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The Electron Microscopes: Concise History and Review

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Abstract

Since its invention, electron microscope (EM) has been a valuable tool in the development of scientific theory and it contributed greatly to biology, medicine and material sciences. This wide spread use of EMs is based on the fact that they permit the observation and characterization of materials on a nanometer (nm) to micrometer (µm) scale. In this review article, the transmission electron microscope (TEM) and the scanning electron microscope (SEM) were defined and reviewed. The EMs functions and types were discussed, in addition to clarifying the parts and components of TEM, SEM and optical microscopes for neophyte, starting from the sample's preparation through imaging of the samples. Also, this review will point out the limitations and advantages of each type and issues to be considered during experimental design. Advanced EM techniques are listed as well.in this review, Diagrammatically, identify the various parts of a microscopes, what is the differences between TEM, SEM and optical microscopes and finally the methods of samples preparation have been mentioned.

Keywords Electron microscopy, SEM, TEM, sectioning, staining

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List of abbreviations: EM = Electron microscope, FS = Freeze substitution, HPF = High pressure freezing, LM = Light microscope, STEM = Scanning electron microscope, TEM = Transmission electron microscope

Introduction

Electron microscopies (EMs) are an instruments for gaining high resolution images of biological specimens and non-biological specimens. It is utilized in biomedical field to examine the structure of tissues, cells, any organized or specialized structures within a living cell and molecules as shown in figure (1), which shows mast cell by EM. At 19th century, physicists realized that to upgrade the light microscope (LM) using shorter wavelengths. Thompson in 1897 found out that the electron has wave-like properties [(1)]. In 1924, de Broglie showed that a beam of electrons moving in a vacuum behaves as a very short wavelength radiation, but Ruska had been used these properties of electrons to build the first EM [(2)]. Improvements are made in forty years within the part of oral biology. The first potential within the development of transmission electron microscopies (TEM) is linked with the next names: Brüche and Johannson (1932) [(3)], Knoll and Ruska (1932) [(4)], Glaser (1933) [(5)], von Borries and Ruska (1938) [(6)]. Significant progression is done, within the technology which come near or nearer to a resolution power of 1 A, and also within the preparation techniques.

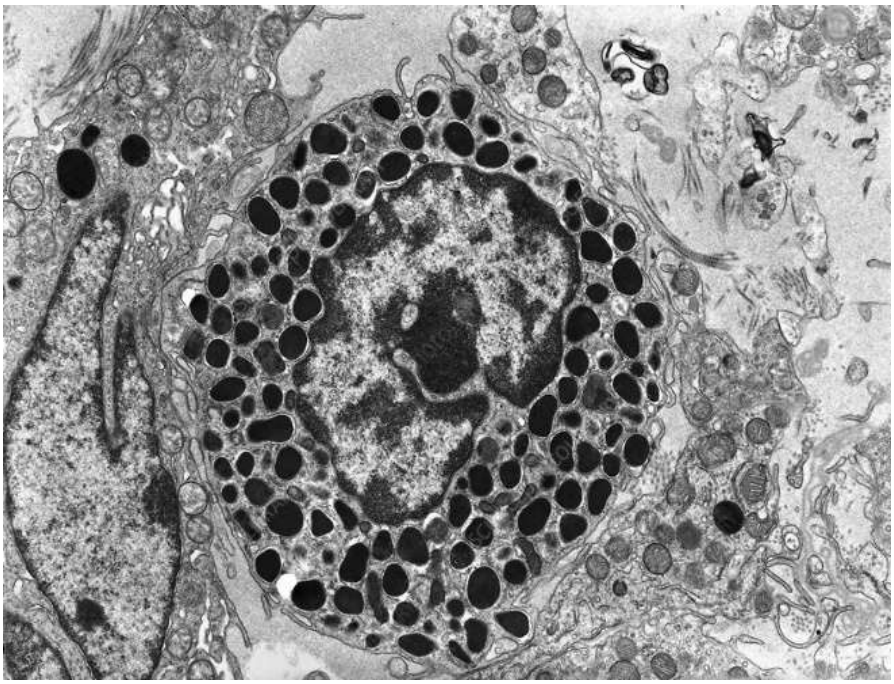


Figure 1. Mast cell (TEM) [7]

