inside the sample chamber. Above is a diagram of the electron column and a description of each of the components of the electron column.

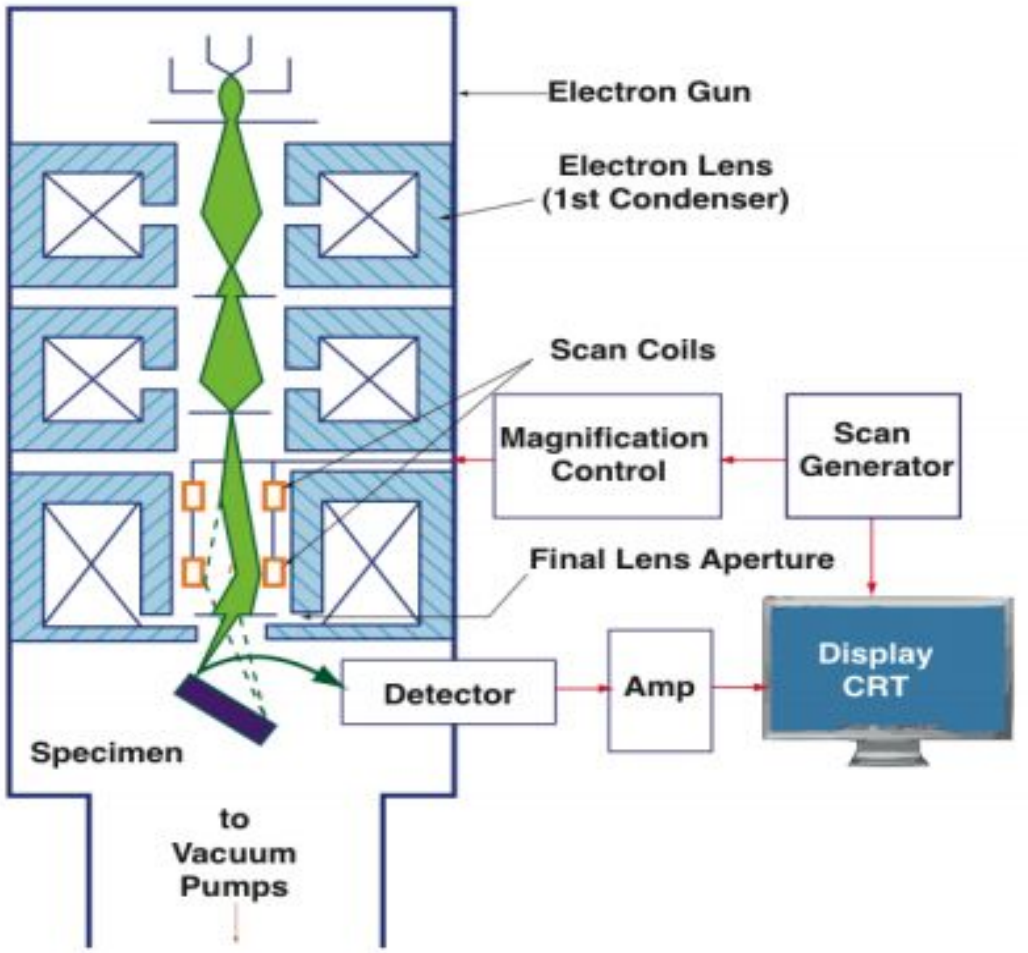


FIG. 4.3.3: Schematic of main components of SEM

Electron Guns

The electron gun provides the SEM with an electron beam of adjustable current and energy. There are two most frequently used methods for producing electrons in the electron guns used for electron microscopy: One is thermionic electron emission and another is field emission. Most electron microscopes use thermionic emission of electrons from a heated filament. Being one of the cheapest and simplest thermionic sources,

