Lesson: Electron Microscopy

Lesson Developer: Anuradha Sharma

College/Department: Botany Dept., Hindu College, University of Delhi

Table of Contents

Chapter: Electron Microscopy

Introduction

Principle of Microscopy

The prokaryotic and eukaryotic cells fall within the size range of 1-100 μm. Unaided human eye cannot resolve objects smaller than 100 μm size. Therefore, microscopes are needed for visualization of subcellular architecture. Microscope not only magnifies the image of objects but also increases the resolution, which refers to ability to distinguish closely adjacent objects as separate entities. The greater is the resolving power of the microscope, the greater is the clarity of the image produced.

The lower limit of resolution for any optical system can be calculated from the following relationship.

$r = 0.61\lambda / n \sin \alpha$

where r, or resolving power, is the minimum distance between two points that can be recognized as separate, λ is the wavelength of light (or other radiation) used to illuminate the object, n is the refractive index of the medium in which the object is placed, and sin α is the sine of half the angle between the specimen and the objective lens. The entire term n sin α is often referred to as the numerical aperture.

Frequently asked question

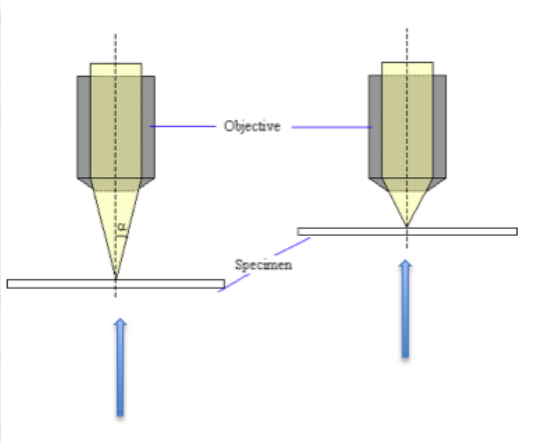
What do you understand by numerical aperture?

The numerical aperture of the objective a microscope is a measure of its resolving power. T value of numerical aperture is given by NA = n sin α.

n refers to the refractive index (1 for air)

α is half the angle subtended by the rays entering into the objective lens

Higher the NA higher the resolving power



Low NA= Low resolving power High NA= High resolving power

Source: http://www.doitpoms.ac.uk/tlplib/optical-microscopy/images/diagram6.gif

There are only a small number of variables affect the resolving power of a microscope. The refractive index can be increased by immersing the sample in oil (n = 1.5) rather than air (n = 1.0), and moving the lens closer to the specimen to increase α. The upper theoretical limit of α is 90 °, meaning that the value of sin α cannot exceed 1. Hence the maximum numerical aperture of an optical system employing an oil immersion lens will be 1.5 X 1 = 1.5. A microscope using white light, which has an average wavelength of about 550 nm, will therefore, have a resolving power of 550/1.5, or about 220 nm. This means that objects closer to one another or smaller than 220 nm cannot be distinguished. A resolving power of 220 nm is adequate to see some details of subcellular structure, but many organelles, such as ribosomes, cellular membranes, microtubules, microfilaments, intermediate filaments, and chromatin fibers, cannot be resolved at this level .The wavelength of an electron is much shorter than that of

visible light, the electron microscope has a theoretical limit of resolution much lower that of the light microscope—about 0.1-0.2 nm instead of 200-300 nm. Because of problems of specimen preparation of biological samples, the practical limit of resolution is almost about 2 nm which means 100 times more resolution than that of light microscope. Electron microscopes thus offers the possibility of increasing the resolving power many folds. There are two types of electron microscopes:

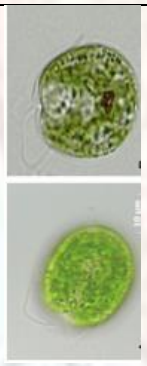
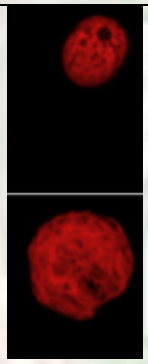
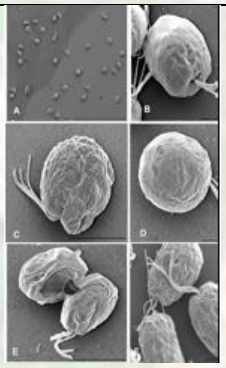
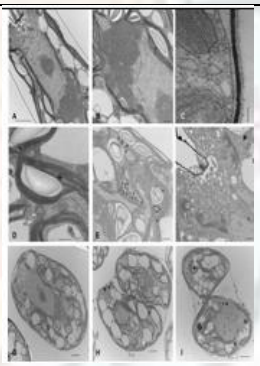
- Transmission electron microscope
- Scanning electron microscope

The electrostatic and electromagnetic lenses are used in an electron microscope to control the electron beam and focus it to form an image. In Transmission electron microscope (TEM), the electrons are transmitted through an object and then focused by the lenses to form the image. In Scanning electron microscope (SEM), the electrons are reflected by the object in a scanned pattern which are then used to form the image. SEM is becoming increasingly popular with cell biologists because of its remarkable ability to study surface topography, along with improved resolution (30-100 Å) and its ability to show 3D structure.

Table: Comparative account of different types of microscopes

Source: Author, Images courtsey: Dr Mani Arora

Description	Compound	Confocal Microsco	Scanning Electro Microscope (SEM	Transmission Electron Microscope (TEM)
Source of illumination for Image Formation	visible light	laser light	electrons	electrons
Types of cells visualized	Individual cells can be visualised, even living ones.	Individual cells c be visualised, eve living ones.	The specimen is coated with gold and the electrons are reflected back and give the deta of surface topography of the specimen.	Thin sections of the specimen are obtained. T electron beams pass through the sections an form an image with high magnification and high resolution.
Image	Two dimensiona		3-D	2-D

Nature of Lenses	glass	glass lenses with dichromatic mirro	one electrostatic lens with a few electromagnetic lenses	one electrostatic lens and few electromagnetic lenses
Medium	air	air	vacuum	vacuum
Specimen mounting	glass slides	glass slides with dyed samples	Mounted on aluminum stubs and are coated in gold	Mounted on coated or uncoated copper grid
Focusing and Magnification Adjustments	changing objectives	digitally enhanc	Electrical	Electrical i.e.changing current of the projecto lens coil
Means for obtaining specimen Contrast	Light Absorption	laser light with dichromatic mirro concentrated at pinhole	electron scatterin	Electron scattering
Comparative account of the various micrographs of an algal cell				

Basic Components of an Electron Microscope

1. The vacuum system—A strong vacuum must be maintained in the entire column along the path of electron beam, since electrons cannot travel very far in air. There are two types of vacuum pumps which work together to create vacuum

2. The Electron gun----The electron beam is emitted by an electron gun which consist of

a) The cathode, a filament made of tungsten emits electrons maintained at50-100kv

b) The anode, to shape the beam maintained at 0 kv

The difference in voltage is called accelerating voltage.

3. Electromagnetic Lenses and image formation—There are many lenses arranged together to control illumination, focus, and magnification

a) The condenser lens-to control the electron beam

b) The objective lens, intermediate lens and projector lens—in concert with each other produce a final image on the viewing screen

4. The photographic system—In addition to viewing, the image can be recorded photographically as an electron micrograph.

5. The cooling system—since a high voltage is used for the emission of electrons, a cooling system is also attached to the column so that it does not get heated up.

