MODULE IV

TRANSMISSION ELECTRON MICROSCOPY (TEM)

Transmission electron microscopy (TEM):

- TEM is also known as conventional transmission electron microscopy or CTEM.
- Max Knoll and Ernst Ruska invented it in 1933 in Berlin.
- Recentelectron microscopy (based on transmission) commonlycontains a beam column which is around 2.5m tall and has a 30cmdiameter, and has an ability to attain a 2Å resolution.
- Thistechniqueis utilized for analysing the surface structure, i.e.,morphology, surface imperfection, i.e.,defects, crystal structure of the atom, size of the particle and alsosample's composition.

Design of TEM is similar to a light microscope-

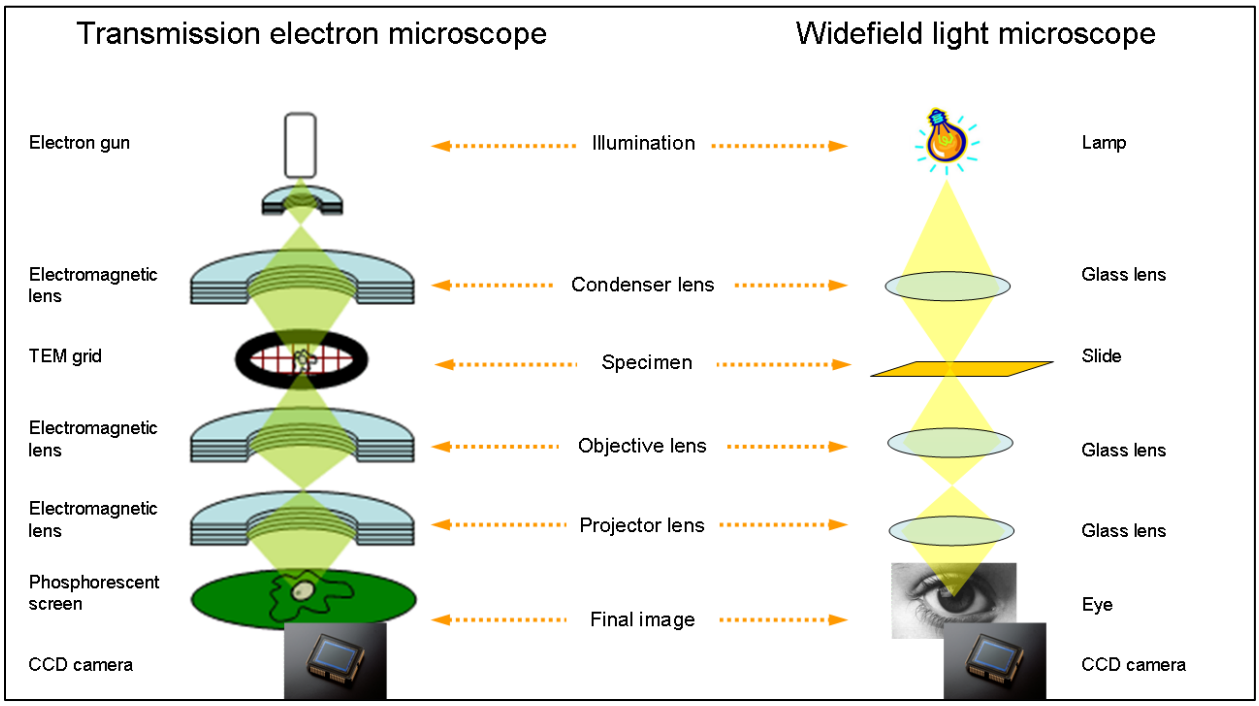

Figure 1 Similarity of a transmission electron microscope with a wide field light microscope.

Working Principle-

An extremely thin sample is required for scanning in TEM from which electron beam is passed through rendering its interaction with the sample as a result of which image is produced.This image can be magnified and focused on the device used for imaging, like a fluorescent screen, on a photographic filmlayer, or to be identified by a sensor like a CCD camera.

Instrumentation of TEM-

- Source of electron
- Gun based on Thermionic Emission

- Beam of Electron
- Electromagnetic lenses
- Vacuum chamber
- Two Condensers lenses, objective and intermediate lens
- Sample holder and stage
- (Imaging Device) Phosphor or fluorescent screen
- Computer

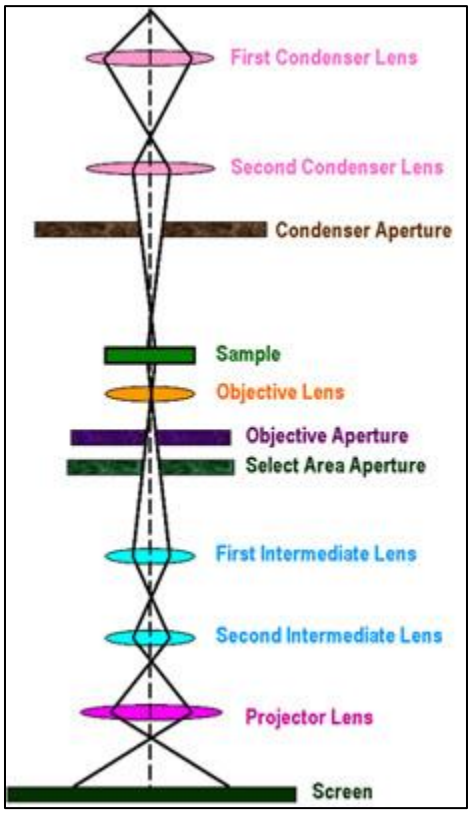

Figure 2 Instrumentation of TEM.

