Welcome to my online session. Myself Priya Rawat, Assistant Professor from Government College of Arts, Science and Commerce, Sanquelim.

Our today's module is from,

Unit 8 : Instrumentation &

Model Name : Phase Contrast & Electron Microscope.

In this session we will be discussing phase contrast microscope, electron microscope and we will list few advantages and disadvantages of both.

By the end of this session you will be able to,

- Explain functions and applications of phase contrast microscope also
- Explain and give functions and applications of electron microscope.

Let us begin. Until the discovery of phase Contrast Microscope, scientists were limited to bright field illumination and did not have the ability to view live microorganisms. This invention of phase contrast microscope has proved to be the most useful in observing transparent, colorless or unstained specimens referred to as phase objects. Most importantly, many phase objects are living biological samples.

Today there are two main types of phase contrast available that is positive phase contrast and negative phase contrast.

Positive phase contrast - Reveals medium to Gray images on a lighter Gray background.
- These images often have a bright Halo along the edge of the sample.

Whereas,

Negative phase contrast **-** The specimen appears lighter with a dark background and they also have a dark hallow outlining the image. We can Simply put it as, it is exactly opposite that of positive phase contrast.

Phase contrast microscopy.

Let us discuss different parts of Phase contrast microscope. The phase contrast microscope is similar to an ordinary compound microscope in its optical composition. Similar to a normal microscope, it possesses a light source, condenser system, objective lens system and ocular lens system. The Phase contrast only differs from a normal microscope in having two additional components which are called as **Sub - stage Annular Diaphragm** and **Phase Plat**e.

Sub-stage Annular Diaphragm is located below the sub-stage condenser of the microscope. The sub-stage annular diaphragm helps to create a narrow, hollow cone or ring of light to illuminate the object. Here in the figure, we can see the diagram of Phase plate. The Phase plate is also called as diffraction plate or phase retardation plate. It is located at the back of the focal plane of the objective lens. The phase retarding components are coated on this plate. The Phase plate is a transparent glass disc with one of few channels. The channel is coated with a material that can absorb light but cannot retard it. The other portion I.e the other channel has Phase plate coated with light retarding material such as Magnesium fluoride. (Here, which is shown in light purple color.) Phase plate helps to reduce the phase of the incident light.

Working of Phase Contrast Microscope.

In phase contrast microscope, in order to get a contrast, the diffracted waves have to be separated from the direct light waves. This separation is achieved by sub- stage annular diaphragm. It is based on the principle that the small phase changes in the light rays, induced by the differences in the thickness and refractive index of different parts of the object can be transformed into differences in brightness or light intensity. And hence we can see that light on passing from annular diaphragm goes through the condenser as shown in the figure, which then passes through the specimen. Here the dotted line represents retarded light rays. And thus we can see that image is formed on the plate.

Let us discuss few advantages and disadvantages or phase contrast microscope. They are listed as below. Advantages: Phase contrast microscope produces high contrast and high resolution

images. It has the capacity to observe living cells and also is able to examine cells in a natural state I.e. it has the ability to combine with other means, such as fluorescence.

Let us list few disadvantages.

- This method of observation through phase contrast microscope is not ideal for thick organisms or particles.
- So also images may appear Gray or Green if white or Green light are used, resulting in poor photomicrography
- Annuli or ring limits the aperture to some extent, which decreases the resolution.

Let us move on to Electron Microscope. As the name suggests, Electron Microscopes, emit electron beams and not light beams towards the target to visualize a magnified image. We have two types of electron microscopes. One is Transmission Electron Microscope and the other one is Scanning Electron Microscope. Transmission Electron Microscope, in short, is also called as TEM. Whereas Scanning Electron Microscope in short is called as SEM. Transmission Electron Microscope has some of the following features. It is capable of producing Image of 1 nanometre size and it is a popular choice for nanotechnology, in semiconductor analysis and production. Whereas Scanning Electron Microscope is 10 times more powerful than Transmission Electron Microscope, which produces high resolution, sharp, black and white 3D images.

Let us discuss working principle of Electron Microscope. It has an electron source, which is nothing but a stream of high voltage electrons produced and are accelerated in vacuum towards the specimen using a positive electrical potential. It usually has high energy bearing from 5 - 100 KeV. Then using metal apertures and magnetic lenses, this stream is confined and focused into a thin and focused monochromatic beam. Later this beam is then focused onto the sample using a magnetic lens. Interactions occur inside the irradiated sample, affecting the electron beam and these interaction effects are detected and transformed into an image. Thus Creating an image through electron microscope.

Let us discuss few advantages and disadvantages of electron microscope. Electron microscopes provide very powerful magnification. They have vast applications in scientific fields including biology, gemology, medical and forensic sciences, metallurgy and nanotechnology. In technology and industrial application they are used for semiconductor inspection, computer chip manufacturing, quality control, etc. Even though the list is quite big I have listed only a couple of advantages here. If we talk about the disadvantages, the major drawback is, its cost, size, maintenance and researcher training. It is quite large, cumbersome and expensive equipment which is extremely sensitive to vibrations and external magnetic field. Due to which it demands large area to contain and protect the microscope. Thus, it is necessary to undergo professional training to learn the involved processes of specimen preparation to minimize and recognize preparation related artifacts and to operate the microscope itself.

Well these are the few references referred in this section. I hope you've enjoyed this session on phase contrast and electron microscope.

Thank you.