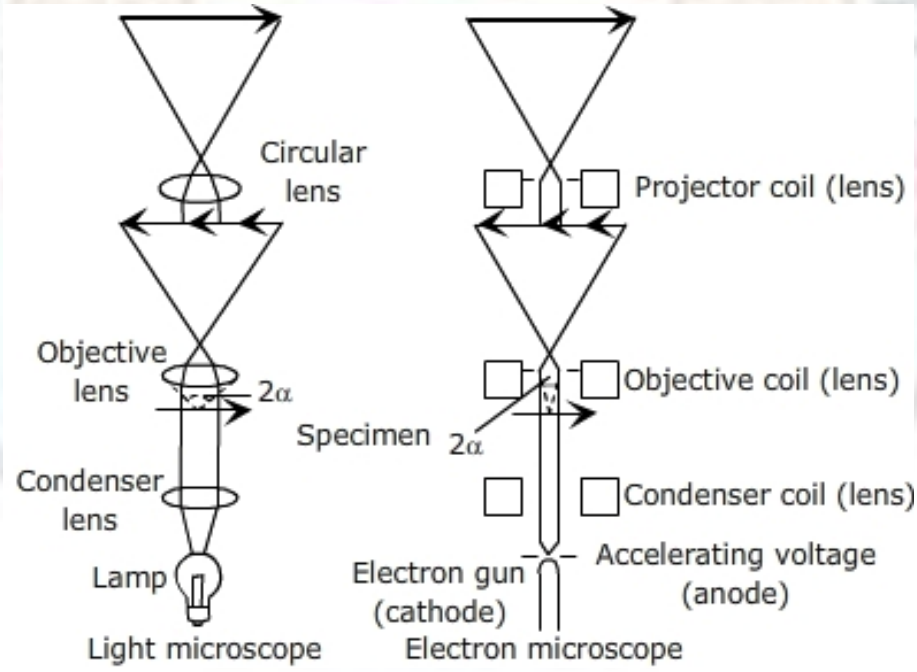


Figure: Schematic diagram of Light Microscope and Electron Microscope

Source: Author

Types Of Electron Microscope

Transmission Electron Microscope (Tem)

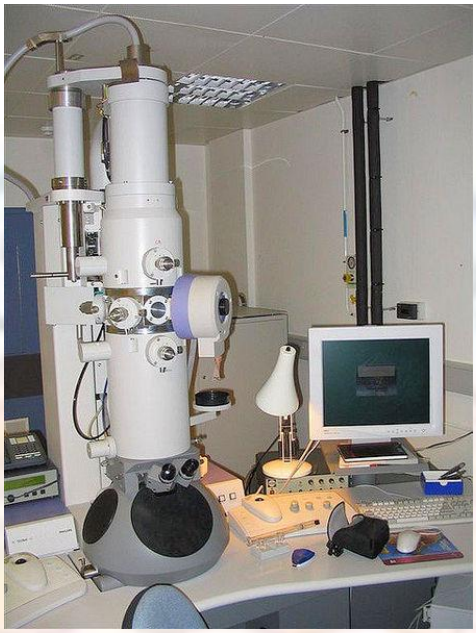


Figure: Transmission electron microscope

Source: http://en.wikipedia.org/wiki/File:Electron_Microscope.jpg

The prototype electron microscope was invented in 1931 by German physicist E. Ruska and the electrical engineer M. Knoll .In 1933; Ruska built an electron microscope that exceeded the resolution of an optical microscope. E. F.Burton and students C. Hall, J. Hillier, and A. Prebus1938. at the University of Toronto, constructed the first practical electron microscope. In 1939, Siemens produced the first commercial Transmission Electron Microscope (TEM).

In Transmission Electron Microscope (TEM), a beam of highly focused electrons is directed towards a thin section of the specimen (<200 nm) and allowed to pass through it. These highly energetic incident electrons interact with the atoms in the sample and produce characteristic radiation and particles which form image. Images are obtained from transmitted electrons, backscattered and secondary electrons, and emitted photons.

TEM uses a high voltage electron beam which is emitted by electron gun to create an image. The electron gun is made up of a tungsten filament cathode as the electron source. The electron beam is accelerated by an anode and then is focused by electrostatic and electromagnetic lenses. The electron beam is then transmitted through the specimen. As the electron beam emerges from the specimen, it carries information about the structure of the specimen that is

magnified by the objective lens of the microscope. The transmitted electrons hit a fluorescent screen at the bottom of the microscope and give rise to a "shadow image" of the specimen with its different parts displayed in varying darkness according to their density. Image is viewed by projecting the magnified electron image onto a fluorescent viewing screen coated with a phosphor or scintillator material. The image can also be photographically recorded by exposing a photographic film or plate directly to the electron beam or a fibre optic light-guide to the sensor of a CCD camera. The image detected by the CCD may be visualized on a monitor or computer.

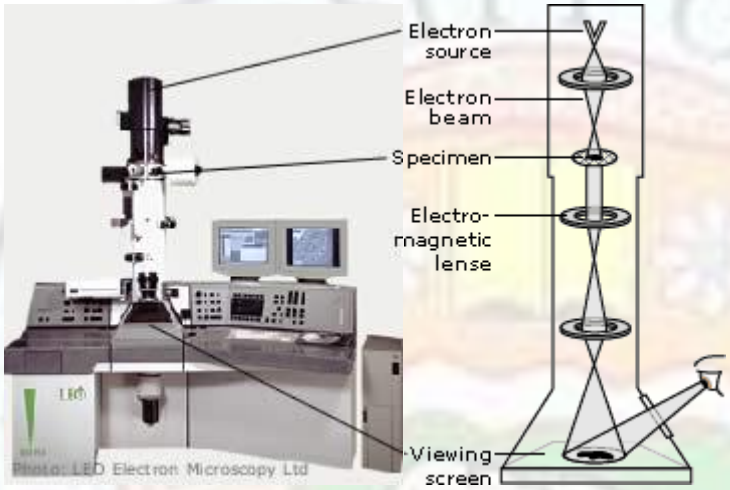


Figure: TEM and Its schematic diagram
Source:http://www.nobelprize.org/educational/physics/microscopes/tem/ex.html

There are different ways to prepare the material for TEM. One way is to cut very thin sections of the specimen from a piece of tissue either by fixing it in resin or working with it as frozen material. Another way to prepare the specimen is to isolate it and study a solution after doing negative staining, for example viruses or molecules in the TEM.

Sample Preparation: Biological material contains large quantities of water. Since the transmission electron microscope works in vacuum, the water must be removed. The tissue is preserved with different fixatives to avoid any disruption due to loss of water. These fixatives also aim to stabilize the specimen's mobile macromolecular structure by chemical crosslinking of proteins with aldehydes such as formaldehyde and glutaraldehyde, and lipids with osmium tetroxide. The tissue is then dehydrated in alcohol or acetone after dehydration. The tissue is then embedded so that it can be sectioned. To do this, the tissue is passed through a 'transition solvent' such as propylene

oxide and then infiltrated with an epoxy resin such as Araldite, Epon, or Durcupan;. After the resin has been polymerized (hardened), the sample is thin sectioned (ultrathin sections) by a diamond or glass knife in an instrument called ultramicrotome .Since the sections are very thin it becomes difficult to hold the sections .To pick up sections a boat is made around the glass knife,which is then filled with water .When sections are cut ,they float on the surface of water. The sections are then picked up directly on to surface of copper grid by touching the grid to the surface of water in boat. Once the sections are placed on the copper grid , the staining is done with heavy metals such as lead, uranium or tungsten to scatter imaging electrons and to produce contrast between different structures because many (especially biological) materials are nearly "transparent" to electrons (weak phase objects). The specimens can be stained "en bloc" before embedding or later after sectioning. Typically thin sections are stained for several minutes with uranyl acetate followed by aqueous lead citrate, which can then be studied under the electron microscope.

