Phase Contrast Microscope

- The phase contrast microscope is a ***light microscope.***
- It is an ***optical instrument.***
- It is used to see living cells.
- It magnifies object.
- It is a modification of ***compound microscope***.
- It magnifies not only objects but also changes in brightness.

Principle

Phase contrast microscope uses an ***optical illumination technique.***

The phase contrast microscope separates the illuminating background light and the specimen scattering light.

This technique changes the brightness passing through a transparent specimen in the image. This change of brightness is called ***phase shift***. The phase shifts are not visible to the naked eye because the changes are very small. So phase contrast microscope is used to magnify the changes.

Phase contrast microscope is used to visualize ***transparent, colourless, unstained, living*** biological specimens. These objects are called ***phase objects.***

It is a modification of compound microscope. It contains all the components of a compound microscope in addition to an ***annular ring*** and a ***phase plate***.

Light is bend (diffracted) and retarded based on the refractive index of the object. Highly refractive structures bend and retard light much. This principle is used in phase contrast microscope.

The following are the components of a phase contrast microscope.

1. *Light source.*
2. *Annular diaphragm*
3. *Condenser*
4. *Object*
5. *Objective lens*
6. *Annular phase plate*
7. *Eye piece*

The object is concentrically illuminated by the apex of a cone of light.

Some of the illuminating light is scattered by the object. The remaining light is unaffected by the specimen and forms the background light.

The light rays diffracted by the specimen pass through the objective lens. The diffracted (bend) rays pass at various angles based on the refractive index and thickness of the specimen.

The other light components corresponding to the background, pass through the phase ring of the objective. This produces an additional phase difference.

The phase differences between the specimen and the background, are amplified in the final image. So slight differences in refractive index are visible.

There are two types of phase contrast microscopy, based on the configuration and properties of the phase ring in the objective. They are;

1. *Positive phase contrast or Dark contrast*
2. *Negative phase contrast or Bright contrast.*

In positive phase contrast, the specimen is ***dark*** and the background is ***bright***. The specimen is surrounded by a ***bright halo.***

In negative phase contrast, the specimen is ***bright*** and the background is ***dark***. The specimen is surrounded by a ***dark halo***.

Components of PCM

Phase contrast microscope consists of a ***light source, annular diaphragm, condenser, objective lens*** with ***phase plate, eye piece, body tube, coarse adjustment, fine adjustment, stage, stage clips, nose piece, arm, inclination joint*** and a ***base.***

It looks like a compound microscope in all features. But in addition it contains ***annular diaphragm and annular phase plate.***

