Transmission Electron Microscopy

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1. **Introduction.** In 1931, while conducting research for his masters at the Technical College of Berlin, Ernst Ruska and Max Knoll design the first Transmission Electron Microscope (TEM). The initial designs were able to magnify specimens up to seventeen times greater than that of a light microscope; modern designs have a resolution at most of 10,000 times greater. One of the major historical limitations of TEM was that electrons were largely unable to pass through thick specimens; until the diamond knife and ultra-microtome were designed in 1951 it was largely impossible to utilize this instrument to full capacity.

While the theoretical upper limit of the transmission electron microscope is estimated to be as high as 10,000 times that of a light microscope, flaws in the equipment used lowers the real limit. Combined with difficulty in preparing specimens, it is realistically only possible to resolve an object to about 0.025nm. A light microscope, in contrast, can resolve an object to about 2nm at maxium resolution.

2. **Function and Design of TEM.** A Transmission Electron Microscope is similar in design to an ordinary light microscope with one key difference: instead of using light, it uses *electrons*. Using a cathode ray tube or filament (a source to generate highly excited electrons) in a vacuum, electrons are accelerated toward a given specimen by creating a potential difference. A series of magnets and metal apertures are used to focus this steam of electrons into a monochromatic beam, which then collide with the specimen and interact depending on the density and charge of the material. These interactions are greatly affected by how your specimen is prepared.

3. **Preparation of Specimen.** Electrons, in general, are scattered by particles in the air, which necessitates that the excited (and accelerated) electrons be kept in a vacuum to prevent unwanted interactions. As such, it is impossible to view live specimens under a TEM. Also, electrons cannot penetrate specimens very deeply and at most can penetrate 50-100nm.

Preparation of thin sections: preservation of samples with osmium tetroxide (stabilizes lipid bilayers and proteins) and glutaldehyde (usually done first; covalently cross links proteins) allows the specimen to be further dehydrated and permeated with a monomeric resin. The specimen in this form can be sliced with either a diamond knife or an ultra-microtome to create *thin sections* that are free of water and volatile substances. This procedure, while sound, is antiquitated and has been replaced by rapid freezing.

Rapid freezing: while creation of thin sections allows for study of a given specimen under TEM, it does not ensure that the specimen seen under the microscope resembles the actual structure in any shape or form (covalent bonding of proteins is problematic). To ensure specimens are fully preserved without damaging bonds it is possible to rapidly freeze a given specimen in such a manner that prevents the water molecules from rearranging themselves. By slamming a specimen into a polished copper block cooled with helium, the water is supercooled into a vitreous ice. These specimens can then be sliced with an ultramicrotome.

3. **Staining Methods.** After preparing specimens so that they are thin enough to be penetrated by electrons, it is necessary to stain said specimen to increase the contrast and isolate certain areas of interest. A TEM resolves objects with greater atomic number with higher contrast. As biological specimens usually are composed of molecules with low atomic numbers, it is necessary to impregnate cells with heavy salts, such as uranium or lead.

Immunogold Electron Microscopy: in a fluorescent microscope, antibodies with fluorescent markers are used to identify and locate specific macromolecules. An analogous method to this is the immunogold electron microscopy, in which a secondary antibody is tagged with a colloidal gold particle. This antibody is specific for a primary antibody that

attaches to a macromolecule of interest. As the gold particle is electron dense, it will appear as a black dot on the TEM. This method will only detect tagged antibodies on the surface of the cut.

Metal Shadowing: by spraying on a coat of a heavy metal (such as platinum) at an oblique angle, it is possible to examine the surface of a specimen. This techniques creates a three dimensional view of the specimen with the specmen itself appearing darker.

Freeze Fracture: This technique is primarily used to examine cellular membranes. Previously rapid frozen cells are cracked open with a blade knife. It is probable that the fracture line will trace the hydrophobic middle of the cellular membrane.

Negative Staining: Whereas metal shadowing showed the specimen outlined with a higher contrast, the negative stain will do the opposite of a standard metal shadowing technique. Instead of the object appearing higher in contrast, it will be outlined in higher contrast, as it the heavy metal salt pools around it, allowing the electrons to pass through the specimen itself easier than the region around it.

Composite Images: To increase detail, it is possible to combine the statistical averages of many images from identical molecules (several hundred ribosome scans or viral coats). It is allow possible to reconstruct a three dimensional image of a macromolecule by combining TEM images taken from different directions. This method is widely used to determine both crystalline and non crystalline structures.

Bibliography; Resources.

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