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Unit 1	**: Microscopy**
Module Name	**: Phase-contrast Microscopy**
Module No	**: 01**
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Notes

Phase-contrast Microscopy

- An ingenious technique to view highly transparent objects without killing them.
- Invented by the Dutch physicist Frederick Zernike in 1930 and was later awarded the Nobel Prize in physics.
- The Phase-contrast microscope causes amplitude differences in the degrees of phase retardation of light rays passing through a translucent material to create an image with increased contrast.
- The technique provides a high contrast image of bacterial cells based on changes in refractive index rather than differential absorption as in bright-field microscopy.
- These minute differences in refractive index within cellular components and between unstained cells produce contrast in transparent specimens
- Light wave transmitted by the material exists in two forms: i) Unretarded; and ii) Retarded.
- Retardation called as phase change can result in transmission of many different rays with different angles.
- The degree of intensity of contrast in image depends upon light absorbing ability and phase change of transmitted light wave by the object.

Components of Phase Contrast Microscope

Annular disc (aperture stop)

- It is a disc with a transparent circular ring (annulus).
- Placed just below the condenser it functions to transmit the hollow beam of light or to produce an annulus of light (a ring of light).
- It avoids hitting off bright light rays on the object.

Phase plate or phase ring

- It is a black ring placed in the rear focal plane of the objective lens.
- It blocks the unretarded rays entering into the objective lens.
- It can advance the phase of light traversing it.
- The diameter of the annular disc and phase plate should be identical for the phase plate to be superimposed on annular disc.
- Phase plate when superimposed on the annular disc produces a hollow cone of light.
- The primary image formed by the objective lens has only retarded light rays as unretarded light rays are blocked by the phase plate.
- An advantage of this selective nature of the retarded light rays is that objects with poor intensity contrast which fail to appear clearly against high intensity un-retarded rays can be viewed.
- Phase contrast produces an image with highly contrasting bright and dark areas against a neutral grey background.
- Hence the internal structure of cells is often better visualised by phase contrast microscopy over bright field optics.
- Recombination of deviated and un-deviated light by the objective causes a difference in phase by one half wavelength, as a result the background appears dark.
- The object introduces phase difference as its RI differs from that of the medium.
- This phase difference, when superimposed on the difference created by the phase plate, results in loss of destructive interference which makes the object appear brighter than the background.

Applications

I. Objects with poor intensity contrast can be resolved well.

II. Image of the unstained object can be obtained.

III. It is widely used in microbiology and tissue culture research to detect bacteria, cellular organelles and other small entities in living specimens.

IV. It allows the visualization of living unstained cells and their organelles.

V. For the detection of bacterial components, such as endospores and inclusion bodies containing poly-β-hydroxy butyrate, polymetaphosphate, sulphur and other substances.