Electron Gun:

Electrons can be producedeither by thermionic emission or by a process called cold field emission. During thermionic emission, a very fine tip of a tungsten filament, a LaB_6 crystal or a ZrO/W Schottky emitter, is heated by an electrical current flowing through it, thereby enabling the emission of electrons. The electrons leaving the filament have a low energy and, therefore, need to be accelerated to the desired speed before entering the electron column. A high voltage between the electron source (cathode) and an anode plate is applied leading to an electrostatic field through which the electrons are guided and accelerated. During cold field emission, the electrons can escape from an extremely fine tungsten tip without heating (at room temperature). The advantage of cold field emission sources is the very high yield of electrons and the very low chromatic aberration of the electrons allowing imaging at atomic resolutions. These instruments are very costly and require very high vacuum.

The Electron gun working can be controlled based on 3 parameters:

- The accelerating voltage,
- Current of the filament (and therefore its temperature),
- And the Wehnelt cap bias voltage.

The temperature of the filament tip is controlled by the filament current which in turn controls the amount of emitted electrons. The filament current is increased till the number of emitted electrons no longer increases, which actually means that filament is saturated in order to maximize the emission.

The passing current between the system having high voltage andground is controlled by bias resistor setting which in turn is controlled by the gun bias.When small bias voltage is used, the wehnelt negative potential isineffective in comparison to the filament,leading to poor focusing of the electrons that are accelerated toward the anode. This results in beam spreading, causing it to appear weak on the screen.When the biasing is increased, the focusing action is improved therefore the effective beam brightness is also increased; but, beyond a certain value the Wehnelt is so negative in comparison to the filamentthat the brightness starts to decrease because electrons are not permitted to emit fromthe filament or, in a case they are emitted, they are repelled back in the direction of the filament.

The point at which the finest brightness of the beam is attained is determined by the distance between the Wehnelt and filament.

Electromagnetic lenses:

Electromagnetic lenses consist of a huge bundle of windings of insulated copper wire, a soft iron cast and pole piece (**Figure 3A**). A magnetic field is induced by the current in the winding and reaches its main strength at the pole piece of the lens. The accelerated electrons entering the magnetic field are deviated by Lorentz forces. The direction of both magnetic field as well as electrons defines the resultant force which is always perpendicular to the plane.In conclusion, the electrons take a circular path through the lens system (**Figure 3B**).

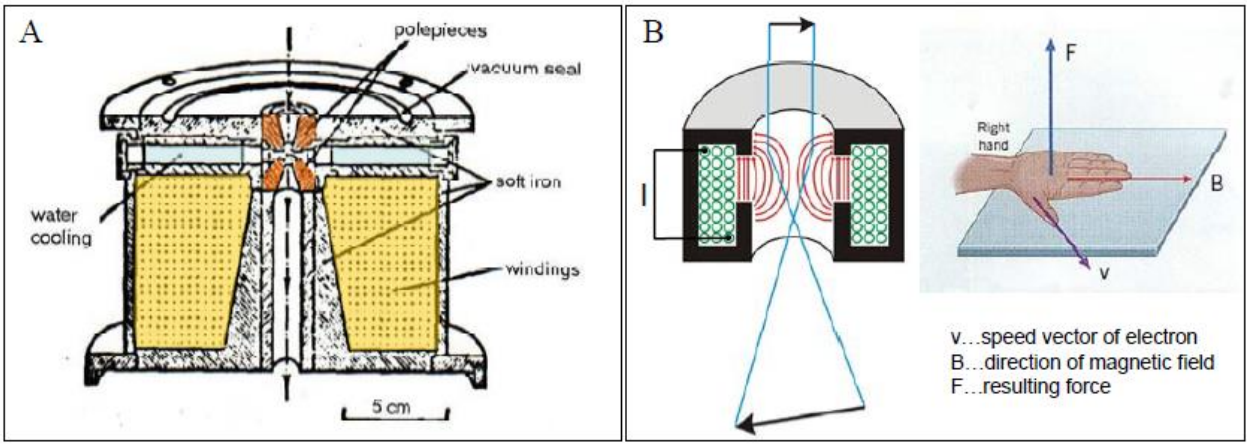

Figure 3 Electromagnetic lens. A) The magnetic field is strongest in the area of the pole piece; B) Electrons passing the magnetic field are deviated perpendicular to the plane defined by the magnetic field B and the velocity vector v.

