

Phase Contrast Microscopy

Phase contrast is a light microscopy technique used to enhance the contrast of images of transparent and colourless specimens. It enables visualisation of cells and cell components that would be difficult to see using an ordinary light microscope.

Principle

The phase contrast microscopy is based

on the principle that small phase changes in the light rays, induced by differences in the thickness and refractive index of the different parts of an object, can be transformed into differences in brightness or light intensity. The phase changes are not detectable to human eye whereas the brightness or light intensity can be easily detected.

Parts of Phase contrast Microscopy

Phase-contrast microscopy is basically a specially designed light microscope with all the basic parts in addition to which an annular phase plate and annular diaphragm are fitted.

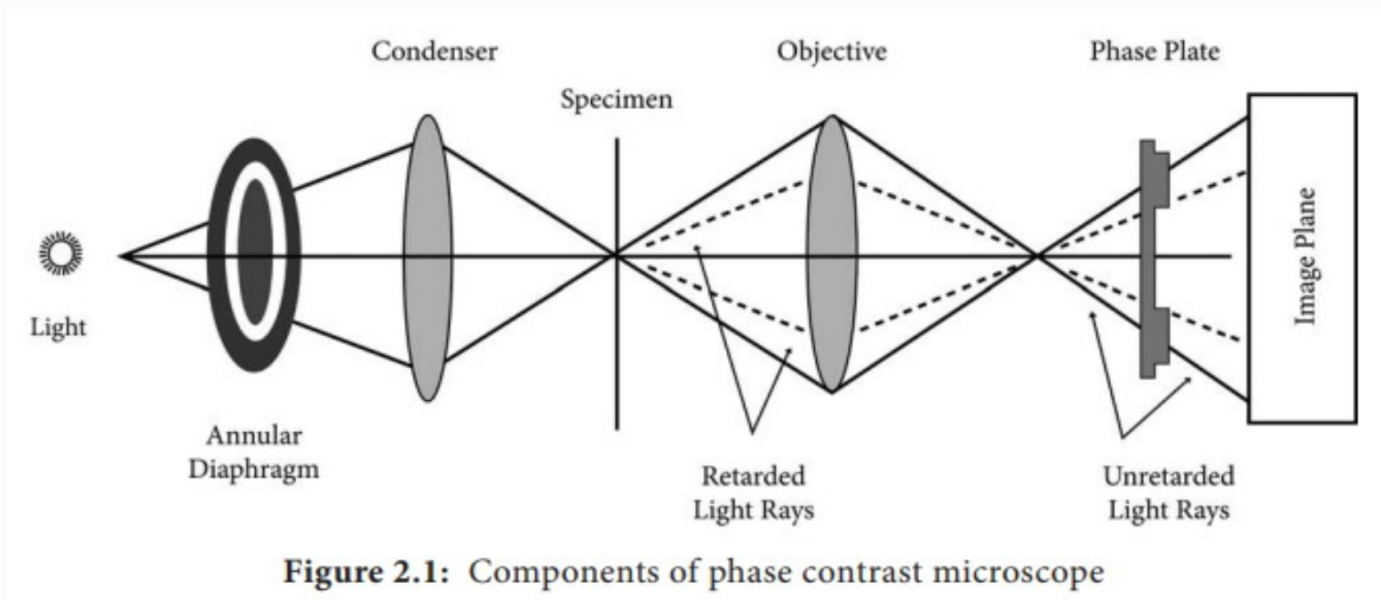
The annular diaphragm

- It is situated below the condenser.
- It is made up of a circular disc having a circular annular groove.
- The light rays are allowed to pass through the annular groove.
- Through the annular groove of the annular diaphragm, the light rays fall on the specimen or object to be studied.

The phase plate

- It is either a negative phase plate having a thick circular area or a positive phase plate having a thin circular groove.
- This thick or thin area in the phase plate is called the conjugate area.

- With the help of the annular diaphragm and the phase plate, the phase contrast is obtained in this microscope.
- **Working Mechanism of**



Phase Contrast Microscopy

The unstained cells cannot create contrast under the normal microscope. However, when the light passes through an unstained cell, it encounters regions in the cell with different refractive indexes and thickness. When light rays pass through an area of high refractive index, it deviates from its normal path and such light rays

experience phase change or phase retardation (deviation). Light rays pass through the area of less refractive index remain non-deviated (no phase change). Figure 2.3 shows the light path in phase contrast microscope

Advantages of Phase contrast Microscopy

- Living cells can be observed in their natural state without previous fixation or labeling.
- Examining intracellular components of living cells at relatively high resolution. eg: The dynamic motility of **mitochondria**, mitotic chromosomes & vacuoles.

- It made it possible for biologists to study living cells and how they proliferate through cell division.