We could compare two sorts of microscopes light (optical) and EM. The optical microscopes (Figure 2) use beam of light while EMs use electron beams to magnify objects details, which can't be seen by an unaided eye clearly. By wave-like characteristic of electrons, the EMs can magnify an object's image, unlike the LMs that use light beam to magnify images. Checking up virus by EM relies on the detection and identification of virus on the basis of their characteristic morphology. Earlier attempts to visualize viruses with even the foremost powerful optical microscopes of the day was largely unsuccessful. This was so because visible light with a mean wavelength of about 5500 A° were unable to inspect the finer and detailed aspects of virus particles, which are comparatively smaller in size. The light wavelengths are relatively long. Therefore, particles having smaller size can't be properly resolved. This problem was solved with the event of EM by Ruska and Knoll in 1931 [8]. These instruments don't use electromagnetic waves with longer wavelengths. Instead, strong electron beams are projected from a source to resolve the thing under observation. The wavelengths of such electron beams were very small, often but 1A°. On the opposite hand, the space between different atoms during a molecule is more than that. Therefore, it is theoretically possible to get resolutions at the atomic level with the assistance of those. Once resolution at such a fine degree is obtained, it is possible to enlarged and magnified images to the specified extent. We can achieve a magnification of up to 2,000,000X where-as from ordinary LM, it is up to 2000X. The parts of the EM are an electron gun, column, electro-magnetic lenses and a fluorescent screen as shown in figure (3). Electron gun is source of electrons. The gun consists of tungsten filament at 30 KV to 200 KV or more then that potential. It's surrounded by a negative shield with an aperture through which a beam is drawn off.

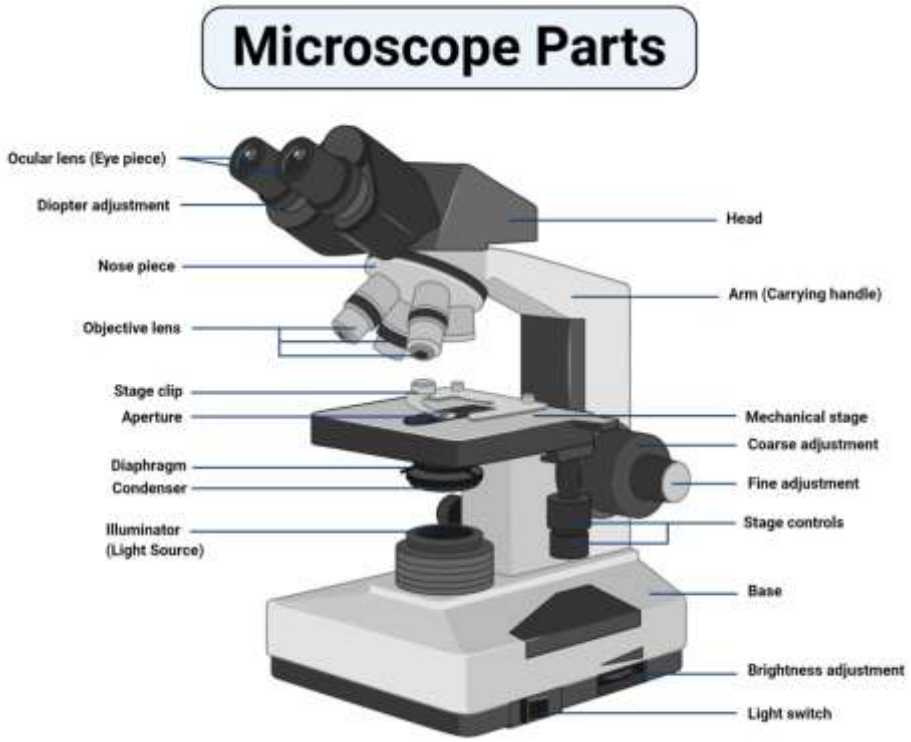


Figure 2. Parts of a microscope [9]