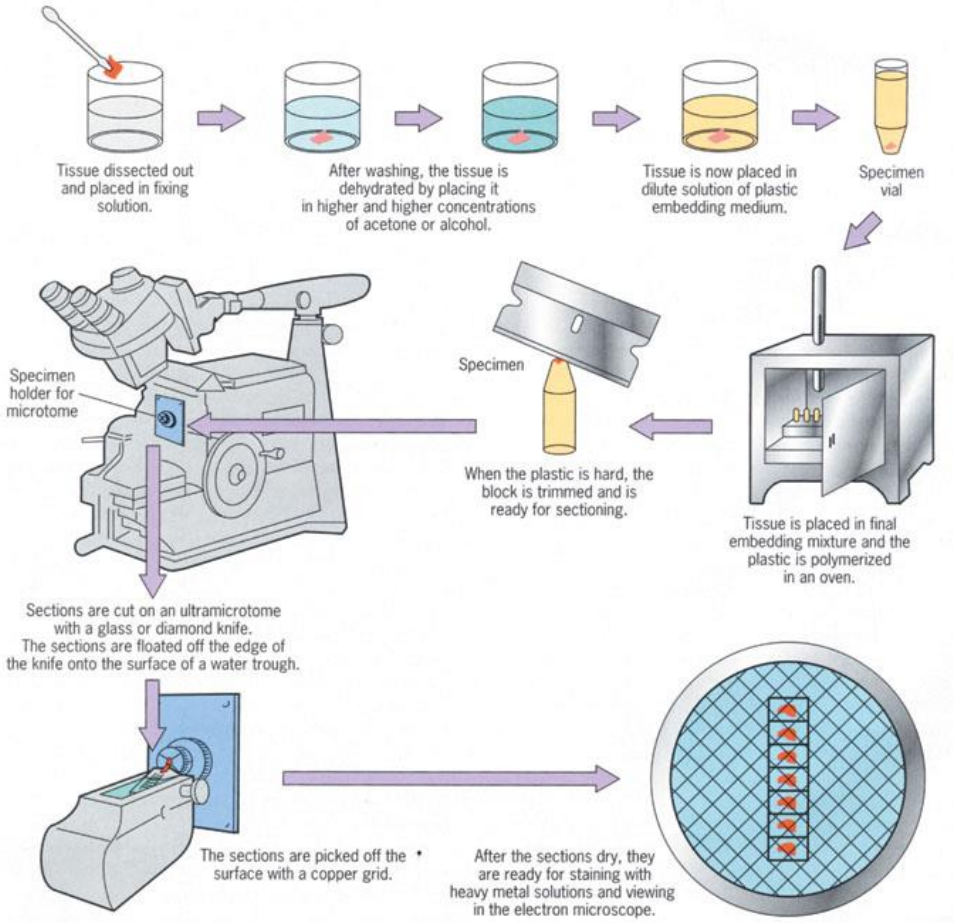


Figure: Procedure for sample preparation for electron microscopy

Source: http://yxsj.baiduyy.com/whole/image/chapter18/18.13.jpg

AN ULTRA -MICROTOME

A microtome (from the Greek mikros, meaning "small", and temnein, meaning "to cut") is a tool used to cut extremely thin sections. An ultra-microtome is used for the preparation of ultrathin sections (50-100 A) for observation under transmission electron microscope. Glass and diamond knives are used to cut very thin sections for electron microscopy.

Figure: An Ultra-microtome

Source:http://upload.wikimedia.org/wikipedia/commons/thumb/4/48/Ultramicrotome_2265_EM_GD_MB.jpg/220px-Ultramicrotome_2265_EM_GD_MB.jpg

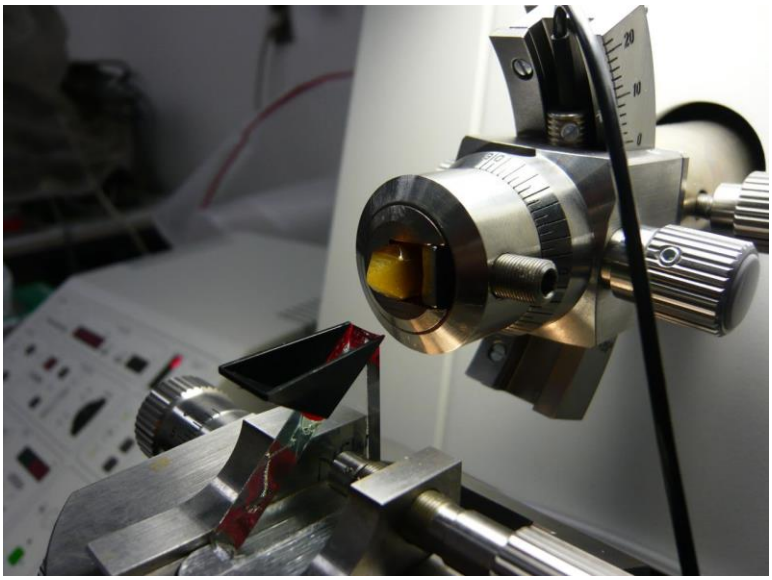


Figure: A close up of the ultramicrotome with the sample,boat and knife.

Source : http://electronmicroscopy.org/images/UCT_Up.jpg (CC-BY-SA)

Embed Interactive video: http://electronmicroscopy.org/Leica_Ultracut_UCTPartsTour.swf (CC-BY-SA)

In spite of the enhanced resolution made possible by use of electron microscope, it is not without its inherent limitations .An electron beam is too weak to pass an appreciable distance through air, so a high vacuum is needed inside the internal chamber of electron microscope. This lack of penetrating power also limits specimen thickness to a few hundred nanometers. Such restrictions create many technical problems in preparing biological material for observation.

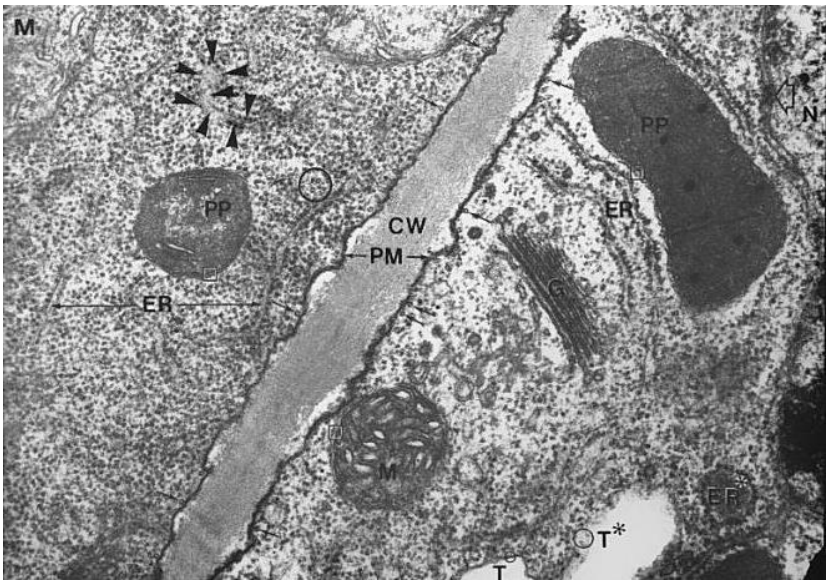


Figure: X 45, 000, EM of parts of two plant cells

Source: Author

Scanning electron microscope (SEM)

Figure: Scanning Electron Microscope

Source: http://electronmicroscopy.org/images/FE_SEM_Microscopesm.jpg (CC-BY-SA)

The Scanning Electron Microscope was invented by Manfred von Ardenne in 1937 .In Scanning electron microscope the image of the specimen is produced with a focused electron beam that is scanned across the area of the specimen. In SEM, a magnetic lens system focuses the beam of electron into an intense spot on the surface of specimen. The spot is moved back and forth across the specimen by charged plates called beam deflectors located between the condenser lens and the specimen. The beam deflectors attract or repel the beam according to signals sent by the deflector circuitory. As the electron beam sweeps rapidly over the specimen molecules in the specimen are excited to high energy level and emit secondary electrons which are then used to form an image of the specimen surface. Secondary electrons are captured by a detector located immediately above and to one side of the specimen. The essential component of the detector is the scintillator, which when excited by electrons incident upon it emit photons of light. These photons are used to generate an electronic signal onto the video screen. As the beam traverses the surface of the object electrons are deflected to varying degrees. The deflected and emitted electrons are detected by a Photomultiplier tube and used to form a 3-D image of the object's surface features.