tungsten is most widely used in thermionic electron guns. Figure 6 shows a diagrammatic representation of a tungsten filament electron gun. The filament is placed in a cylindrical case called a Wehnelt cylinder or Wehnelt cap. Wehnelt cap (Fig. 4.3.4) has an aperture and the filament is situated immediately above the aperture. Below the The most classic electron gun is the triode gun based on thermionic emission from a tungsten filament heated to about Tc = 2700 K .The filament has a diameter of about 0.1 mm and is bent in the shape of a V hairpin to localize the emission area on the tip. The size of this area is around 100 × 150 µm. By thermionic excitation the electrons overcome the work function Φ of the tungsten tip and a current with the density j_c is emitted according to the Richardson law:

$$j_c = AT_c^2 \exp(-\Phi/kT_c)$$

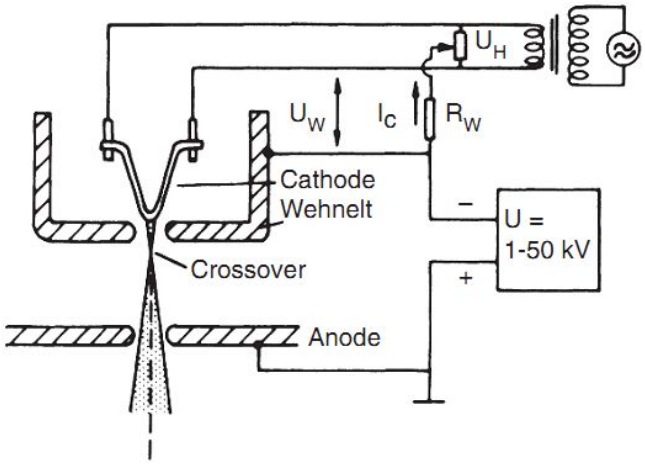


FIG.:4.3.4

Condenser Lenses: After the beam passes the anode it is influenced by two condenser lenses that cause the beam to converge and pass through a focal point. What occurs is that the electron beam is essentially focused down to 1000 times its original size. In conjunction with the selected accelerating voltage the condenser lenses are primarily responsible for determining the intensity of the electron beam when it strikes the specimen.

Apertures: Depending on the microscope one or more apertures may be found in the electron column. The function of these apertures is to reduce and exclude extraneous electrons in the lenses. The final lens aperture located below the scanning coils

determines the diameter or spot size of the beam at the specimen. The spot size on the specimen will in part determine the resolution and depth of field. Decreasing the spot size will allow for an increase in resolution and depth of field with a loss of brightness .

Scanning System: Images are formed by rastering the electron beam across the specimen using deflection coils inside the objective lens. The stigmator or astigmatism corrector is located in the objective lens and uses a magnetic field in order to reduce aberrations of the electron beam. The electron beam should have a circular cross section when it strikes the specimen however it is usually elliptical thus the stigmator acts to control this problem.

Specimen Chamber: At the lower portion of the column the specimen stage and controls are located. The secondary electrons from the specimen are attracted to the detector by a positive charge.

Vacuum System

The ability for a SEM to provide a controlled electron beam requires that the electronic column be under vacuum at a pressure of at least 5x10-5 Torr. A high vacuum pressure is required for a variety of reasons. First, the current that passes through the filament causes the filament to reach temperatures around 2700K. A hot tungsten filament will oxidize and burn out in the presence of air at atmospheric pressure. Secondly, the ability of the column optics to operate properly requires a fairly clean, dust-free environment. Third, air particles and dust inside the column can interfere and block the electrons before the ever reach the specimen in the sample chamber. In order to provide adequate vacuum pressure inside the column, a vacuum system consisting of two or more pumps is typically present.