Condenser lens system:

The beam diameter isreduced and controlled by condenser lens system. The purpose of the first condenser C1 lens (or spot size) which is a strong lens is to de-magnify the electronsource image by around X1/100 to provide a small “point” source at the “crossover” that is more coherent than the large (50 µm diameter) tip of the filament. The purpose of the second condenser C2 lens (brightness or intensity) which is a weaker lens is to project the de-magnified image of the source on top of the samplebya magnification of X2, giving an overall demagnification of X1/50. Illumination spread onto the screen is controlled by this lens.A part named condenser aperture is positioned just below or sometimes between the condenser lenses; its role is to collimate (i.e. making parallel) the beam of the electron as well as modification in its intensity.

Objective and intermediate lenses:

The reason behind the back focal plane being very close to the lens itself is because the magnification factor of the objective lens is larger. Aperture of the objective (it is the middle aperture on the column) is mounted in the back-focal plane.The selected area aperture sits in the first image plane below the specimen, which is below both the objective lens and the objective aperture.
By altering the first projector lensexcitation (also known as intermediate lens or diffraction lens), either an image or a diffraction pattern is produced.

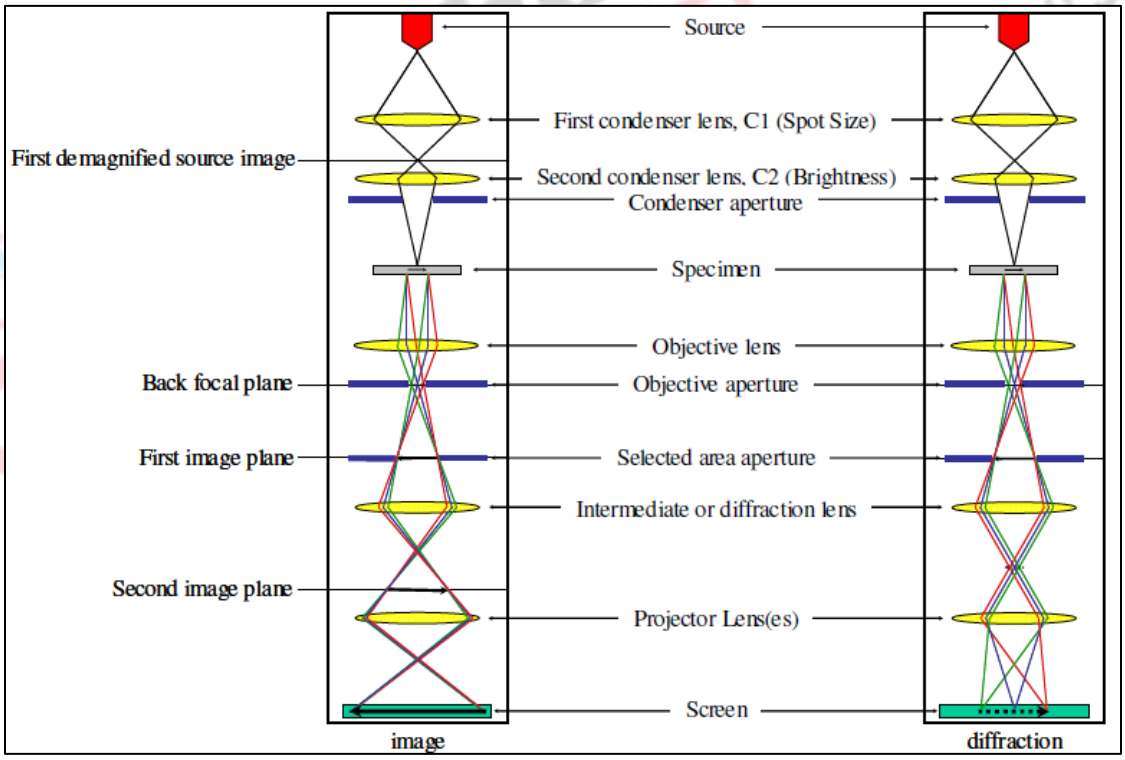

Figure 4 Diagram showing the formation of both an image and a diffraction pattern.

Specimen holders and stages:

In TEM, the electron column does not offer a lot of space for the sample. Further, the sample should be fine (thin) so that the electrons can penetrate the specimen to produce an image. The average thickness of a biological specimen should be around 70 nm for a TEM with an acceleration voltage for the electrons of ~100 kV (higher voltages allow the investigation of thicker samples). Thin sections of thesample are

mounted on copper grids of 3 mm diameter, which are available in a wide variety of materials and mesh sizes. The grids with the sections on top are attached in a holder and introduced into the goniometer of the TEM through a vacuum lock, since the system always stays under high vacuum. The goniometer is the mechanical setup which enables highlyprecise and stable control of the specimen holder during imaging. Any drift or instability results in an blurredimage, particularly at high magnifications (**Figure 5**).