The resolving power of the SEM is less than that of the TEM. However since the image formaion by SEM is dependent of surface properties it can magnify samples up to many centimeters and has a greater depth of field. It can thus produce good representative images of the three dimensional shape of the sample.

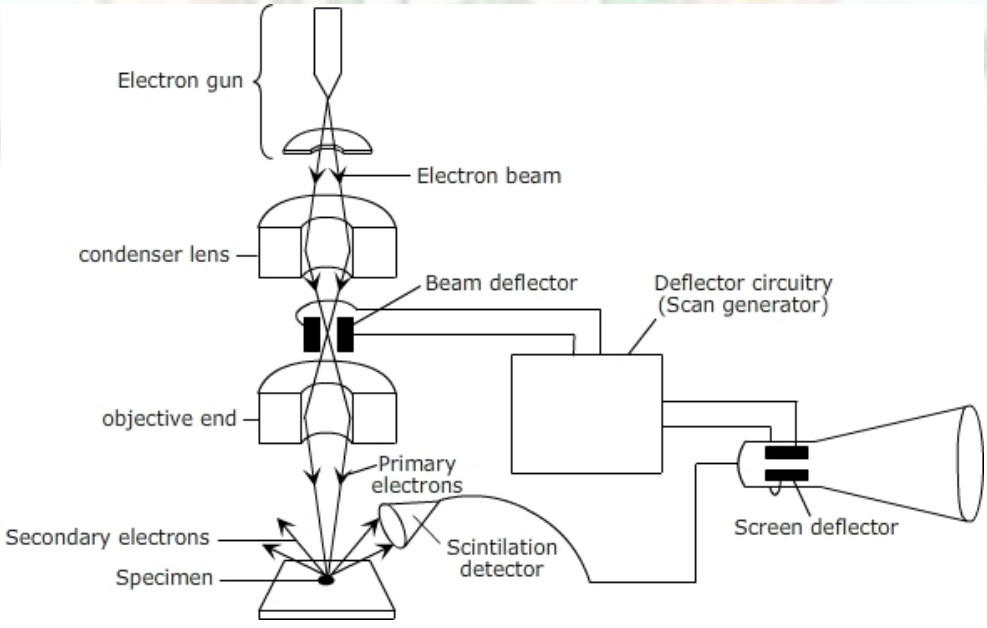


Figure: Schematic diagram of an SEM

Source: Author

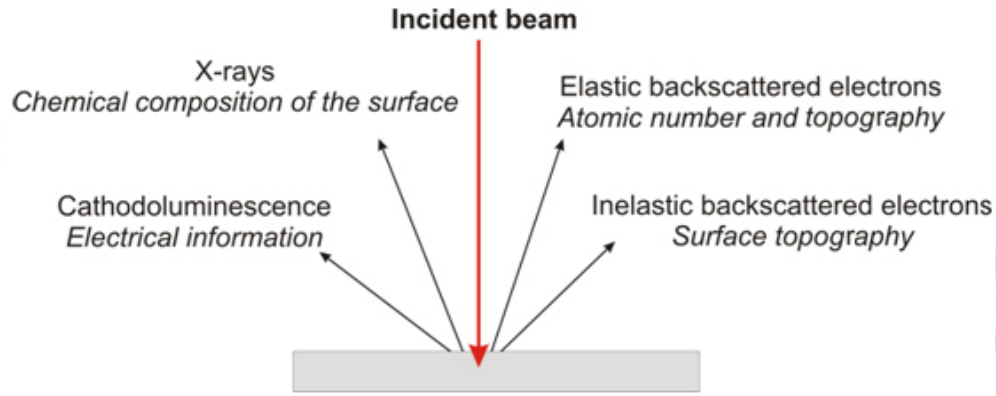


Figure: SEM allows us to observe the surface topography of the samples with resolution higher than the compound microscope. The diagram shows how the incident beam of electrons interact with sample in SEM analysis.

Source: http://cnx.org/content/m22326/latest/graphics6.jpg (CC-BY-SA)

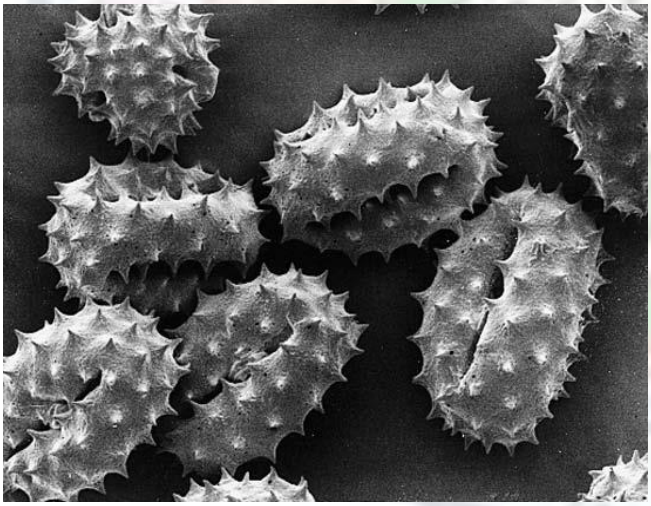


Figure:Magnified 1,500 times by a scanning electron microscope, pollen from the *Cineraria* plant has spiny bumps.

Source: http://media.web.britannica.com/eb-media/10/109410-004-B2D57355.jpg

Sample preparation

The material is primary fixed by Immersing in 2.8% glutaraldehyde in 0.1M Hepes buffer, pH 7.2 (with 0.02% Triton X-100), for several hours at room temperature or overnight at 4°C.

The material is then washed thrice (each 5 to 10 minute duration) in 0.1 M Hepes buffer, pH 7.2. Dehydration is done for 10 min. in 25% ethanol, 10 min. in 50% ethanol, 10 min. in 70% ethanol, 10 min. in 85% ethanol, and 10 min. in 95% ethanol, 2 x 10 min. in 100% ethanol, and 10 min. in 100% ethanol (EM grade). This is followed by Critical Point Drying which is an automated process and takes approximately 40 minutes to complete. The sample is then mounted onto metal stub with double-sided carbon tape. Finally a Sputter Coating is done by apply a thin layer of metals (gold and palladium) over the sample using an automated sputter coater.

