Hello students, we are going to study electron microscopy-

introduction and parts from Unit 3 that is microscopy.

In this module we are going to study introduction to electron

microscopy; types, that is scanning electron microscopy

and transmission electron microscopy abbreviated as

SEM and TEM, sample preparation and staining

techniques for electron microscope.

After studying this module,student will be able to

distinguish between light and electron microscopy, and

explain the difference between SEM and TEM. Student will be

able to understand different methods of sample preparation

in an electron microscope.

Light microscope is an important instrument for studying

microorganisms. However, it does not permit study of detailed

structures of microorganisms.

This is because the resolution limit of light microscope is

0.2 microns. This limitation arises from the wavelength of

the visible light.

Resolution of a microscope increases with decrease in

the wavelength, so this problem can be overcome by

using an electron microscope.

Electron microscope uses a beam of electrons which can be focused like visible light.

Wavelength of electron beam is 0.005 nanometers, which is approximately one lakh times shorter than visible light.

Resolution of the electron microscope is 0.5 nanometers.

Electron microscopes are of two types scanning electron microscopes and transmission electron microscope. SEM creates an image by detecting reflected or knocked off electrons, whereas TEM that is transmission electron microscope uses transmitted electrons, that is, electrons passing through the sample to create an image.

Coming to the parts of electron microscope. Electron gun consists of a heated tungsten filament which generates a beam of electrons which is focused on the specimen by the condenser.

Lenses that are used here are magnetic lenses which are donut shaped Electromagnets.

We cannot use glass lenses in electron microscope because electrons cannot pass through glass. The column containing the lenses and specimen is maintained under vacuum to obtain a clear image. Because

electrons are deflected by collisions with air molecules.

To move electrons down the column, an accelerating voltage varying between hundred to 1000 KVs applied between tungsten filament and anode.

In electron microscopy it requires sample preparation. So as with brightfield microscopy, samples usually must be stained in an electron microscope. This is because biological molecules are composed primarily of atoms with low atomic numbers, that is hydrogen, carbon, nitrogen and oxygen. Electron scattering is fairly constant throughout an unstained cell or virus.

Therefore specimens are prepared for observation by soaking thin sections, specially in case of TEM we need thin sections.

So these thin sections are soaked in solutions of heavy metal salts, such as lead citrate, and uranyl acetate to make them electron opaque.

Coming to sample preparation in TEM.

Sample is first fixed with glutaraldehyde or osmium tetroxide. It is then dehydrated with organic solvents like acetone or ethanol. Then it is soaked in unpolymerized liquid epoxy plastic until it permeates completely and hardens to form a solid block.

Thin sections are cut from the block with a glass or a diamond knife using a special instrument. An ultra microtome.

Then staining is carried out with solutions of heavy metal salts like lead citrate and uranyl acetate to make them electron opaque.

Coming to the other specific staining techniques which are used in TEM, the first one is negative staining.

In negative staining, the specimen is spread out in a thin film with either phosphotungstic acid or uranyl acetate.

Heavy metals do not penetrate the specimen, but render the background dark, whereas the specimen appears bright in photograph. In this figure we can see poliovirus with negative staining. So negative staining is used to see the structure of viruses, bacterial gas vacuoles and other similar materials.

Second technique is shadowing.

In shadowing, sample is coated with a thin film of platinum or other heavy metal by evaporation at an angle of about 45 degrees.

Matter strikes the microorganism on only one side, as we can see in this image.

The area coated with metal scatters electrons and appears

light, the uncoated side and the shadow region created by the object is dark.

The third technique is freeze etching in which the cells are rapidly frozen in liquid nitrogen and then warmed to -100 degrees Celsius in a vacuum chamber.

Precooled Knife which is cooled to -196 degrees Celsius fractures the frozen cells. In this picture we can see cell membrane that is fractured.

Specimen is then left in high vacuum for a minute to allow ice to sublimate. The exposed surfaces are shadowed and coated with layers of platinum and carbon to form a replica of the surface. The specimen is dissolved chemically and replica is studied in TEM. So in this picture we can see fractured surface and these exposed cell organelles. In the next step,platinum and carbon shadowing is carried out and this replica is then viewed under TEM.

Sample preparation for SEM is relatively easy as compared to TEM. Air dried materials can be examined directly. However, most often sample is fixed,dehydrated and dried to preserve surface structure and to prevent collapse of these cells when exposed to high vacuum.

Dried samples are mounted and coated with a thin layer of metal to prevent the buildup of an electrical charge on the surface and to give a better image. So in this module we have seen the difference between SEM and TEM we have seen the sample preparation for SEM andTEM and these are the references for this module.

Thank you.