Separate pumps are required because one pump isn't really capable of doing all the work but, in conjunction they can provide a good vacuum pressure relatively quickly and efficiently. A majority of the initial pumping is done by the action of a mechanical pump often called a roughing pump. The roughing pump operates first during the pump-down process and has excellent efficiency above 10^{-2} Torr. Although many mechanical pumps used in SEMs are capable of producing pressures better than $5x10^{-5}$Torr, a very long pump down time would mostly be required. Pressures lower than 10^{-2} Torr are more easily acquired by the action of a turbo-molecular pump. Turbo-molecular pumps make

use of a turbine that rotates at 20,000 to 50,000 rotations per minute to evacuate gas molecules and particulates found inside the column. Turbo-molecular pumps are expensive and sensitive to vibrations thus it is important to remember that sudden jolts to the instrument can not only affect the beam but, the severely damage turbo pumps.

SEM operation:

In a typical SEM an electron beam is thermoionically emitted from an electron gun fitted with a tungsten filament cathode. Tungsten is normally used in thermionic electron guns because it has the highest melting point and lowest vapor pressure of all metals, thereby allowing it to be heated for electron emission, and because of its low cost. Other types of electron emitters include lanthanum hexaboride (LaB) cathodes, which can be used in a standard tungsten filament SEM if the vacuum systems is upgraded and field emission guns (FEG), which may be of the cold-cathode type using tungsten single crystal emitters or the thermally-assisted Schottky type using emitters of zirconium oxide. The electron beam, which typically has an energy ranging from 0.5 keV to 40 keV is focused by one or two condenser lenses (as shown in Fig.4.3.3.) to a spot about 0.4nm to 5 nm in diameter. The beam passes through pairs of scanning coils or pairs of deflector plates on the electron column, typically in the final lens, which deflect the beam in the x and y axes so that it scans in a raster fashion over a rectangular area of the sample surface.

When the primary electron beam interacts with the sample, the electrons lose energy by repeated random scattering and absorption with in a teardrop-shaped volume of the specimen known as the interaction volume, which extends from less than 100nm to around 5nm into the surface. The size of the interaction volume depends on the electron's landing energy, the atomic number of the specimen and the specimen's density. 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. Electronic amplifiers of various types are used to amplify the signals which are displayed as variations in brightness on a cathode ray tube. The raster scanning of the CRT display is

synchronized with that of the beam on the specimen in the microscope, and the resulting image is therefore a distribution map of the intensity of the signal being emitted from the scanned area of the specimen. The image may be captured by photography from a high resolution cathode ray tube, but in modern machines it is digitally captured and displayed on a computer monitor and saved to a computer's hard disk. Resolution is the ability to resolve two closely spaced points. Resolution is NOT the same as magnification. One way to improve resolution is by reducing the size of the electron beam that strikes the sample. Resolution can also be improved by

• Increasing the strength of the condenser lens

• decreasing the size of the objective aperture

• decreasing the working distance (WD = the distance of the sample from the objective lens). Increasing the strength of the condenser lens

• decreasing the size of the objective aperture

• decreasing the working distance (WD = the distance of the sample from the objective lens).

4.3.4. Transmission Electron Microscope (TEM)

The Transmission Electron Microscope (TEM) is used to study samples at extremely high magnifications. It probes the internal structure of solids and gives us access to microstructure. This allows for a more detailed study of samples that are at or beyond the resolution of the light microscope or SEM. The TEM provides both morphological information through imaging and structural information through electron diffraction.

Principle of TEM

The signals from interaction of electrons like Unscattered electrons (transmitted beam), elastically scattered electrons (diffracted beam) and inelastically scattered electrons are utilized in the TEM. The transmission of unscattered electrons is inversely proportional to the specimen thickness. The thicker parts of specimen under investigation will have fewer transmitted unscattered electrons and so will appear darker; conversely the thinner parts will have more transmitted electrons and thus will appear lighter. Another part of the incident electrons, are elastically scattered by atoms in the specimen. These scattered

electrons are then transmitted through the remaining portions of the material. All electrons follow Bragg's Law and thus are scattered according to

$$2dSin\theta = n\lambda$$

Where d= inter atomic plane spacing

λ=wavelength of electron beam

θ= angle of incident

All electrons enter the specimen normal to its surface with same energy/wavelength. All incidents that are scattered by the same atomic spacing will be scattered by the same angle. These scattered electrons can be collated using magnetic lenses to form a pattern of spots; each spot corresponding to a specific atomic plane. This pattern can then yield information about the orientation, atomic arrangements and phases present in the area being examined. Fig. 4.3.5. shows the diffraction pattern of a monocrystalline sample.