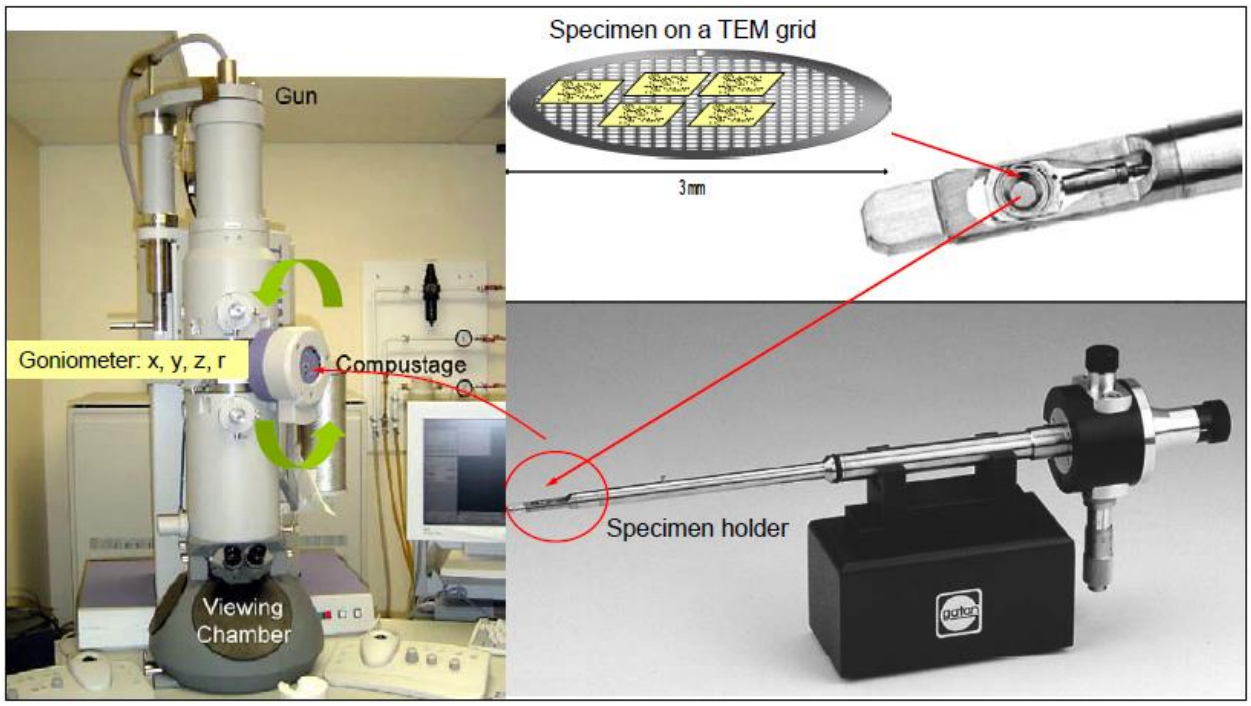

Figure 5 Thin sections of a specimen on a TEM grid, holder tip and complete specimen holder, which is introduced into the goniometer of the TEM through a vacuum lock.

Vacuum System:

Vacuum system is employed in electron microscopes for 4 reasons:

- As electrons are readily scattered,electrons have amean free path of ~1cm at atmospheric pressure; however, at 10^{-6} Pa they can have mean free path as high as 6.5m.
- The purpose of the vacuum system is to provide insulationbetween the filament of both anode and cathode as well as in the region around the field emitters, thus hamperingundesirabledischarge of the electron gun.
- In order to inhibit the oxidation and 'burning out' of the filament, oxygen is eliminated around the filament.
- Samples contamination is decreased by reducing the interaction amongst electron beam and molecules of the gas.

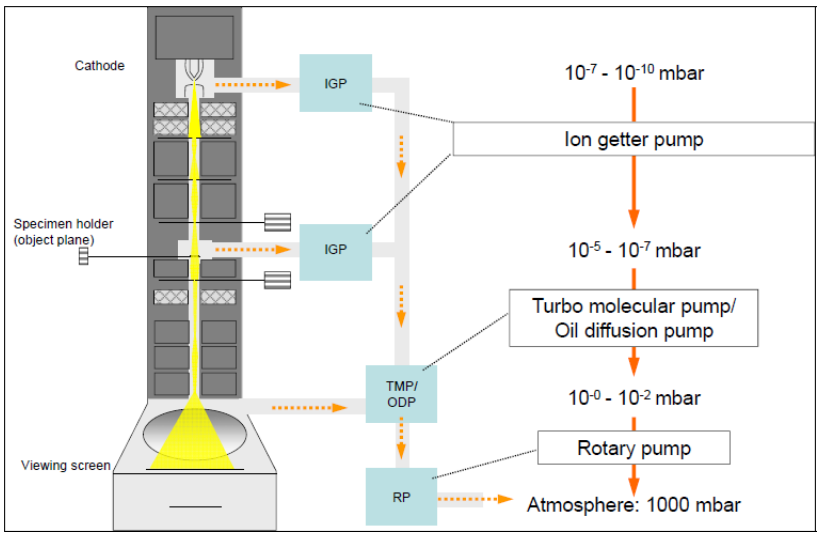

Figure 6A typical vacuum system for a TEM. RP-rotary pump, TMP-turbo molecular pump, IGP-ion getter pump, ODF-oil diffusion pump.