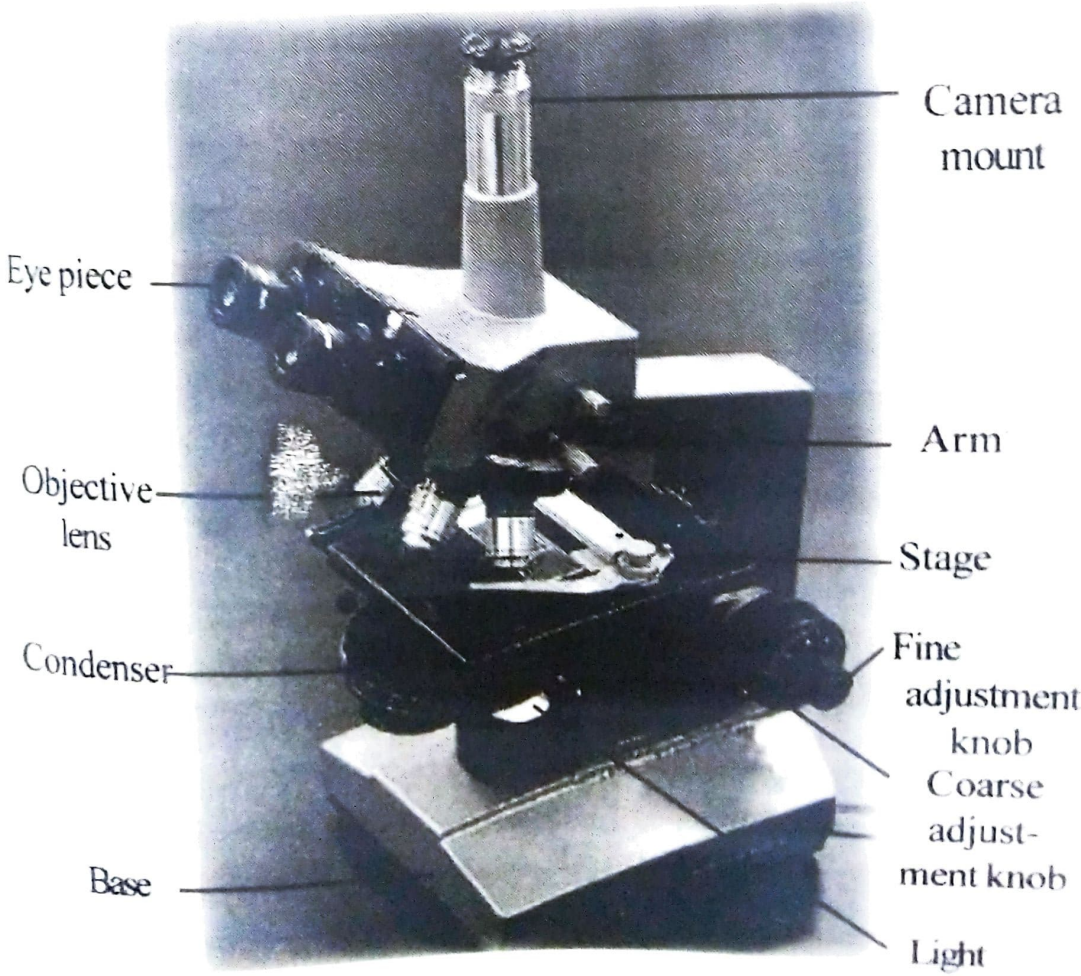

Fig. 12.5: Photograph of a phase contrast microscope.

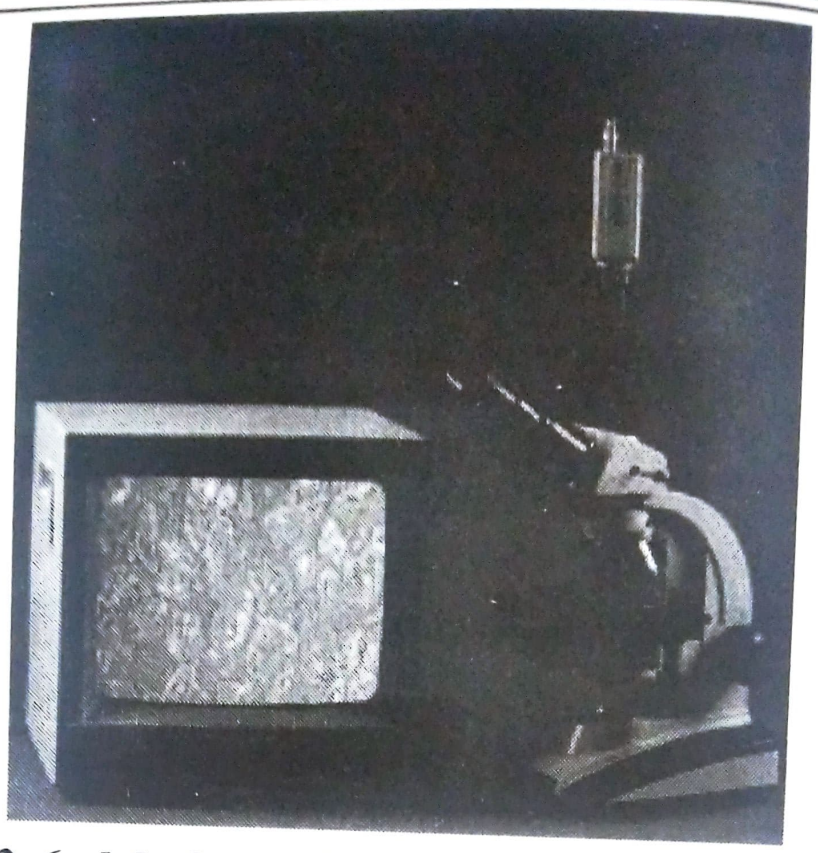

Fig.12.6: Modern phase contrast microscope with CCD camera and computer display.