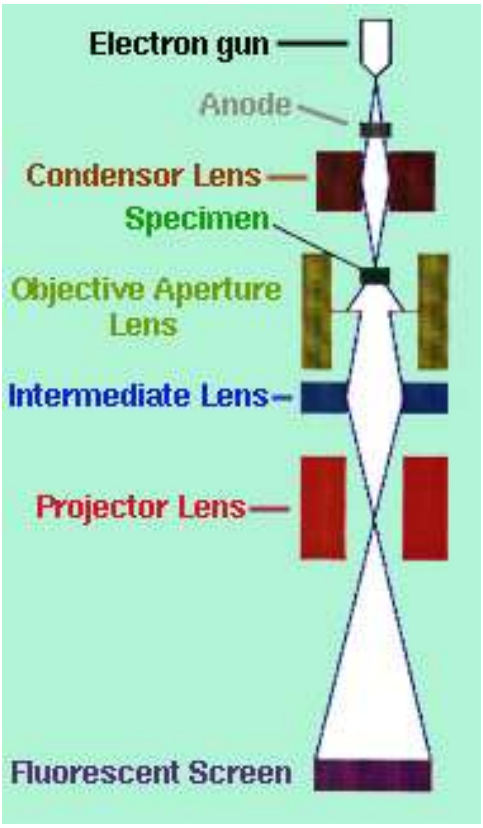


Figure 3. Parts of electron microscope [10]

We can brief the differences between optical microscope and EM by 35 differences as shown in table (1).

Table 1. The differences between optical microscope and electron microscope [11]

Character	Light microscope	Electron microscope
Famed as	Optical microscope	Electron beam microscope
Developed by	Dutch Zacharius Jansen and Hans were the first to invent the compound microscope in the 16th century	In 1931 physicist Ernst Ruska and German engineer Max Knoll
Source	By light (wavelength 400-700 nm) to illuminate the objects under study	By a beam of electrons (equivalent wavelength 1 nm)
Precept	The images created by absorption of light waves	The image created by scattering or transmission of electrons
Size	Smaller and lighter	Heavier and larger
Lenses	Glass lenses	Electromagnets lenses
Vacuum	No vacuum used	Need a high vacuum
Specimen	Fixed, unfixed, stained, unstained, living and non-living	Fixed, stained and non-living
Specimen	Both live and dead specimens can be examined.	Only dead specimens can be examined
Specimen preparation	Less tiresome and simple	It involves hard processes, e.g. Using corrosive chemicals and skill required to prepare specimens
Time of preparation	Takes a few minutes to hours	Takes a few days
Specimen thickness	5 µm or thicker	Ultra-thin, 0.1 µm or below
Specimen dehydration	No need dehydrated before viewing	Used only dehydrated
Coating	Stained by colored dyes	Coated with heavy metals
Specimen	Mounted on the glass slide	Mounted on the metallic grid
Focusing adjustment	Done mechanically	Adjusting by electromagnetic lenses
Magnification limit	Low magnification of up to 2000X	High magnification of up to 1,000,000X
Resolving power	Low power of resolving, below 0.3 µm	The high resolving power about 0.001 µm
Viewing the image	Images are viewed directly. Images are viewed by the eyes through the eyepiece	Images are viewed on a photographic plate or zinc sulfate fluorescent screen
Nature of image	Poor surface view	Good surface view and internal details
Color of the image	Colored images	Grayscale or black and white
Dimension of the image	Plane (2D)	2D only in a transmission electron microscope, 3D images in scanning electron microscope
Living processes	Visualization of living processes and even cell division is possible.	Living processes cannot be viewed.
Room	No special settings required.	Used in a room where humidity, pressure, and temperature are controlled
Simplicity	Simple to use	Users must have technical skills
Voltage requirement	No high voltage is need	High voltage is needed (50,000 v or above)
Filaments	No filaments used	Tungsten filaments
Cooling system	No cooling or chiller system	Cooling system need to cool down the

