P.G. 2nd Semester ZOOLOGY Paper VI
By Dr. V. Kumar.

Topic : Phase Contrast Microscope

Phase Contrast microscope was invented by Zernicke in 1935, and was awarded Nobel Prize in 1953. This microscope is used to observe living cells and tissues without fixing and staining them. It enables biologists to study the effect of various chemical and physical agents on the living cells.

Principle

Phase Contrast microscope works on the Principle that different cell organelles have different densities and hence, different refractive indices. The refractive index of the cell and its surrounding medium are also different.

The two light waves when passing through a cell will emerge in Phase if they encounter the same refractive index and thickness of the medium.

If one light wave encounters an organelle and the other one passes through the ground substance, the first light ray is retarded and emerges slightly out of phase. The denser parts of the cells or the cell organelles alter the path of light rays more than the less dense Cytoplasm or the ground substance.

The Phase Contrast microscope selectively retards the out of Phase light wave with respect to other. Normally the Phase shifts are 0.25 of a wavelength

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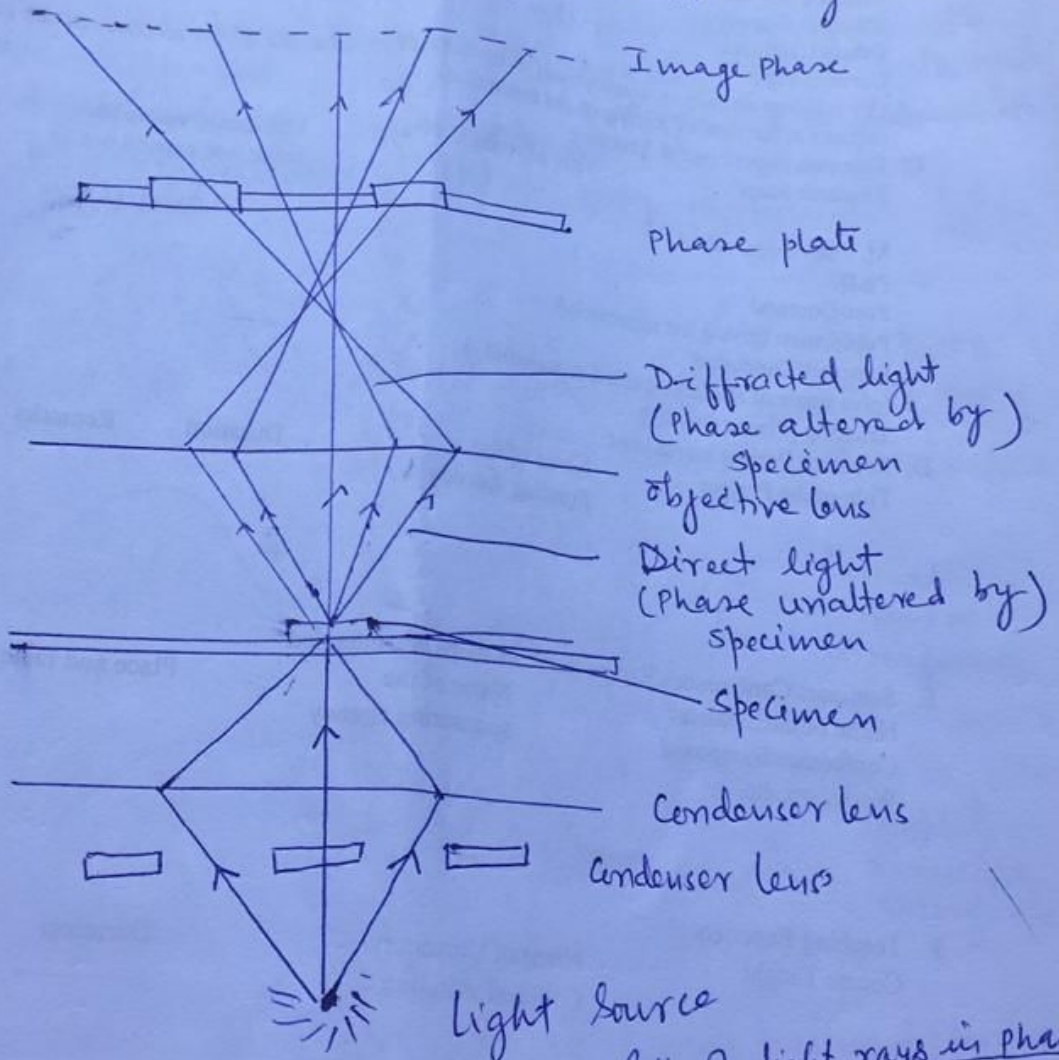
By Phase contrast microscope it is further retarded to 0.25 λ leading to complete destructive interference.

The two light waves cancel each other producing darkness or added up producing brightness.

Thus, Phase Contrast microscope intensifies the Phase difference and transforms them into Variation in brightness

The cell is thus observed in shades of grey, depending upon the thickness and refractive index of the surrounding medium



Path of light rays in phase Contrast Microscope

Working of Phase Contrast microscope

In Phase Contrast microscope an annular Phase Plate is placed in the back focal plane of the objective and an annular diaphragm is placed in the substage Condenser.

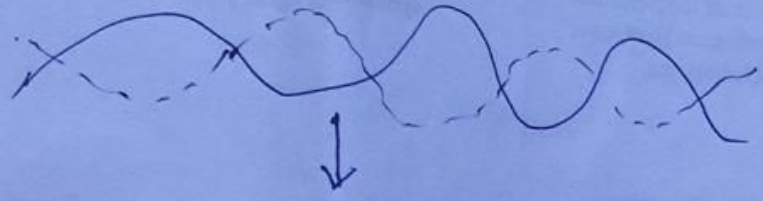
The Phase Plate is a transparent disc containing an annular groove or elevation, whose shape and size coincide with the direct image of substage condenser.

The light rays which pass through the central part of objective and then through the annular groove of the Phase Plate form the geometric image of the object.

The light rays that fall laterally on the objective pass through the annular phase plate and are different either being advanced or ~~reduced~~ retarded by 1/4 wavelength ($\frac{\lambda}{4}$) and forms a different image. These may produce two types of Contrast.

A & B are out of phase by 0.25 λ

Partial distructive interference



Complete distructive interference

Interaction between two light rays having different phases

(a) Bright or negative contrast
when two sets of rays are added, the object appears brighter than the surroundings

(b) Dark or Positive Contrast
when two sets of rays are subtracted making the image darker than the surroundings.

Uses of Phase contrast Microscope

→ Phase contrast microscope is used to observe living cells and tissues.
→ It is used to observe cells cultured in vitro.
→ It is also used to study the effect of different chemical and Physical agents on the living cells and to examine the artifacts introduced by different methods of fixation & staining.
→ By using time lapse motion picture, the following Phenomena can be studied under Phase contrast microscope -

(a) Different nuclear and cytoplasmic changes occurring during cell division and cell movement.
(b) Formation of fibrillar expansions and fine membranes.
(c) Formation of water Vacuoles by the process of pino-cytosis.

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