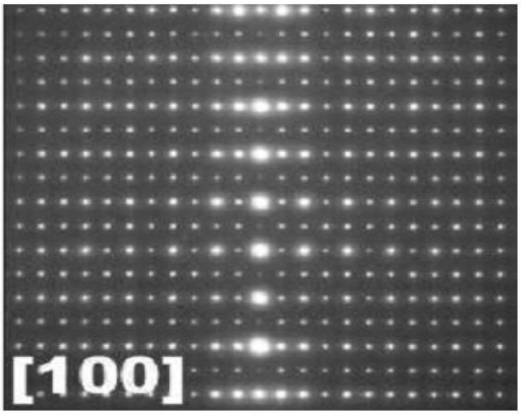


FIG.:.4.3.5

TEM operation

In TEM the sample is placed on grid. The grid is then placed into the TEM through an airlock and bombarded with a focused electron beam. The instrument of transmission electron microscope is shown in Fig.7. As may be noticed, a typical TEM has three sections.

1. Illumination system. It takes the electrons from the gun and transfers them to the specimen giving either a broad beam or a focused beam. In Fig.4.3.6., the parts above the

specimen belong to illumination system.

2. The objective lens and stage.
3. The TEM imaging system. It includes the intermediate lens and projector lens.

The diffraction pattern and image of the object are formed at back focus plane image plane of the objective lens respectively. By focusing the intermediate lens and projector lens on the back focus plane of the objective lens, a diffraction pattern is obtained on screen. It is known as the diffraction mode. By focusing the intermediate lens and projector lens on the image plane of the objective lens, image is obtained on the screen. It is the image mode. The operations in modern TEM are carried out by pressing a single button, as computer attached has pre- recorded values of current, which changes the focal length of these lenses suitably to toggle between these two modes.