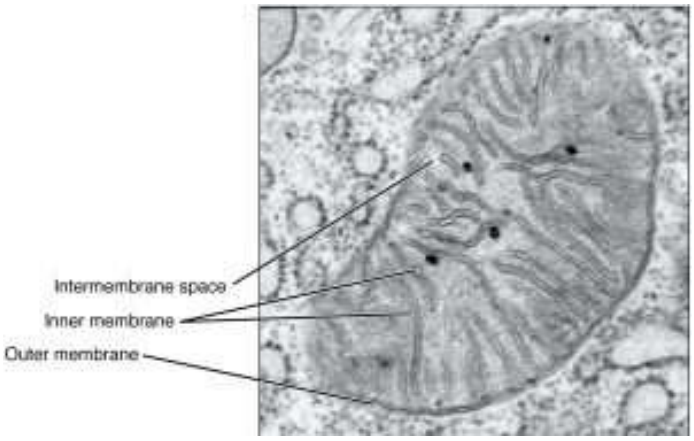
		heat generated due to high voltage electric current
Radiation risk	No risk	Risk of radiation leakage
Complexity	Less complex	Complex
Cost	Cheap and low maintenance costs	Very expensive as well as to maintain
Convenience	Suitable for schools and for learning institutions	Is for limited to specialized use such as research
Advantages	• Easy • Cheap • Real color and sometimes require staining • Live specimens	• More resolution • Give images of surface and interior structures • More magnification • 3d images
Disadvantages	• Low resolution (0.2 nm) • Low magnification • The specimen used is thin	• Expensive • Requires extensive training • Sample must be dead • Black and white
Types	• Dark-field microscope • phase-contrast microscope • Fluorescent microscope confocal microscope polarized microscope • Differential interference contrast microscope	• Transmission electron microscope (TEM) • Scanning electron microscope (SEM)

Types of EM

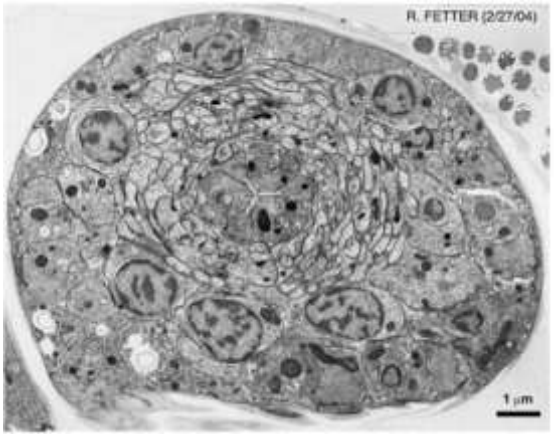
There are two kinds of EM [12]:

(a) Transmission Electron Microscope (TEM)

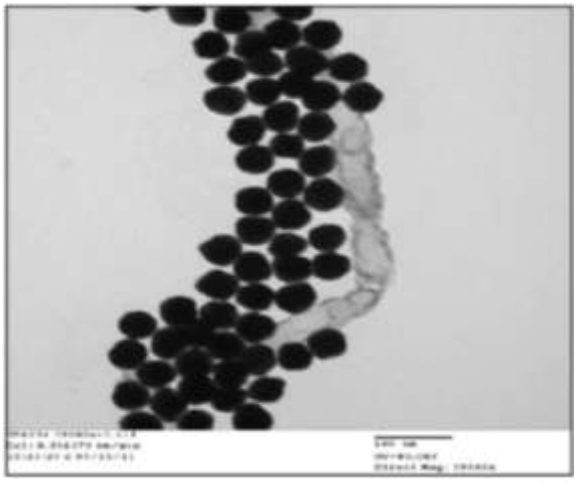
This EM is often compared with a LM [11]. It uses transmitted electrons which will penetrate the thin sample (usually no more than 100 nm thick). TEM is employed to look at very thin specimens (tissue, molecules, etc.) through which electrons can pass after the sample, the electrons hit a fluorescence screen that forms an image with the electrons that were transmitted. Figure (4) shows some images obtained in TEM. TEM is analogous to the conventional (compound) LM. TEM is used also to image the interior of cells.