Variousmicroscope parts are differently vacuumed as per their requirements. The gun requires 10^{-9} Pa vacuum, while the specimen requires 10^{-6} Pa and the projection chamber plus camera requires 10^{-5} Pa.

Vacuums can be categorized as: rough (100 - 0.1 Pa), low (10^{-1} - 10^{-4} Pa), high (10^{-4} - 10^{-7} Pa), or ultrahigh (< 10^{-7} Pa).

Phosphor or fluorescent screen (Imaging Device):

There are 2 procedures for specimen observation in TEM as shown in **Figure 4**.

1. Image mode

2. Diffraction mode

In case of image mode, the electron beam hitting the sample is controlled by condenser lens and aperture, the beam which is transmitted will be focused and enlarged by objective and projector lenses,and the image is formed on the screen with identifiableinformationin relation to the microstructure of the sample. In case of diffraction mode, at the fluorescent screen a diffraction pattern (of electron) is obtained which originates from the electron beam illuminated sample region. The pattern of diffraction is completelysimilar to that of apattern of X−ray diffraction. The spot pattern is produced by a single crystal on the screen whereas poly-crystal produces a pattern of powder or ring. The purpose of the image mode is to analyse microstructure, e.g. the grain size, and lattice defects, whereas the use of diffraction mode is to examine crystalline structure.

Image Modes of TEM-

In TEM, the 2 primary image modes vary in the manner of using the objective aperture as filter in electron optics system. These modes are:

1. Bright field microscopy

2. Dark field microscopy

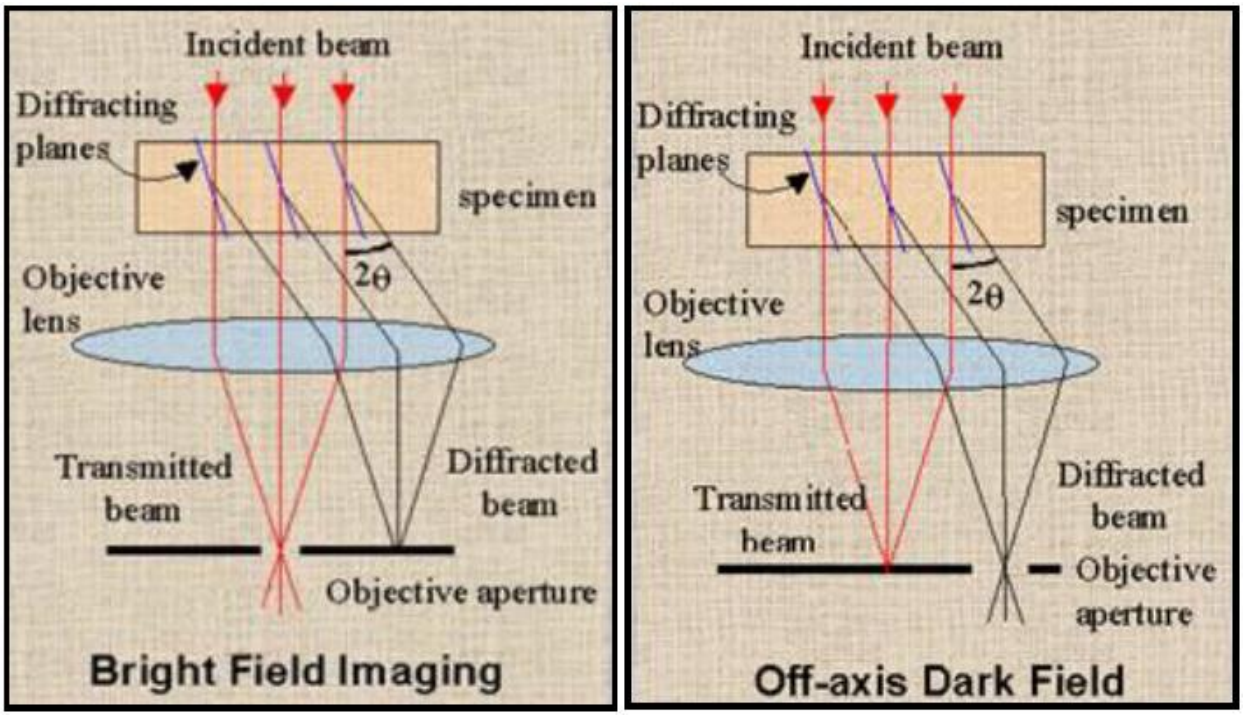

Figure 7Two image modes of TEM.

In bright field imaging, the image of the sample is created by the electrons that pass through the film without diffracting. Adiaphragm is used to stop the diffracted electrons. In the corresponding dark field imaging mode, the image is formed by the diffracted beam. The technique is called as bright Field which is mainly sensitive to extended crystal lattice defects in an otherwise ordered crystal, e.g., dislocations. The electron rays corresponding to bright field and dark field imaging are shown in **Figure 7**.