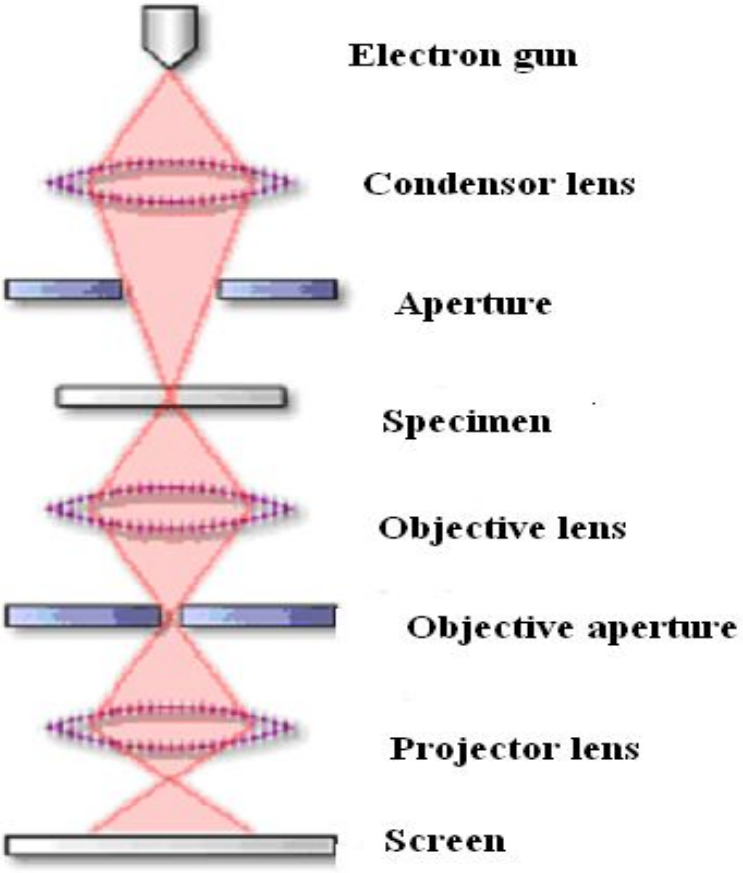


FIG.4.3.6.: Components of TEM

Sample Preparation

The first step is to decide whether the sample is useful to be observed and in which view, plan or cross-section. Due to the strong interaction between electrons and matter, the specimens have to be rather thin, less than 100nm. This is achieved with several methods, depending on the material. In general, mechanical thinning is used to thin and polish the sample. Then it is glued with epoxy glue on a really small and round holder. Whereas TEM data come from the edges of a hole in the centre of the specimen, in sample preparation, the hole is created by the method of ion thinning. Ion thinning is a method where a specimen is irradiated with beams of Ar ions (usually), and after a period of time a hole is created. To minimize the damage created during focus ion beam milling, the embedded sample can first be coated with a metal deposition layer. Consequently, sample preparation is a precise and a severe procedure, which may affects the results of the microscopic analysis and study.

4.3.5. Atomic Force Microscopy (AFM)

Introduction

Typically, when we think of microscopes, we think of optical or electron microscopes. Such microscopes create a magnified image of an object by focusing electromagnetic radiation, such as photons or electrons, on its surface. Optical and electron microscopes can easily generate two-dimensional magnified images of an object's surface, with a magnification as great as 1000X for an optical microscope, and as large as 100,000X for an electron microscope. Although these are powerful tools, the images obtained are typically in the plane horizontal to the surface of the object. Such microscopes do not readily supply the vertical dimensions of an object's surface, the height and depth of the surface features.

Then atomic force microscope (AFM), developed in the mid 1980's, uses a sharp probe to magnify surface features. With the AFM, it is possible to image an object's surface topography with extremely high magnifications, up to 1,000,000X. Further, the

magnification of an AFM is made in three dimensions, the horizontal X-Y plane and the vertical Z dimension.

Basic principle of AFM

AFM is similar, in design, to Scanning tunneling microscope (STM), but measures the force between the sharp microscope tip and surface atoms(see Fig.4.3.7). In STM, a sharp microscope tip is scanned over the specimen surface without touching it, and at the same time, the tunneling current between the tip and the surface atoms, proportional to the distance between them, is recorded. But STM tip may interact physically with the surface in such a way that disruption of the surface structure occurs. In fact, there is usually a finite interaction force between the tip and the sample surface. Even at relatively low tunnelling currents, the interaction force may be substantial when measured against the strengths of molecular interactions. Knowledge of the existence of these forces led Binnig et al., to develop the AFM, in which the probe becomes a cantilever, placed parallel to the surface rather than normal to it. The cantilever of the AFM has a sharp, force-sensing tip at its end, and it is this that interacts with the surface. As the interaction force between the cantilever tip and the surface varies, deflections are produced in the cantilever. These deflections may be measured, and used to compile a topographic image of the surface.