This transmission electron micrograph shows a mitochondrion as viewed with an electron microscope [13]



TEM section through an embryo fixed using High pressure freezing (HPF) [14]

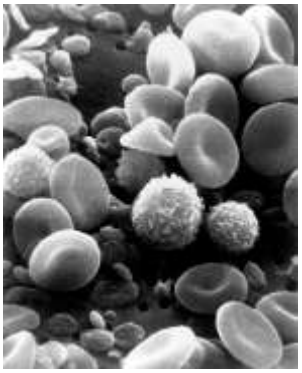


TEM image of 50 nm gold nanoparticles [15]

Figure 4. Transmission electron microscopy (TEM) images of some samples

(b) Scanning Electron Microscope (SEM)

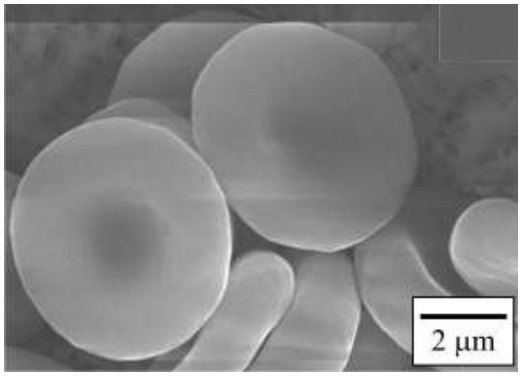
SEM utilize scattered electrons and secondary or back scattered from the surface of the sample, thus showing a three-dimensional image. SEM depends on the secondary electrons that emitted from the surface of a specimen. It gives detailed images of the surfaces of samples like cells and organisms that aren't possible by TEM as shown in figure (5). It is also use to count particles and measure particles size. It is named SEM because the image is made by scanning a focused beam of electrons onto the surface of the sample during a raster pattern. The interaction of this beam with the atoms near the surface causes emission of electrons at each point within the raster such as low energy secondary electrons, high energy back scattered electrons, X-rays and photons. These are often collected with a spread of detectors, and converted to brightness at each equivalent point on a cathode ray tube (CRT). Because the dimensions of the raster at the specimen is smaller than the viewing screen of the cathode ray tube, equipped SEMs with secondary, backscatter and X-ray detectors are often use to study the topography and composition of specimens. Figure (6) shows the SEM and figure (7) shows part of SEM.



A

SEM blood cells conventional pretreatment

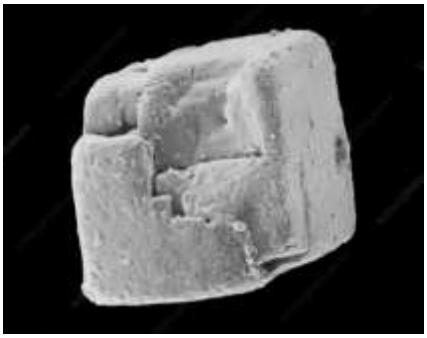
(16)



B

SEM images of RBCs

(16)



C

Table salt crystal (NaCl) [17]

Figure 5. A, B and C scanning electron microscopy images

A
Q250 Analytical SEM for materials science

B
SU3500 Scanning electron microscope

Figure 6. A and B models of scanning electron microscopes [18]

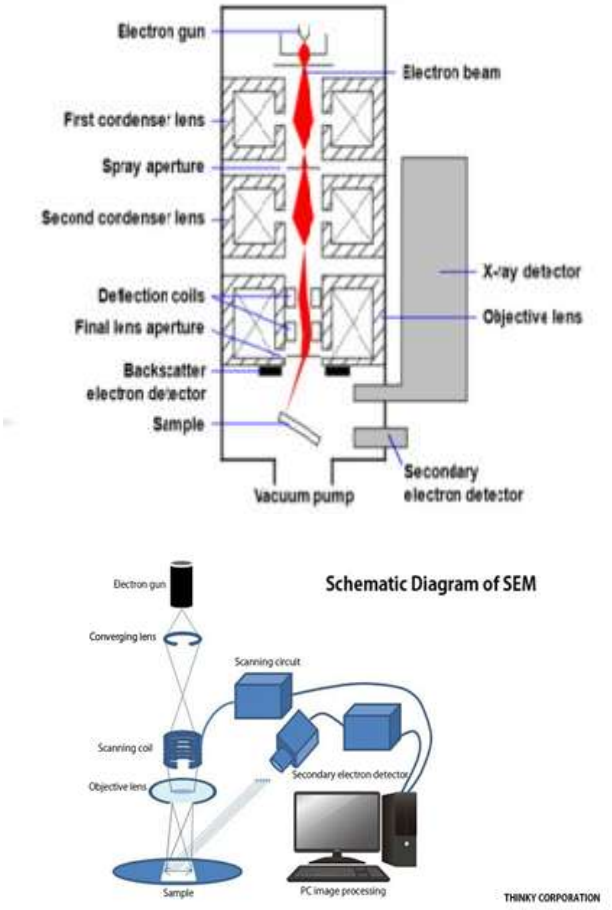


Figure 7. Schematic and parts of scanning electron microscope (SEM) [19]