Electron interaction with matter-

The interaction between the electron beamand the sample is coulombic. The negatively charged electrons can interact strongly with the electron cloud in the solid and also the positively charged nucleus.In contrast, X-rays are EM radiation and they only interact with the electron cloud. In TEM, for imaging purposes, only the forward scattered electrons are of interest. There are two main types of scattered radiation:

- Elastic - this represents coherent scattering (mainly) with no loss of energy. There is also a phase relation with the incident radiation.
- Inelastic - the energy of the scattered electrons is lower than the incident beam. These are also incoherent radiation with no phase relationwith the incident radiation.

TEM sample preparation-

Significant part of TEM is its sample preparation for the analysis. There are twomain conditions for TEM sample preparation:

- Electron transparent sample must be used. If not the whole sampleat least the ROI should be thin. The allowed thickness value for the metallicsamples is 30 - 50 nm. Usually, 100 nm is an upper limit for thesample thickness.

- The sample must be mechanically strong for treatment.

TEM samples are either self-supported or mounted on a grid for analysis. Copper grids are the most commonly used, though for high temperature work Mo grids are used. For nanoparticles and thin films a-C film is used as support. A-C has low contrast in the TEM and will not obscure the contrast arising from the specimen. Some typical TEM grids are shown in figure 7.

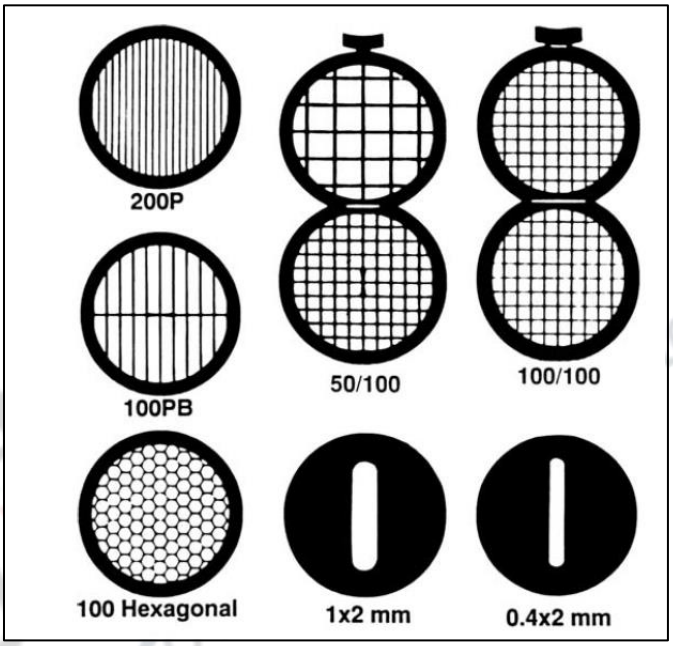

Figure8Typical TEM grids.

Thinning the sample by different techniques-

Electrolytic polishing:

Electrolytic polishing is used for conducting samples like metals/alloys in order to produce samples that are electron transparent. The initial sheet thickness can be around a few hundred μm. This can be prepared by rolling or grinding bulk specimens. Similarly, metal coatings on substrates can be peeled off and used for the final thinning. Thin discs can also be cut from bulk specimens. This process is called coring.These discs are thinned by electrolytic polishing.Electrolytic polishing technique is the window technique.The sample is made the anode and a thin stainless sheet is made the cathode. The sample is immersed in the electrolyte, which is usually cooled by water or liquid nitrogen. Perchloric acid is usually used as the electrolyte. The sample edges are covered by lacquer to expose a 'window', hence the name. The experimental setup and the hole generation are shown in **Figure 9**.

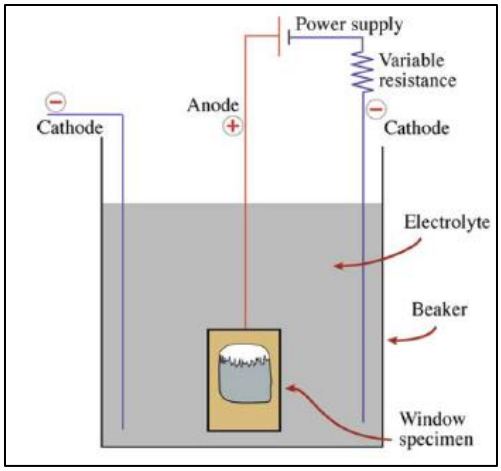

Figure 9 Window polishing technique.

When a current is applied, the material is dissolved from the anode (sample) and deposits on the cathode. The rate of dissolution depends on the current and applied voltage. The I - V characteristics are shown in **Figure 10**. Depending on the current and voltage, there are three regimes - etching, polishing, and pitting. The edges are coated so that material removal will start within the window. Once a hole is formed within the window, the sample is pulled out. The region around the hole is usually electron transparent and can be mounted on a TEM grid.

