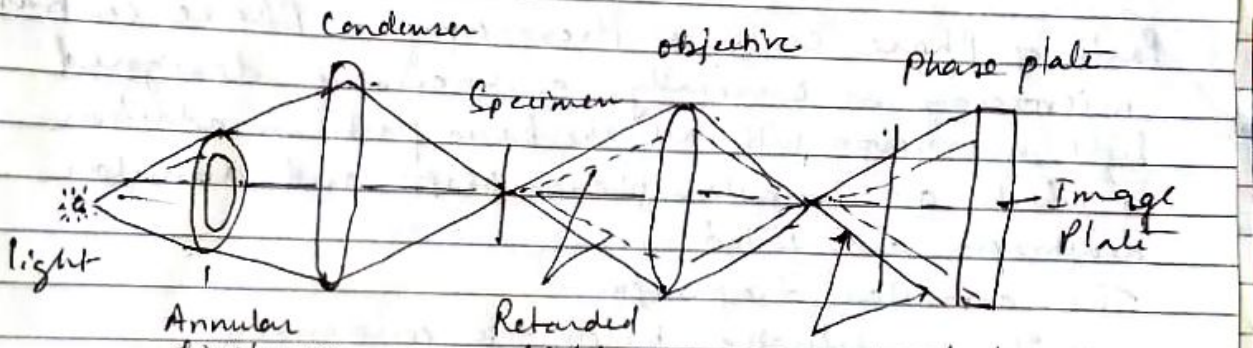
Phase Contrast Microscopy

Unstained living cells absorb practically no light. Poor light absorption results in extremely small differences in the intensity distribution in the image. This makes the cells barely or not at all visible in a bright field microscope. Phase contrast microscopy is an optical microscopy techniques that converts phase shifts in the light passing through a transparent specimen to brightness changes in the image.

It was first described in 1934 by Dutch Physicist Frits Zernike.



Schematic diagram of Phase contrast microscopy

Principle of Phase contrast microscopy –

When light passes through cells small phase shifts occur, which are invisible to human eye. In a phase contrast microscope these phase shifts are converted into changes in amplitude, which can be observed as differences in image contrast.

The working of Phase contrast Microscopy –

1. 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 substage condenser front focal plane.

2. Wave fronts passing through the annulus illumina the specimen and either pass through undeviat or are diffracted and retarded in phase by Structure and phase gradients present in the specimen.

3. Undeviated and diffracted light collected by objective is segregated at the rear focal plane by a phase plate and focussed at the intermediate image plane to form the final phase contrast image observed in the eye pieces.

Parts of Phase Contrast Microscopy - Phase cont 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 -

1) It is situated below the condenser

2) It is made up of a circular disc having a circular annular groove

3. The light rays are allowed to pass through th annular groove.

4. Through the annular groove of the annular diaphrag the light rays fall on the specimen or object to b studied.

5. At the back focal plane of the objective develop an image

6. The annular phase plate is placed at this ba focal plane

The Phase Plate -

1) It is either a negative phase plate having a thick circular area or positive phase plate having a thin circular groove

2. This think or thin area in the phase plate is called the conjugate area
3. The phase plate is a transparent disc.
4. With the help of the annular diaphragm and the phase plate, the phase contrast is obtained in this microscope.
5. This is obtained by separating the direct rays from the diffracted rays.
6. The direct light pass through the annular groove whereas the diffracted light rays pass through the region outside the groove.
7. Depending upon the different refractive indices of different cell components, the object to be studied shows a different degree of contrast in this microscope.

Applications of Phase Contrast Microscopy –
To produce high contrast images of transparent specimens such as –

1) living cells (cultures)
2) Microorganisms
3. Thin tissue slide
4. lithographic Patterns
5. ~~Fib~~ Fibres
6 latex dispersions
7. glass fragments
8 Sub cellular Particles (Nuclei and other organelles)

Advantages –

1) living cells can be observed in their natural state without Previous fixation or labelling.
2. It makes a highly transparent object more visible
3. No fixation or staining is needed
4. It gives a high resolution and dynamic Motility of cell organelles.
5. It helps in study of living cells and their proliferations of cells during cell division.

Limitations –

1) Phase contrast condensers and objective lenses add considerable cost to a microscope, and so phase contrast is often not used in teaching labs except perhaps in classes in health professions.
2. To use phase contrast the light Path must be aligned.
3. Generally more light is needed for phase contrast than for corresponding bright feild viewing, since the technique is based on the diminishment of the brightness of most objects.

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