Technique

The first plant virus to be observed under the microscope was tobacco mosaic (Williams and Wykoff, 1943) [20]. Since then, microscopy technology has improved considerably. Many techniques associated with EM have brought out reasonably clear pictures and these techniques are:

Ultrathin sectioning

This technique is useful in studying the particle within the host cell. It is also useful for studying the crystal structure. Thin sections (25-90 nm) are created from fixed, dehydrated and embedded biological materials, using special microtomes thermal or mechanical advance microtomes and glass or diamond knives with very sharp and hard cutting edges.

Negative Staining

Negative staining is a simple way for observe the structures of isolated organelles, individual macromolecules and viruses by Hall (1955) [21] was the first to explain the effectiveness of negative staining during a study in which panicles were being positively stained with phosphotungstic acid. Instead of looking like dark on a light background, they would be seen light on a dark background. Huxley (1956) [22] also observed a same effect with tobacco mosaic Virus. Brenner and Horne (1959) [23] noticed an equivalent event and named its negative staining. Some negative stains with normal pH are:

Sodium or Phosphotungstate (PTA) 5 to 8
Uranyl acetate 4.2 to 4.5.
Ammonium molybdate 5 to 7
Methylamine tungstate 5 to 7

The significance of negative staining is to surround or embed the biological samples within electron dense material which gives high contrast.

Positive staining

In positive staining method, significant metal salts attach to the various organ or macromolecules inside the sections to rise their electron density to be dark against a lighter background. Some positive stains are: chemical group acetate (UA), Reynold's lead citrate. Uranyl ions react with phosphate and amino groups, in order that the nucleic acids and certain proteins are extremely stained [24].

Comparison between negative and positive staining

Negative staining may be a technique utilized in preparing specimens for microscopical examination. The sample is mixed with an electron dense material that penetrates the interstices of the sample but not the material of the sample itself. The specimen then appears transparent against an opaque background. The positive stain-stick with specimen and provides its color where-as negative stain doesn't mix with the specimen but settle around its outer boundary and forming a silhouette (outline). The negative stain produces a dark background round the cell.

Freeze drying

To pull out the moisture (e.g., from food) by freezing firstly then subjecting to a high vacuum used as a way for drying foods and chemicals while causing little decomposition is named freezing drying or process of drying food or blood plasma or pharmaceuticals or tissues without damage their physical structures, material is frozen and then after that warmed in a vacuum in order that ice sublimes for biochemical the term lyophilized is usually used. Freeze drying technique helps in getting an accurate idea about the shape of the particles [25].

Method

The freezing dying technique has been described intimately by Williams (1952) [26]. This technique includes the rapid freezing of the specimen and thus the layer of water covering it on the grid to which it had adsorbed followed by sublimation of the ice around it. A thin layer of heavily metal is then precipitate on the dehydrated surface to provide contrast.

Carbon replica

Replication is a means of depicting the topography of an object, such as a tissue surface. Even before the introduction of the SEM replication techniques had been used for light and TEM [27,28]. Preparation of carbon replica almost like plaster of Paris molds are prepared in many cases to bring out the surface characteristics of virus particles.