Scanning Transmission Electron Microscope (STEM)

Figure: Scanning transmission electron microscopy
Source:http://ncem.lbl.gov/images/CM300.jpg

STEM contains elements of both TEM and SEM. Like SEM, it uses an electron beam that sweeps over the specimen. The image is formed by the electrons transmitted through the specimen as with a TEM. A STEM is capable of distinguishing specific characteristics of the electron that are transmitted by the specimen, thus deriving information about the specimen not obtainable with

the conventional TEM. However a STEM is technically sophisticated and requires a very high vacuum and is much more electronically complex than a TEM or a SEM

Environmental Scanning Electron Microscope (ESEM)

Figure: Image of an ESEM facility

Source: http://www.technicalsalessolutions.com/Instruments/SEM/ESEM.jpg

Environmental Scanning Electron Microscope (ESEM) is a specialized SEM , which makes possible to perform microanalysis on uncoated specimens. ESEM allows the specimen to be observed without freezing, coating, fixing, embedding.

Environmental SEM was first commercially developed in the late 1980s and allowed the samples to be observed in low-pressure gaseous and high relative humidity (up to 100 %) environments. The first commercial ESEM was produced by the Electro Scan Corporation in USA in 1988.

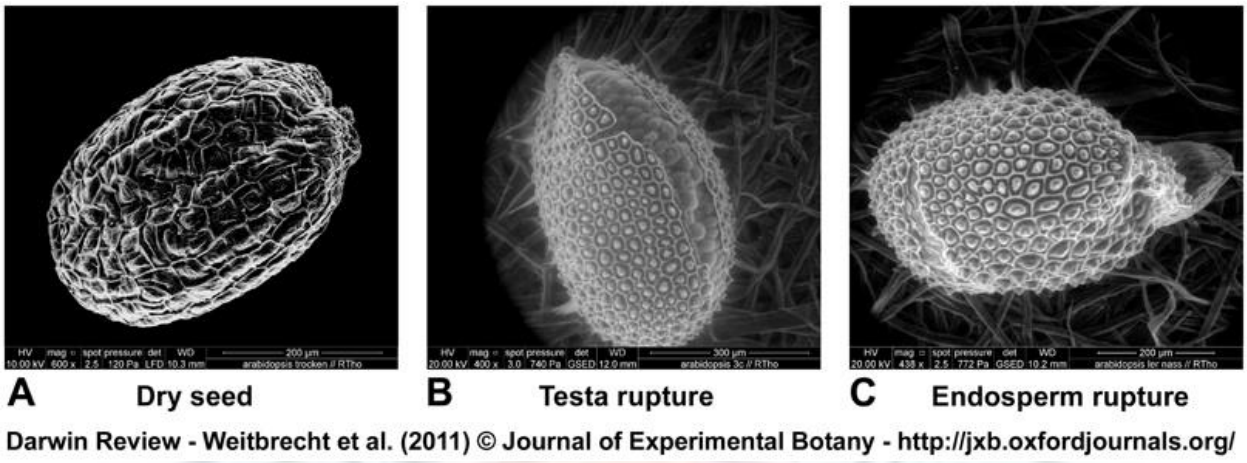


Figure: A. SEM image of dried *Arabidopsis* seed. **B-C.** An ESEM image of an imbibed germinating seed. ESEM allows the specimen to be observed without freezing, coating, fixing, embedding.

Source: http://www.seedbiology.eu/html4images/weitbrecht-fig4.jpg

Non -conducting specimens may also be imaged without any coating using ESEM. It can also produce quality images and resolution with the samples being wet or contained in low vacuum or gas. Hence, it greatly facilitates imaging of those biological samples that are unstable in the high vacuum of conventional electron microscopes. This is made possible due to development of a secondary-electron detector, which is capable of operating in the presence of water vapour and also by the use of pressure-limiting apertures to separate the vacuum region (around the gun and lenses) from the sample chamber. In Environmental SEM the specimen is placed in a relatively high-pressure chamber where the working distance is short and the electron optical column is differentially pumped to keep vacuum adequately low at the electron gun.

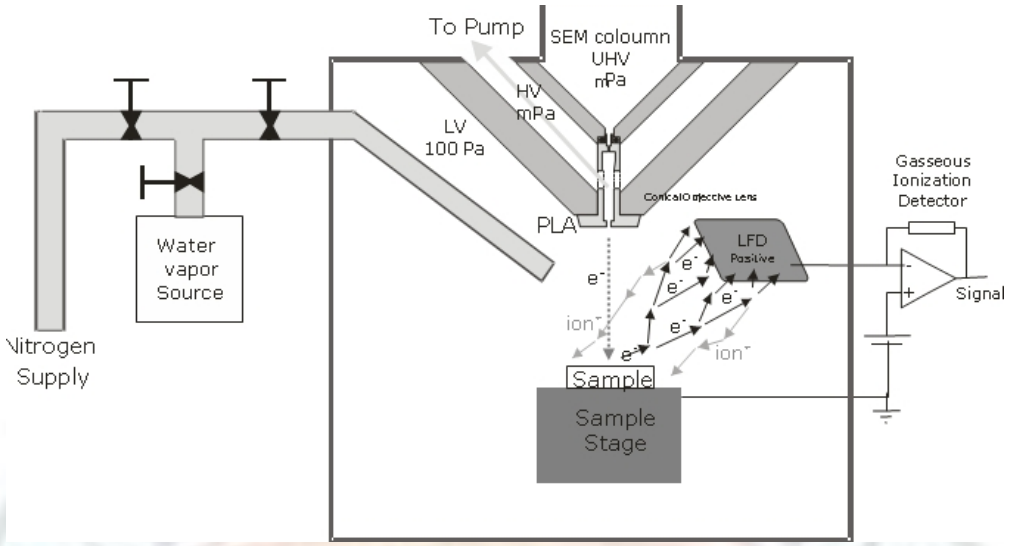


Figure: Environmental SEM canning Electron Microscope (ESEM), where a differential pumping system with two pressure limiting apertures between the ultra high vacuum SEM column and the low vacuum sample chamber allows high pressures up to 10 hPa around the sample. This is enough to have liquid water at moderate cooling of 5^0C.

Source:http://en.wikipedia.org/wiki/File:ESEMsystem.jpg, http://en.wikibooks.org/wiki/nanotechnology

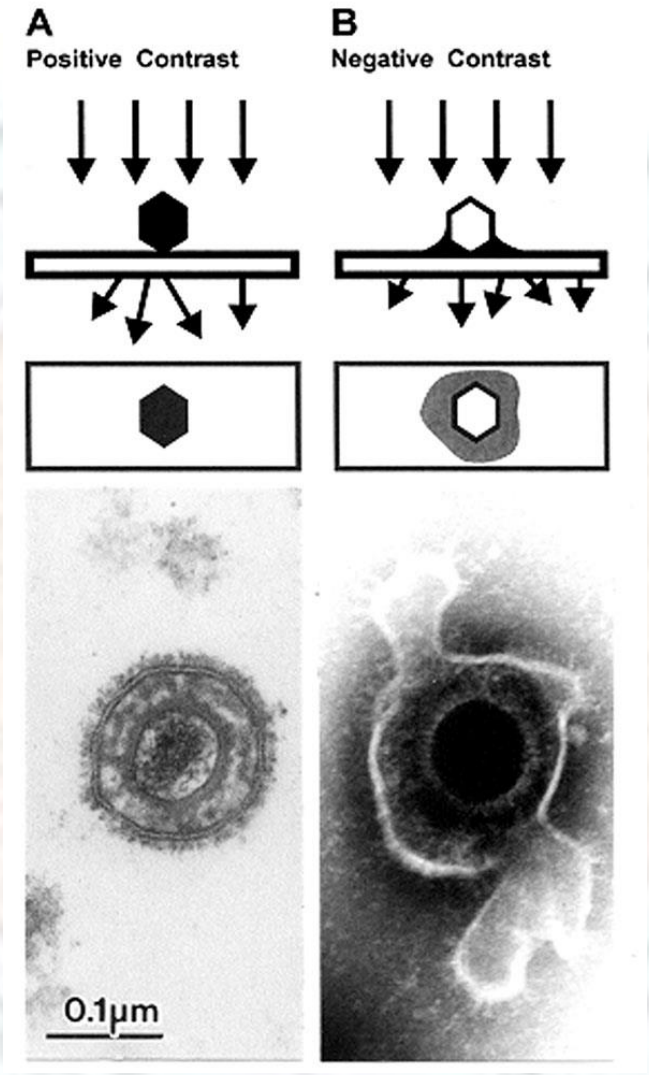
The main preparation techniques are not required in the environmental SEM but some biological specimens can benefit from fixation.Embedding in a resin with further polishing can be used for specimens when imaging in backscattered electrons or when doing quantitative X-ray microanalysis. ESEM is especially useful for non-metallic and biological materials because coating with carbon or gold is unnecessary. .