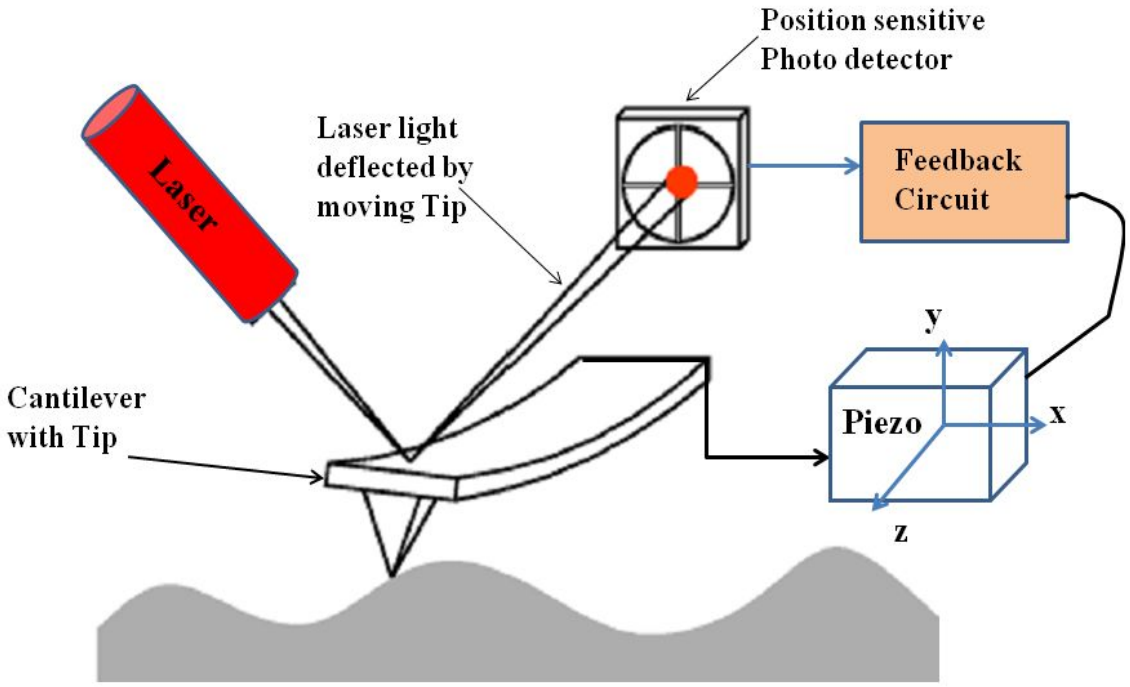


FIG. 4.3.7.: A schematic illustration of operation of an atomic force microscope.

Unlike traditional microscopes, scanned-probe systems do not use lenses, so the size of the probe rather than diffraction effects generally limits their resolution. The atomic force microscope measures topography with a force probe. AFM can image the sample surface in either constant height or constant force modes. In constant height mode, the height of the scanner is fixed as it scans. For small cantilever deflections (< 500 nm) on hard surfaces, the error signal (in volts) is used to generate an image that is sensitive to small changes in topography.

Construction and Working

The AFM consists of a cantilever with a sharp tip at its end, typically composed of silicon or silicon nitride with tip sizes on the order of nanometers. The tip is brought into close proximity of a sample surface. The Vander Waals force between the tip and the sample leads to a deflection of the cantilever according to Hooke's law, where the spring constant of the cantilever is known. Typically, the deflection is measured using a laser spot reflected from the top of the cantilever into an array of photodiodes. However a laser detection system can be expensive and bulky; an alternative method in determining cantilever deflection is by using piezoresistive AFM probes. These probes are fabricated with piezoresistive elements that act as a strain gage. Using a Wheatstone bridge, strain in the AFM probe due to deflection can be measured, but this method is not as sensitive as the laser deflection method.

In an AFM a constant force is maintained between the probe and sample while the probe is raster scanned across the surface. By monitoring the motion of the probe as it is scanned across the surface, a three dimensional image of the surface is constructed. The constant force is maintained by measuring the force with the "light lever" sensor and using a feedback control electronic circuit to control the position of the piezoelectric ceramic (see Fig.4.3.7).

Modes of Operation

Over the years several modes of operation have been developed for the AFM. The

primary modes of operation are

• Contact mode

• Non-contact mode, and

• Dynamic contact mode.

Contact mode: The microscope can operate in constant force mode also known as contact mode in which the cantilever height is effectively adjusted continuously so that a constant tip–sample interaction forces. When the total force becomes positive (repulsive), the atoms are in the “contact” regime. In this imaging the probe remains in contact with the sample all the times. As a result, the probe and sample interaction occurs in repulsive regime.