4- Samples preparation

Commonly, cells are fixed with chemicals using glutaraldehyde, followed by (Osmium) Os tetroxide. Glutaraldehyde primarily cross-links proteins. Os tetroxide reacts powerfully with membrane lipids and collectively with proteins. As a result of this methodology and due to the diffusion of the fixative into the cell, slow infiltration of the fixative and extraction of cellular contents. Both of these problems will cause fixation artifacts, like malformed cellular membranes or organelles, and to loss of material, making the cell appear less dense than it is really. A premium resource for protocols and procedures for EMs sample preparation are generally found among the sensible ways (practical method) for microscopy series (e.g., Glauert 1975) [29]. When fixation with liquid fixatives, samples dehydrated in increasing concentrations of a solvent. General solvents used are acetone or ethanol, usually followed by propene oxide. There are wondrous reviews on the principles, practice, and utility of high-pressure freezing sample preparation (Moor, 1987) [30] and (McDonald et al., 2007) [31]. Most freeze substitution (FS) protocols include a progressive warming to temperatures that let fixation chemistry to occur at a reasonable rate. Embedding is that the strategy of

infiltrating the specimen with resins that is ready to be polymerized into a tough plastic applicable for thin sectioning. A variety of embedding resins can be offered. Epoxy resins such as Epon are the best to section and permit for wonderful post staining. Epoxy resins are typically polymerized at 60-70°C and don't seem to be contributing to immune labeling. Methacrylate resins similar to the Lowicryls can infiltrate into dehydrated specimens and be polymerized at temperature by ultraviolet light. Combined with HPF and FS, embedding in these resins retains antigenicity, making HPF/FS samples acceptable for post-embedding immunolabeling. In any event, embedding and curing in any resin have to be compelled to yield a tough block where the sample within it.

4-1 Sectioning and staining

To examine the sample among the EM, thin sections (~60–80 nm) ought to be cut from the block [32]. The face of the block ought to be cut with an instrument with a sharp blade or combination of blades or glass microtome knife to a neat trapezoid, usually <1 mm on an aspect. Cutting tiny sections allow the loading of dozens of serial sections on an EM specimen support known as a grid as shown in figure (8). Once cut, the block is mounted in associate ultramicrotome - a specialized machine that cuts sections by slowly advancing the block face by very little, specifically controlled increments over a diamond or glass knife edge to supply sections of a given thickness chosen by the operator. Sections are so small and fragile to be directly manipulated with forceps or different tools. Sections float off the ultramicrotome knife edge onto a little, water-filled reservoir built into the knife, and so the sections ought to be strictly transferred onto metal grids. Grids go along with varied mesh patterns or open slots through that the sections are generally imaged. To hold up the sections over these holes, grids square measure coated with a thin plastic (Formvar is customary) that is in a position to be strengthened with carbon coating. The final step in sample preparation before imaging is post sectioning staining, |this is usually often finished uranylacetate, followed by lead citrate to strengthen contrast, and is well done by floating the grids, sections side down, on droplets of stain, followed by distilled water rinses type of stained structures in varied cells and samples, followed by checking for background with secondary antibody–only controls. An example of this approach square measure found in Rout et al. (2000) [33].

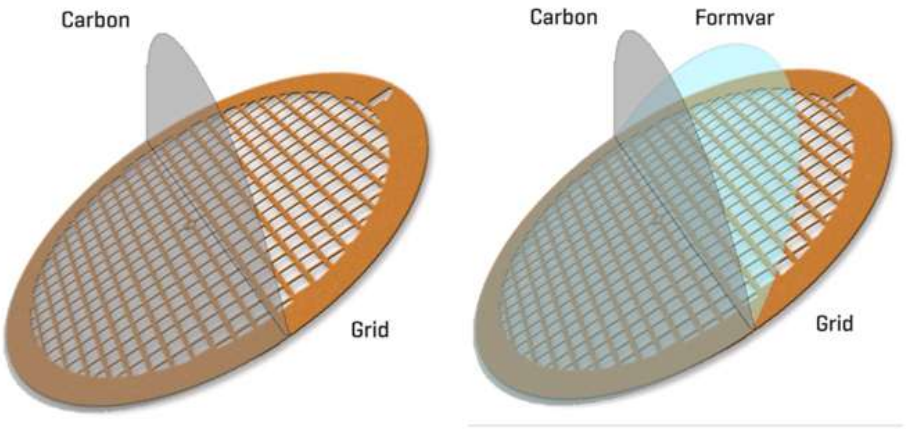


Figure 8. Carbon film supported copper grid and formvar/Carbon supported copper grids [34]