Techniques for preparing tissues for electron microscopy other than sectioning

Negative Staining

In contrast to thin sectioning, negative staining method is the easiest technique used in TEM for examining very small objects. The shape and surface appearance of small particles such as intact organelles or viruses can be examined without cutting these into thin sections. In the negative staining technique, such particles are suspended in a small drop of liquid applied to

copper grid and allowed to dry in air. After drying, a drop of stain such as phosphotungstic acid or uranyl acetate is applied to the surface. When viewed in TEM, specimen is visualized against the stained dark background. In the closely related positive staining technique, a specimen is first reacted with the stain and the stain then is removed, producing a stained sample visible.



POSITIVE STAINING NEGATIVE STAINING

Figure: "Comparison of a herpesvirus appearance after positive and negative staining. Comparison of herpesvirus appearance after positive and negative stain electron microscopic. A. Positive staining. Samples undergo a lengthy process of fixation, incubation with heavy metal ions (osmium, uranyl), dehydration, embedment, ultrathin sectioning, and staining. Chemical moieties in the object show differential affinities for the heavy metal stains, resulting in a clear outline of the viral bilayer envelope, viral envelope proteins, nucleocapsid, and the dense nucleic

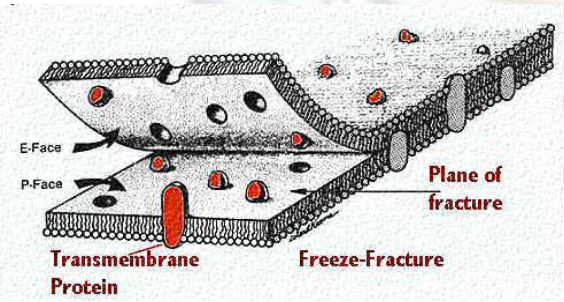
acid containing core. B. Negative staining. After a brief fixation, samples are mounted directly on electron microscopic grids and stained. The electron-dense stain (phosphotungstic acid [phosphotungstic acid], uranyl acetate, and the like) penetrates the virion and embeds the particle in a matrix of stain. Due to density differences between the stain and weakly scattering biological components of the virion, the virion appears as a transparent and detailed reverse (negative) image. Penetration of stain into the nucleocapsid provides a dense core with the crenellated appearance presented by the central channel of capsomeres on the nucleocapsid surface. Viral surface proteins appear as projections from the labile envelope. phosphotungstic acid". Hazelton, P.R. and Gelderblom, H.R. 2003. Electron microscopy for rapid diagnosis of emerging infectious agents. EID Vol 9 (3).

Source: http://wwwnc.cdc.gov/eid/images/02-0327-F5.jpg

FREEZE-FRACTURE TECHNIQUE

The freeze-fracture technique consists of physically breaking apart (fracturing) a frozen biological sample along the planes of natural weakness that run through each cell.These planes occur generally between the two layers of lipid molecules which forms part of limiting membrane around various organelles of the cell. A freeze fracture replica is then made by vaccum deposition of platinum and carbon.

The main steps in making a freeze fracture replica are (i) Pre treatment with glutarladehyde and glycerol for cryoprotection (to reduce ice crystal formation and resulting damage) (ii) rapid freezing, (iii) fracturing, (iv) formation of replica, and (iv) replica cleaning. Images provided by freeze -fracture and other related techniques have profoundly shaped our understanding of the functional morphology of the cell. This technique is used to study membranes and reveal the pattern of integral membrane proteins.



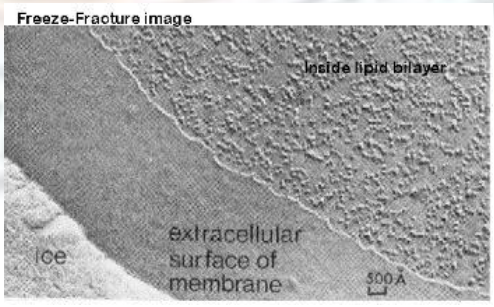


Figure: The membrane fractures along the plane that is the weakest i.e. in between the leaflets of the lipid bilayer revealing the protein distribution. The electron micrograph on the right shows the membrane structure in detail.

Source:http://www.cytochemistry.net/cell-biology/membr9.jpg, http://www.cytochemistry.net/cell-biology/ffimage.jpg

General outline of technique:

1. Cells are quickly frozen in liquid Freon (-130 C), which immobilizes cell components instantly.
2. Block of frozen cells is fractured in an evacuated chamber with a microtome and a steel knife at about -100c. This fracture is irregular and occurs along lines of weakness like the plasma membrane or surfaces of organelles.
3. A replica of the fractured specimen is made by shadowing with platinum.
4. A thin layer of carbon is evaporated vertically onto the surface to produce a carbon replica.5.
5. Organic material is digested away by acid, leaving a replica.
6. The replica can be studied under EM.

