Lec.2

Electron Microscope (EM)

Electron microscopy (EM) base on using an electron beam which is focused into a small probe across the surface of a specimen. The first electromagnetic lens was developed in 1926 by Hans Busch. Electron microscope follows the same principle of compound microscope, but uses electrons beam as a illumination source instead of light.

Electron microscopes allow biologists to explore cells in more details. To observe the organelles such as : Mitochondria, Ribosomes, Endoplasmic reticulum (ER), Golgi apparatus and Lysosomes.

Heavy metals (such as lead) are used to stain cells prior to examine via EM. The stain is more visible in organelles than in the surrounding cytoplasm. Defects in a cell's organelles are easily seen. Electron microscopes are used in science laboratories and in many industries, such as forensics, nanotechnology and mining.

Disadvantages of electron microscopes

1- It is a large machine

2- Require training

3- Very expensive

4- Specimens require a lot of preparation. For example, because electrons have to pass through a specimen to create a TEM image, very thin slices of cells must be prepared. If the slice is too thick, the specimen absorbs all of the electrons and no image is produced. 5- The specimens are mounted in plastic, which means that only dead cells can be viewed.

There are two types of electron microscopes:

1. Scanning electron microscope (SEM)

The mode of action for the SEM slimier to compound microscope however, an electron beams behave like waves which focus via using a magnetic field rather than uses of ordinary lenses. Metallic coating is required for the biological specimens.The electron microscopes are used to achieve up to 100,000x magnification and more than 1000 x resolution than the light microscope. At these limits, sub-cellular structures can be easily observed.

Structure of the SEM:

1- Lens : here are not the optical materials (like glass), but electrical field. Electron optics :

a- condenser lens : focusing the electron beam to the objective lens.

b- objective lens : responsible for size of electron beam impinging on sample surface

2- Electron beam.

3- Transducers (detectors).

Advantages:

1- Almost all kinds of samples, conducting and non-conducting (stain coating needed)

2- Based on surface interaction , no requirement of electron-transparent sample.

3- Imaging at all directions through x-y-z (3D) rotation of sample.

Disadvantages:

1- Low resolution, usually above a few tens of nanometers.

2- Usually required surface stain-coating with metals for electron conducting.

2- Transmission Electron Microscope:

In transmission electron microscopy (TEM), a beam of highly focused electrons are directed toward a thinned sample (<200 nm). Normally no scanning required helps the high resolution, compared to SEM. These highly energetic incident electrons interact with the atoms in the sample producing characteristic radiation and particles providing information for materials characterization.

Advantages:

1- High resolution, as small as 0.2 nm.

2- Direct imaging of crystalline lattice.

3- Delineate the defects inside the sample.

4- No metallic stain-coating needed, thus convenient for structural imaging of organic materials.

5- Electrons can only travel through a vacuum, so the specimen must be completely dehydrated.

6- Electrons have poor penetrating ability. The specimen is usually imbedded in a plastic block and cut into thin sections (no more than 50 nm thick) for viewing.

7- The image contrast results when electrons are scattered by the specimen. Most biological materials scatter electrons poorly. Therefore specimens are usually "stained" with a coat of heavy metal (uranium, osmium, and tungsten) to increase scattering ability.

Disadvantages:

To prepare an electron-transparent sample from the bulk is difficult (due to the conductivity or electron density, and sample thickness).

Newer Techniques in Microscopy:

1- Confocal Microscopy (Confocal Scanning Laser Microscope)

The laser is scanned over a plane on specimen (beam scanning) or the stage is moved (stage scanning) and a detector measures the illumination from each point to produce an image of the optical section. Many sections are scanned, a computer combines them to form a three-dimensional image from the digital signals. Image can then be measured and analyzed quantitatively.

Advantages of using Confocal microscope

1. Illumination of one spot at a time reduces interference from light scattering by the rest of the specimen.
2. The aperture above the objective lens blocks out scatery light. Image has excellent contrast and resolution.

2- Scanning Probe Microscopy (SPM)

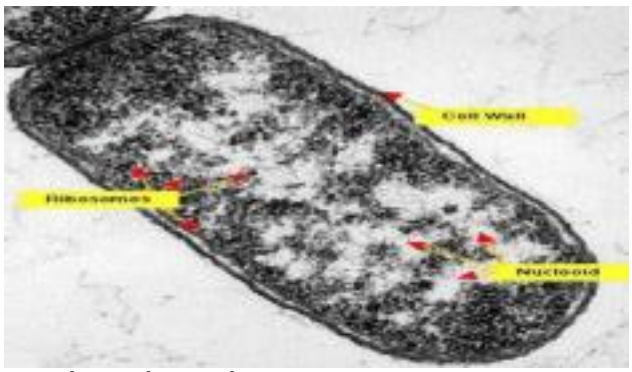
Scanning Probe Microscope measure surface features of the sample by moving a sharp probe over the object's surface.

3- Scanning Tunneling Microscope (STM)

STM able to provide 100 million magnification and allow the viewing of the atom on the surface of a solid.

4- Atomic Force Microscope

Moves a sharp probe over the specimen surface while keeping the distance between the probe tip and the surface constant. Accomplished by exerting a very small amount of force on the tip.

Transmission Electron Microscope (TEM)	Scanning Electron Microscope (SEM)
· Pass a beam of electrons through the specimen. The electrons that pass through the specimen are detected on a fluorescent screen on which the image is displayed. · Thin sections of specimen are needed for transmission electron microscopy as the electrons have to pass through the specimen for the image to be produced. · This is the most common form of electron microscope and has the best resolution	· Pass a beam of electrons over the surface of the specimen in the form of a 'scanning' beam. · Electrons are reflected off the surface of the specimen as it has been previously coated in heavy metals. · It is these reflected electron beams that are focused of the fluorescent screen in order to make up the image. · Larger, thicker structures can thus be seen under the SEM as the electrons do not have to pass through the sample in order to form the image. This gives excellent 3-dimensional images of surfaces · However the resolution of the SEM is lower than that of the TEM.
 Bacterium (TEM)	 A head and the right eye of a fly

	(SEM)

Light Microscope	**Electron Microscope**
Cheap to purchase	Expensive to buy
Cheap to operate.	Expensive to produce electron beam.
Small and portable.	Large and requires special rooms.
Simple and easy sample preparation.	Lengthy and complex sample prep.
Material rarely distorted by preparation.	Preparation distorts material.

Vacuum is not required.	Vacuum is required.
Natural color of sample maintained.	All images in black and white.