5- Imaging

The currently stained sections on grids are prepared for examination within the EM. By using a holder, a grid is inserted into the column of the microscope where the grid is in the beam. Modern EM have a PC interface creating it easy to search out and operate the instruments. Digital cameras also are used for locating areas of interest, focusing, and correcting astigmatism besides record the images and have almost universally replaced film. Figure (9) shows samples of the standard of cell structure as viewed among the TEM from samples prepared by typical chemical fixation, similar to the elaborate cytoskeletal arrays in cultured myocytes (Figure 9A) to the detailed ultrastructure of cellular organelles (Figure 9B). Wonderful ultrastructural preservation, notably of membrane organelles similar to the Golgi, are typically obtained using HPF/FS samples (Figure 9C).

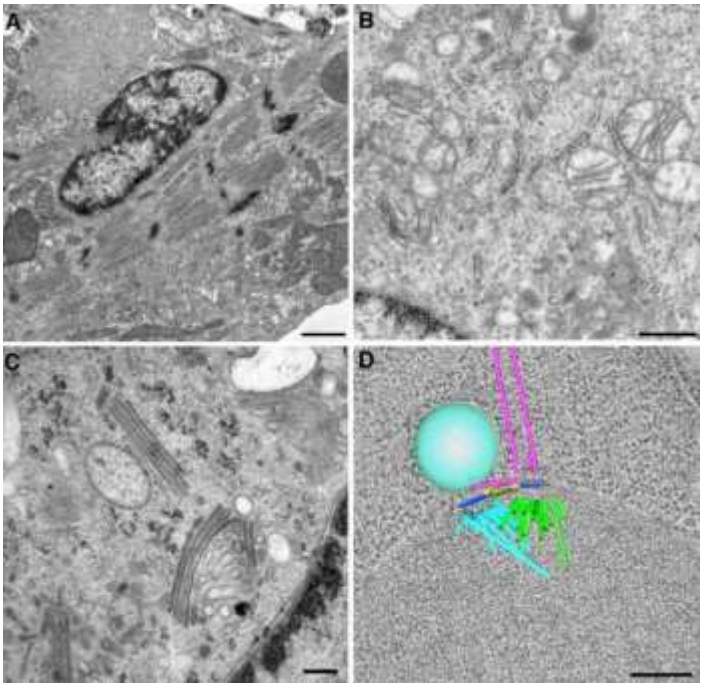


Figure 9. Cell structure as visualized by EM. (A) Actin-myosin cytoskeleton revealed in a cultured cardiomyocyte ready by standard chemical fixation. Scale bar (1 µm). (B) cytoplasmic organelles in a mouse macrophage prepared by standard chemical fixation. Scale bar (700 nm). (C) Golgi membranes in a cultured 3T3 cell prepared by HPF and freeze substitution. Scale bar (200 nm). (D) Three-dimensional tomographic model of a forming mitotic spindle from budding yeast. Scale bar (200 nm) [32]

6- Main parts of EM

EM is in the form of a vacuum column, which is vertically mounted, and it has the following components [35].

1- Electron gun

The electron gun is a tungsten filament, which generates electrons.

2-Electromagnetic lenses

- Condenser lens, which focuses the electron beam on the specimen. A second condenser lens make the electrons into a thin tight beam.
- Objective lens, which has high power and make the intermediate magnified image.

- Projector (ocular) lenses, the third set of magnetic lenses which produce the final further magnified image.

3- Specimen holder

The specimen holder is a thin film of carbon held by a metal grid.

4- Image viewing and recording system.

- The final image is projected on a fluorescent screen.
- There is a camera below the fluorescent screen where the image is recorded.

7- Advantages

- Very high magnification.
- High resolution.
- Material rarely damage or distorted by preparation.
- Can investigate a greater depth of field.

8- Limitations

- The live specimen cannot be determined.
- As the penetration power of the electron beam is low, the object ought to be ultra-thin. For this reason, the specimen is dried and take ultra-thin sections before observation.
- Since the EM works in a vacuum, the specimen should be completely dry.
- Very expensive to build and maintain.
- Need training.
- This microscope is a large, cumbersome and sensitive to vibration and external magnetic fields.

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