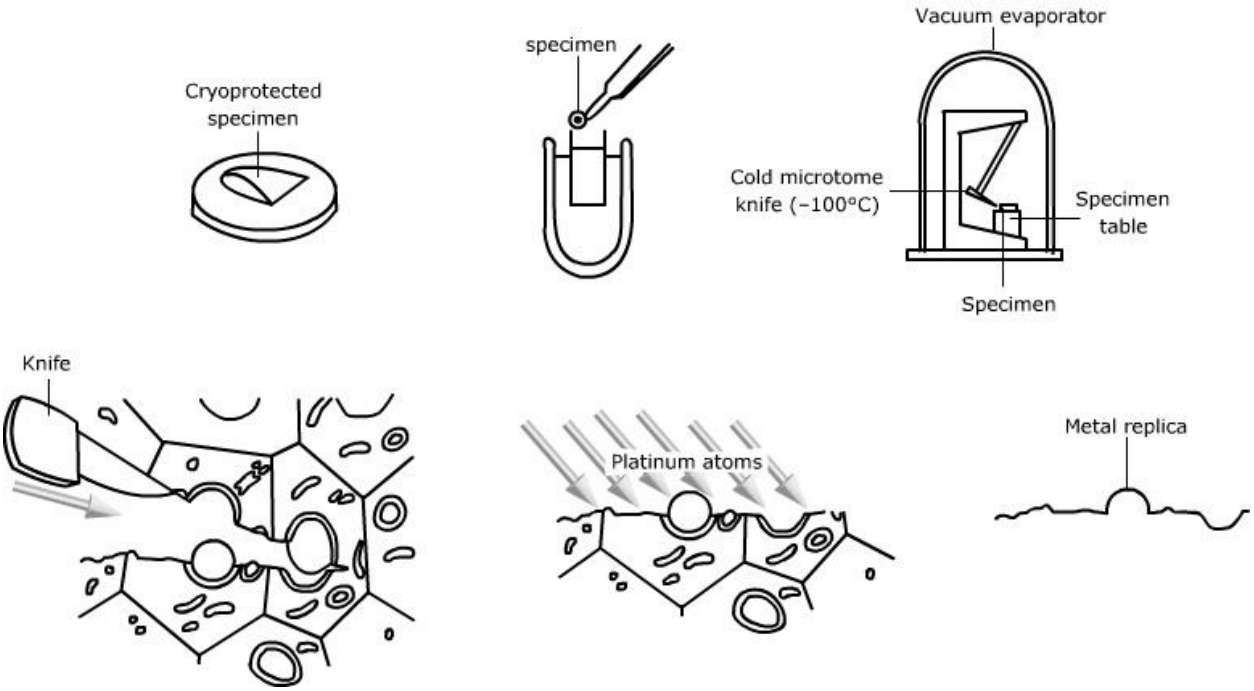


Figure: The technique of freeze fracturing

Source: Author

Freeze- Etching Technique

The freeze-etching technique of sample preparation is related to freeze fracture, but it adds a further step to freeze -fracture procedure, which makes it more informative. Instead of employing fixatives to preserve cell structure, specimens are rapidly frozen in liquid Freon, placed in a vacuum and struck with a sharp knife edge as in freeze fracture. At this temperature biological samples are too hard to be cut and instead fracture along lines of natural weakness. These weak areas are generally associated with biological members. Brief exposure of the broken tissue to vacuum, results in sublimation of water from the fractured surfaces. This removal of water produces an "etching" effect. This etching will cause small areas of the true cell surface around the periphery of the fracture face to stand out against the background. A replica of the freeze-etched specimen is made by heavy metal such as platinum, and then backing it with a carbon film. After dissolving the tissue in strong acid, the remaining metal replica can be viewed with the electron microscope. Such preparations provide a unique picture of cells, particularly where members are studied. Freeze –etching is specifically useful because it avoids exposure to fixatives, embedding agents, and stains, all of which may deform cell ultrastructure. Unlike such treatments, rapid freezing causes minimal tissue distortion and permits immediate arrest of cell function.

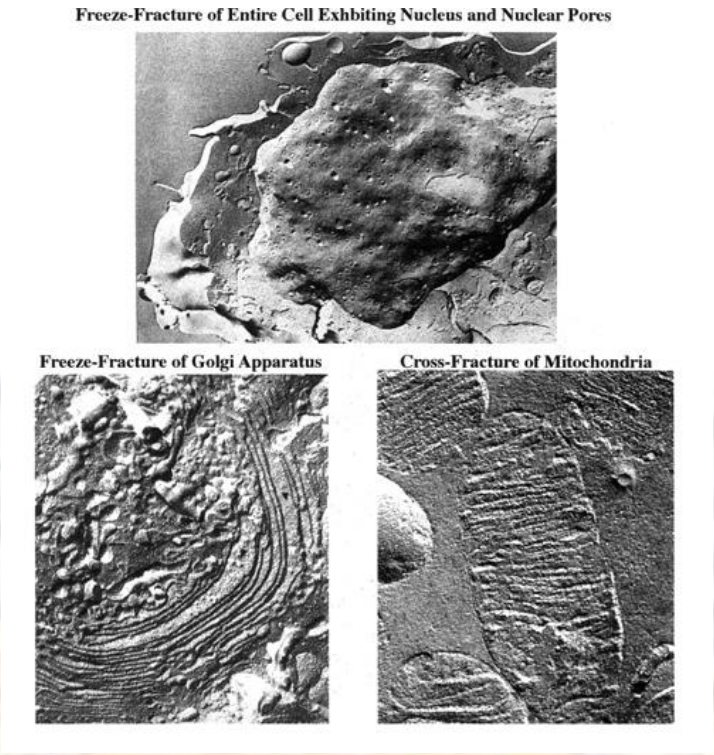


Figure: Freeze fracture views of few cell organelles

Source: Author

Shadow casting

Shadow-casting is a technique which shows the surface texture of microscopic material rather than the routine transparent appearances. Sections or smears may be studied throughout the whole range of microscopic magnification. The method involves the in vacuum deposition of a metallic film on dried specimens. Metal is deposited from an oblique angle so that it coats some surfaces of specimen more than others. This leaves the area to the "leeward" side of the specimen uncoated producing a "shadow" of the specimen.

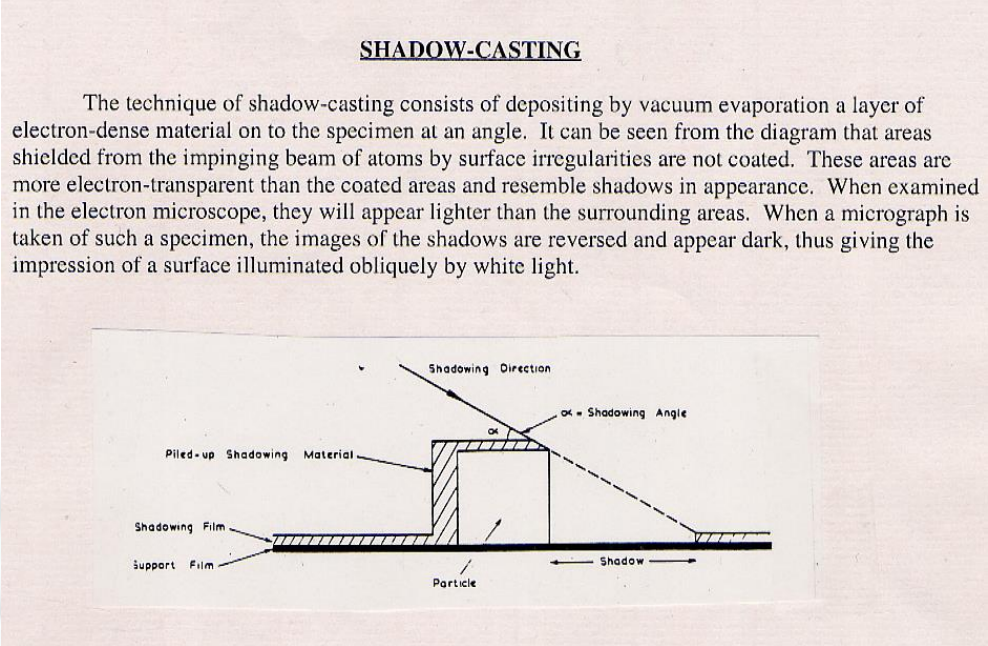
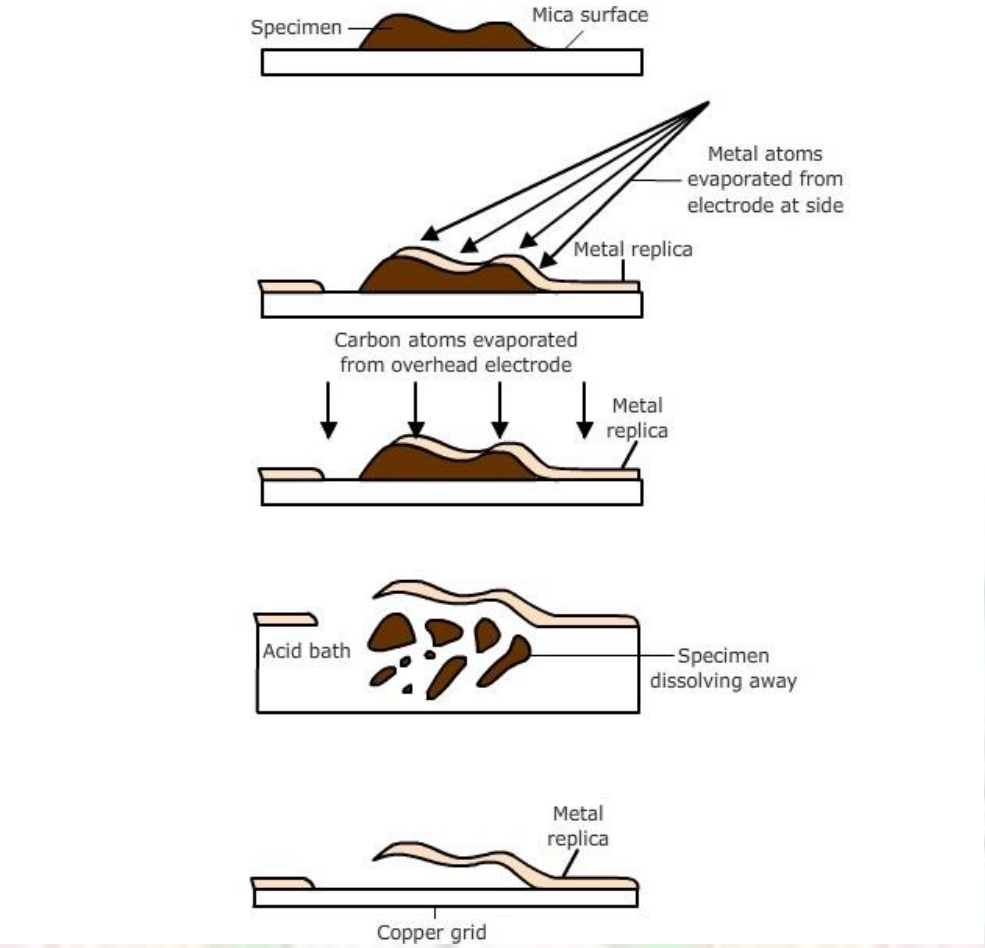
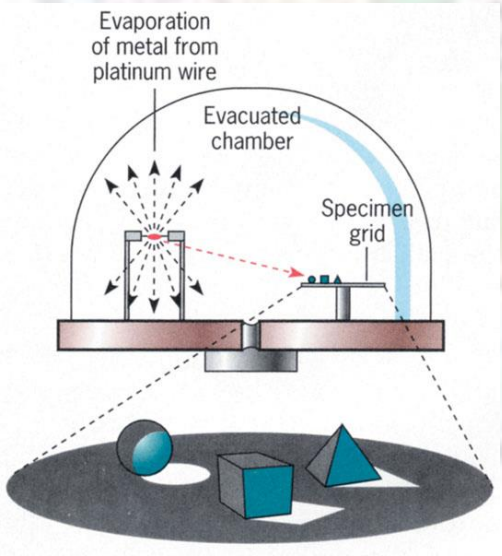


