CHRISTIAN EMINENT COLLEGE, INDORE

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e-Content

On

"Phase Contrast Microscopy"

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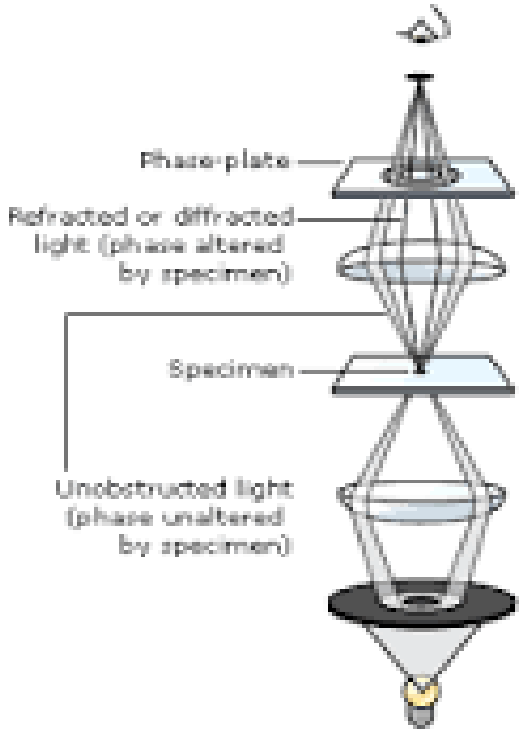
PHASE CONTRAST MIROSCOPY

Phase-contrast microscopy is an optical-***microscopy*** technique that converts ***phase*** shifts in light passing through a transparent specimen to brightness changes in the image. ***Phase*** shifts themselves are invisible, but become visible when shown as brightness variations. Phase *contrast microscopy, was described in 1934 by Dutch physicist* ***Frits Zernike****, is a contrast-enhancing optical technique that can be utilized to produce high-contrast images of transparent specimens. In effect, the phase contrast technique employs an optical mechanism to translate minute variations in phase into corresponding changes in amplitude, which can be visualized as differences in image contrast. One of the major advantages of phase contrast microscopy is that living cells can be examined in their natural state without previously being killed, fixed, and stained. As a result, the dynamics of ongoing biological processes can be observed and recorded in high contrast with sharp clarity of minute specimen detail.*

STRUCTURE OF PHASE CONTRAST MICROSCOPY:

Diagram of a modern upright phase contrast microscope, including a schematic illustration of the phase contrast optical train. Partially coherent illumination produced by the tungsten-halogen lamp is directed through a collector lens and focused on a specialized annulus (labeled condenser annulus) positioned in the substage condenser front focal plane. Wavefronts passing through the annulus illuminate the specimen and either pass through undeviated or are diffracted and retarded in phase by structures and phase gradients present in the specimen. Undeviated and diffracted light collected by the objective is segregated at the rear focal plane by a phase plate and focused at the intermediate image plane to form the final phase contrast image observed in the eyepieces.

The most important concept underlying the design of a phase contrast microscope is the segregation of surround and diffracted wavefronts emerging from the specimen, which are projected onto different locations in the objective rear focal plane (the diffraction plane at the objective rear aperture). In addition, the amplitude of the surround (undeviated) light must be reduced and the phase advanced or retarded (by a quarter wavelength) in order to maximize differences in intensity between the specimen and background in the image plane. The mechanism for generating relative phase retardation is a two-step process, with the diffracted waves being retarded in phase by a quarter wavelength at the specimen, while the surround waves are advanced (or retarded) in phase by a phase plate positioned in or very near the objective rear focal plane. Only two specialized accessories are required to convert a brightfield microscope for phase contrast observation. A specially designed annular diaphragm, which is matched in diameter and optically conjugates to an internal phase plate residing in the objective rear focal plane, is placed in the condenser front focal plane.



APPLICATIONS:

1. *The phase contrast microscope is widely used for examining such specimens as biological tissues. It is a type of light microscopy that enhances contrasts of transparent and colorless objects by influencing the optical path of light.*
2. *It is used in the study of living cells, microorganisms, thin tissue slices, lithographic patterns, fibers, latex dispersions, glass fragments, and subcellular particles (including nuclei and other organelles*