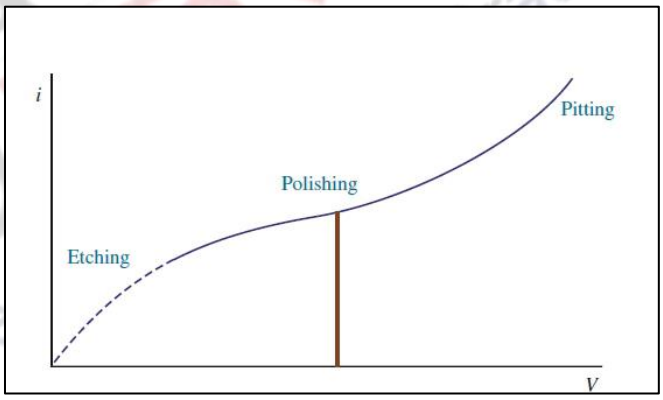

Figure10 I - V characteristics during polishing.

Ion milling technique:

For non-conducting samples, usually, grinding and polishing steps are used in order to reduce sample thickness. Sometimes, an ultramicrotome is used in order to generate thin samples. These can be either electron transparent or can be used as the starting material for further thinning. The schematic of the technique is shown in **Figure 11**. For samples, where ultramicrotome cannot be used then a standard tripod polisher is used in order to thin the sample. This produces samples that are a few nm thick. The final polishing step is done by an ion beam miller.

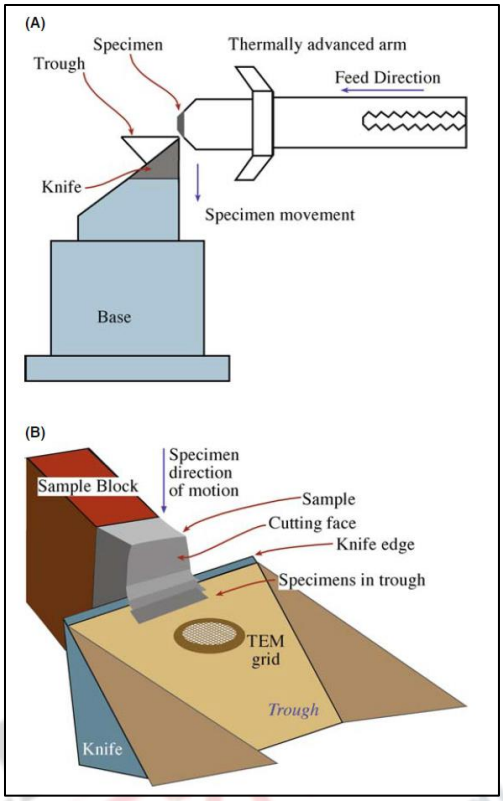

Figure 11 Schematic of ultramicrotome technique.

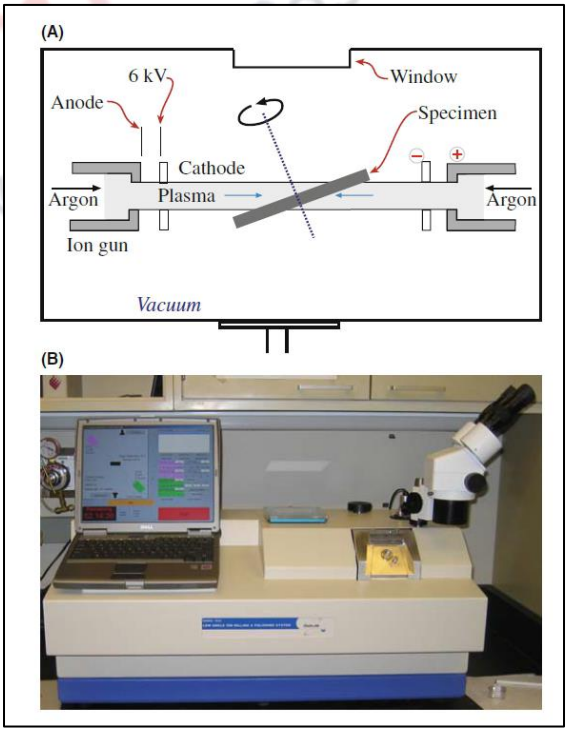

Figure 12 The schematic of the ion beam miller and an actual instrument.

The sample is bombarded with high energy ions or neutral atoms. Usually, Ar ions are used and they are formed by passing the Ar gas though a high voltage (4 - 6 keV). The sample is held in vacuum and also usually cooled by liquid nitrogen. The ions are incident on the sample to sputter the material away. To minimize ion penetration the beam is usually incident at a low angle (≈ 20°), if the angle is very small the sputter rate is small. Ion beam is highly controlled and a localized process but it is time consuming. Sputter rates are usually a few Åper second so that creating an electron transparent sample can take hours, especially if the initial thickness is high.

Cross section sample preparation:
Slices from the sample are cut using a diamond slicer. These slices are placed between spacer layers and then glued on to a grid. The slices are glued in such a way that the interface is parallel to the slot in the grid. This sample is then thinned by standard tripod polishing until it is a few μm thick. The final sample is thinned using an ion beam miller to create an electron transparent sample.

