Microscopy

- **Phase – contrast microscopy:**
- Phase contrast microscope can be used to study internal structures in living microorganisms.
- There is no need to fix or stain the specimen.
- The principle of phase-contrast microscopy is based on slight variations in refractive index.
- As rays pass from the light source through the specimen, their velocity may be altered by differences in the thickness and physical properties of various structures of the specimen.
- Light rays passing through the specimen are diffracted differently and travel in different pathways to reach the eye of the viewer.
- These phase differences are seen through the microscope as different degrees of brightness.
- The internal details of a cell appear as degrees of brightness against a dark background.

Fluorescence and Electron Microscopes

- **Fluorescence Microscope:** Fluorescence microscope exposes a specimen to ultra violet.
- Violet or blue light and forms an image of the object with the resulting fluorescent light.
- Usually a mercury vapour arc lamp produces an intense beam, which passes through a special infrared filter which reduces heat transfer.
- The light passes through an exciter filter that transmits only the desired wave length.
- A dark field condenser provides a black background against which the fluorescent objects glow.

- The microscope is used to visualize objects that emit light when a light of a different wave length strikes the object.
- Usually the specimens are stained with fluorescent dyes, called fluorochromes that fluoresce brightly upon exposure to light of a specific wave length.
- Some microorganisms have the ability to auto-fluoresce also.
- The fluorescence microscope has applications in several fields and can be used for direct detection of microorganisms using fluorescent antibody techniques (FAT) and for direct microscope counts (DMC) after staining them with fluorochromes in ecological studies.

Electron Microscope

- In this microscope, electrons are used to illuminate the specimen for magnification (instead of light).
- The electrons have a wavelength of over 1000 times shorter than visible light and therefore the resolving power increases by about a thousand fold.
- The resolving power of an electron microscope is about 0.3 nm – 0.5 nm.
- There are two different types of electron microscope:

1. Transmission electron microscope (TEM)
2. Scanning electron microscope (SEM)

Transmission Electron Microscope

- A heated tungsten filament in the electron gun generates a beam of electrons that is focused on the specimen by the condenser.
- Electrons cannot pass through a glass lens and therefore, magnetic lenses (electromagnets) are used to focus the beam.
- Electrons deflect by collisions with air molecules and hence the column and specimen must be kept under high vacuum to obtain a clear image.
- The specimen scatters electrons passing through it and the beam is focused by magnetic lenses to form an enlarged visible image of the specimen on a fluorescent screen.
- A denser region in the specimen scatters more electrons and appears darker in the image since fewer electrons strike that area of the screen.
- In contrast, electron-transparent regions are brighter.
- The image can also be captured on photographic film.

The Scanning Electron Microscope

- This microscope is used to study external surface features of microorganisms.
- The specimen need not be sectioned.
- SEM provides three dimensional views of specimens.
- In SEM also, an electron gun provides a finely focused beam of electrons called the primary electron beam.
- These electrons pass through electromagnetic lenses and are directed over the surface of the specimen.
- The primary electron beam knocks electrons out of the surface of the specimen.
- These secondary electrons are transmitted to an electron collector, amplified and used to produce an image on a viewing screen or photographic plate.
- SEM has a resolution of about 20 nm and useful magnification of about 10000x.

- There are also other advanced microscopes with higher resolution and magnification.

1. Acoustic microscopes

- There are microscopes that employ sound waves with a frequency of 3000 mega hertz which have a resolution equal to that of the optical microscope.
- Acoustic waves easily propagate through materials impervious to optical waves.

2. Scanning tunneling microscopes (STM)

- STM are used for studying the surface characteristics.
- Even the arrangement of individual atoms on surfaces of metals can be accurately revealed.