One of the drawbacks of remaining in contact with the sample is that there exist large lateral forces on the sample as the drip is "dragged" over the specimen.

Intermittent contact: Closer still, in the “intermittent contact” regime, the repulsive van der Waals force predominate. Here the cantilever is allowed to oscillate at value close to its resonant frequency. When the oscillations occur close to a sample surface, the probe will repeatedly engage and disengage with the surface restricting the amplitude of oscillation.

Non contact: As they approach, tip and sample atoms first weakly attract each other. This zone of interaction is known as the “non-contact” regime. Cantilever is again oscillated as in intermittent contact mode, but at much smaller amplitude. As the probe approaches the sample surface long range interaction such as the Vander walls and electrostatic force occur between atoms in probe and the sample. This causes detectable shift in frequency of the cantilever.

Advantages

The AFM has several advantages over the electron microscope. Unlike the electron microscope which provides a two-dimensional projection or a two-dimensional image of a sample, the AFM provides a true three-dimensional surface profile. Additionally, samples viewed by an AFM do not require any special treatment that would actually destroy the sample and prevent its reuse. While an electron microscope needs an

expensive vacuum environment for proper operation, most AFM modes can work perfectly well in an ambient or even liquid environment. This makes it an excellent tool for studying live biological samples. Imaging of conducting and non-conducting surfaces down to sub-nanometer resolution without the need for any additional information. Imaging in air and liquid, allowing in-situ measurements and real time imaging of biological and chemical processes. AFM can be used to measure and localize many different force including adhesion strength, magnetic forces and mechanical properties.

Disadvantages

The main disadvantage that the AFM has compared to the scanning electron microscope (SEM) is the image size. The SEM can show an area on the order of millimeters by millimeters and a depth of field on the order of millimeters. The AFM can only show a maximum height on the order of micrometers and a maximum area of around 150 by 150 micrometers. Additionally, the AFM cannot scan images as fast as an SEM. It may take several minutes for a typical region to be scanned with the AFM; however an SEM is capable of scanning at near real-time.

References/Suggested readings:

1. M.T. Postek, K.S. Howard, A.H. Johnson and K.L. McMichael, Scanning Electron Microscopy: A Student's Handbook, (Ladd Research Ind., Inc. Williston, VT., 1980).

2. I.M. Watt, The Principles and Practice of Electron Microscopy, (Cambridge Univ. Press. Cambridge, England, 1985).

3. M.T. Postek, K.S. Howard, A.H. Johnson and K.L. McMichael, Scanning Electron Microscopy: A Student's Handbook, (Ladd Research Ind., Inc. Williston, VT., 1980).

4. C.E. Lyman, D.E. Newbury, J.I. Goldstein, D.B. Williams, A.D. Romig, J.T. Armstrong, P. Echlin, C.E. Fiori, D.C. Joy, E. Lifshin and Klaus-Ruediger Peters, Scanning Electron Microscopy, X-Ray Microanalysis and Analytical Electron Microscopy: A Laboratory Workbook, (Plenum Press. New York, N.Y., 1990).

5. Joachim Mayer, Lucille A. Giannuzzi, Takeo Kamino, and Joseph Michael. TEM Sample Preparation and FIB-Induced Damage. Mrs Bulletin, volume 32, May 2007.

6. G. Binnig, C.F. Quate and C. Gerber, Phys. Rev. Lett. 56, 930 (1986).

Problems:

Q1.A microscope in which an image is formed by passing an electron beam through a spe cimen and focusing the scattered electrons with magnetic lenses is called a

A) Transmission electron microscope

B) Scanning electron microscope

C) Atomic force microscope

D) Optical microscope

Q2. What is the difference between SEM and TEM?

Q3. What are the advantages of AFM over other forms of microscopy?

Q4. What is the principle of working of Atomic force microscopy?