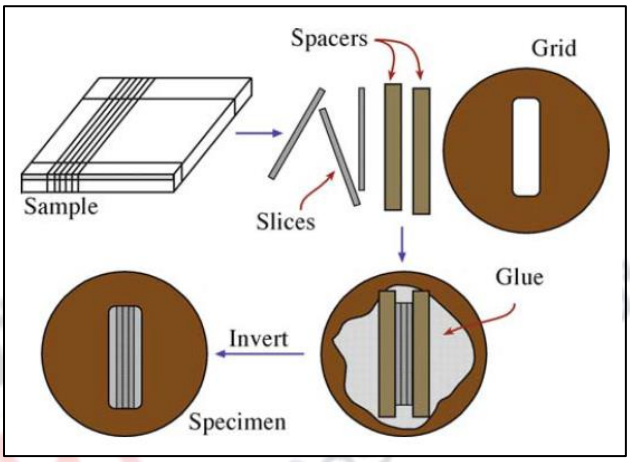

Figure 13 Cross sectional sample preparation.

Replica technique:
Replica technique is used for studying bulk specimens which cannot be destroyed to prepare electron specimens. It is also useful for studying surface topography features and precipitates though SEM techniques have gradually replaced replica sample preparation. A replica of the sample surface is prepared using a plastic mold. The mold is then removed from the surface and the surface of the specimen is replicated by the surface of the plastic. A thin film of carbon or metal like Cr, Pt is evaporated on the surface of the plastic. Sometimes the evaporation is done from an oblique angle, shadow evaporation, to enhance the contrast. The plastic is removed by dissolving in a suitable solvent and the film is then floated on to a grid for analysis.

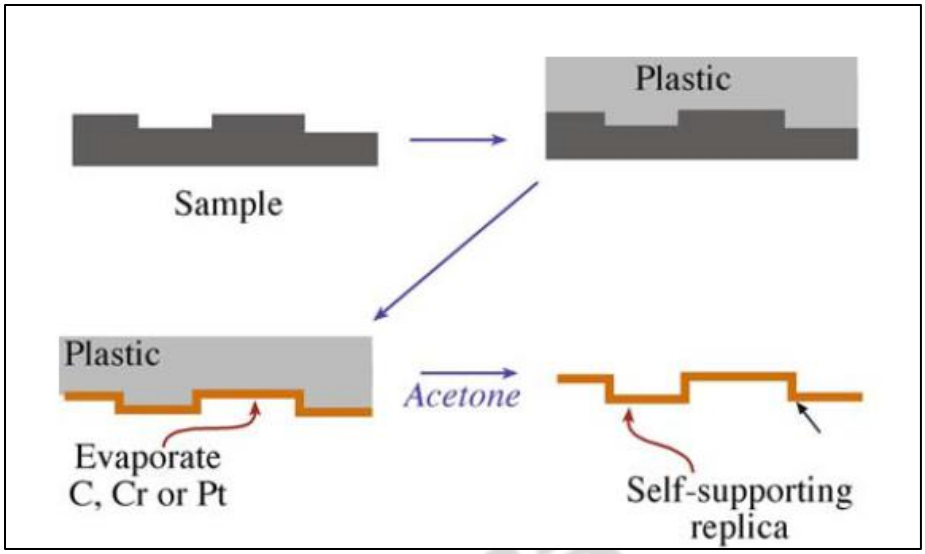

Figure 14 Replica technique for sample preparation.

Review your learning:

1) Why are thin sections of specimens necessary in TEM?

   a) Electrons are negatively charged
   b) Electrons have a wave nature
   c) Electrons have no mass
   d) Electrons have a poor penetrating power.

2) Why TEM images have much higher resolution than images from light microscope?

   a) TEM is much greater in size than light microscope
   b) Electrons travelling as waves have wavelength much shorter than visible light
   c) TEM can achieve greater magnification
   d) The fluorescent screen of TEM can generate high resolution images

3) Which of the following is the correct pathway of electrons in the TEM?

   a) Anode → electromagnetic lens system → sample → fluorescent screen
   b) Anode → electromagnetic lens system → sample → electromagnetic lens system → fluorescent screen
   c) Cathode → electromagnetic lens system → sample → electromagnetic lens system → fluorescent screen
   d) Cathode → electromagnetic lens system → sample → fluorescent screen

4) What should be done right after the TEM column is shown to be evacuated?

   a) Insert the sample holder
   b) Further insert the sample holder
   c) Remove the dummy holder
   d) Shift the beam

5) Before loading the sample, the following softwares have to be turned on, except…

   a) Electron gun tilt/shift
   b) TEM imaging and analysis
   c) Microscope user interface
   d) Digital Micrograph

True/False:

1) Ultra-violet light is applied to the sample to warm up the sample.
2) The main purpose of cutting extremely thin slices of samples is for better observation of intracellular components instead of extracellular components.

3) TEM cannot be used to examine live specimen.

4) We should turn off the light before examination of sample using the fluorescent screen.

5) We need to do the alignment of the electron gun, beam and rotation center every time we use the TEM

Fill in the Blanks:

1) The gun requires the vacuum of the order of …….

2) The specimen requires the vacuum of the order of ……..

3) Projection chamber and camera requires the vacuum of the order of ……

Long type questions:

1) Explain the working principle of TEM
2) Describe various types of pumps used to achieve vacuum?

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