Figure: The principle behind shadow casting

Source: http://www.bgsu.edu/departments/biology/facilities/MnM/TEM/shadowcasting.jpg

The thin metal film is obviously formed on the specimen by condensation after vaporization. It is therefore assumed that the metals with the higher vaporization temperature will condense more quickly after vaporization, and form finer particle sizes. Also, the concurrent evaporation of two or more elements will result in smaller aggregate size by increasing the distance over which any atom must diffuse in order to secure its place within a crystal lattice. The particle size of a film of evaporated gold will therefore be larger than that of evaporated platinum or that of a 60/40 alloy of gold/palladium. The "grain" size of evaporated tungsten is exceedingly fine, but deposition time is very long and temperature is extremely high. Isolated particles can also be visualized by placing them in an evacuated chamber and spraying heavy metal across their surfaces. The shadow-casting process causes metal to be deposited on one side of the specimen, creating a "shadow" and a resulting three-dimensional appearance.





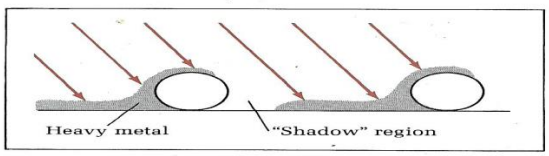


Figure: Technique of shadow casting

Source: http://yxsj.baiduyy.com/whole/image/chapter18/18.15.jpg

Source: Author

Summary

Cell biology is an experimental science which is based on the execution and interpretation of experiments designed to provide information about cell structure and function. Our current understanding of the relationship between cell structure and function has been made possible by a combination of microscopic and biochemical techniques. The light microscope was historically helpful in the discovery of cell and the resolution of about 200 nm severely restricts its usefulness for studying the details of cell architecture. By changing the source of illumination from light to electrons, resolving power was enhanced by several orders of magnitude from 200 nm to about 0.5 nm. The invention of the transmission electron microscope therefore revolutionized our view of cell architecture. Diverse set of procedures for specimen preparation, such as thin sectioning, negative staining, positive staining, shadow casting, whole mounting, and freeze-fracture, has opened our eyes to the existence of an exquisite subcellular architecture and the more recent development of the scanning electron microscope has provided the three dimensional view of the cell surface.

Exercises

1. Define magnification and resolving power of microscope. Discuss the factors which determine the resolving power of a microscope.
2. Describe the different parts of an electron microscope and compare it with a light microscope. Discuss different types of electron microscopes.
3. Discuss the differences between TEM & SEM
4. Write short notes on :

(1) ESEM (ii) STEM (iii) Negative Staining

(iv) Freeze –fracture technique (v)Shadow Casting

References

1) Becker .W.M; Kleinsmith ,L J;.Hardin ,J. andBertoni,G.P.2009.The World of the Cell.7th edition .Pearson Benjamin Cummings Publishing ,San Francisco.

2) Cooper ,G.M .and Hausman,R.E.2009.The Cell: A Molecular Approach.5th edition. ASM Press and Sunderland, Washington,D.C.;Sinauer Associate, MA.

3) De Robertis ,E.D.P. and De Robertis,E.M.F.2006. Cell and Molecular Biology.8th edition .Lippincott Williams and Wiklins, Philadelphia.

4) Sheelar ,P. and Bianchi,D.E.2009. .Cell and Molecular Biology (3rd edition). John Wiley & Sons Inc.UK.

Glossary

. Angstron (A°)- A unit of length usually used to describe molecular dimensions equal to10^{-8}cm

. **Electron Microscope**- A microscope in which a focused beam of electrons is used to produce an enlarged image of the object.

. **Freeze-fracture**- A technique for preparing material for EM by rapid freezing and fracturing of the tissues ;the exposed faces are used to create a replica which is observed and photographed in EM.

. **Micron(micrometer, µm)-** A unit of length used to describe cellular dimensions; it is equal to 10^{-4}cm or 10^{4} A°.

. **SEM**- An electron microscope that permits observation of a specimen's surface structure .The electron beam is not transmitted through the specimen but causes the release of secondary electrons from the surface of the specimen which forms the image.

. **TEM**- An electron microscope in which electron beam is transmitted through the specimen and forms the image on fluorescent screen at the bottom of the microscope.

Web Links

1. http://electronmicroscopy.org/UCT_OP.htm
2. http://electronmicroscopy.org/FE_Form_Function.htm
3. http://wwwnc.cdc.gov/eid/article/9/3/02-0327_article.htm
4. http://www.umassmed.edu/cemf/whatisem.aspx
5. http://www.bgsu.edu/departments/biology/facilities/MnM/protocols.html