The light source is a ***tungsten lamp.***

The ***annular diaphragm*** is a plate having a circular ***thin area*** in the centre surrounded by ***thick areas.*** Hence, light coming from the source can pass through the diaphragm in the form of a ***circle.***

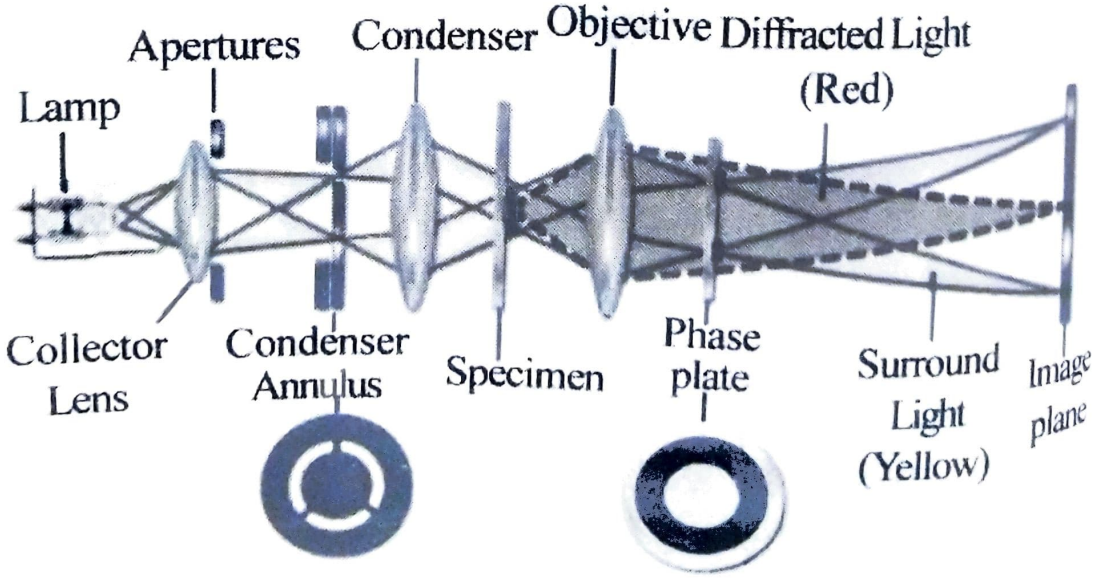

Fig.12.7: Optical path of a phase contrast microscope

The ***condenser*** is located above the diaphragm. It focuses the light on the object. It forms a hollow cone of light. The light rays in and around the light cone are absorbed by the condenser. The light cone is focused on the specimen.

The ***annular phase plate*** is a transparent disc with a ***groove.*** The size of the groove is identical to the circular area of the sub-stage diaphragm. The annular groove of phase plate filters off 70-90% ***S waves*** and leaves ***D waves*** as such. It is placed at the back focal plane of the objective.

The ***specimen stage*** is located above the condenser. The stage is a platform with a ***hole*** in the centre. Light falls on the specimen through the hole. It holds the ***specimen slide.***

The body consists of ***two tubes*** fitted into each other. The small tube can slide up and down along the large tube. The lower end of the large tube is fixed with a ***nose-piece*** and the upper end of the small tube is fitted with ***eye-piece.***

The ***coarse adjustment*** moves the body tube up and down to correct the ***distance*** between the eye-piece and the objective lens for correct focusing.

The nose-piece is a ***rotating disc*** with holes for fitting the objective lenses. A suitable lens (10X, 15X, 20X or 100X) can be fitted with the disc for having maximum resolving power.

The ***arm*** holds the body tube and coarse adjustments.

The ***fine adjustment*** helps in ***exact focusing*** of the specimen by correcting the distance between the eye-piece and objective lens.

Inclination joint connects the upper part of the microscope with the base. It permits the ***tilting*** of the

upper part in any direction to bring the microscope to eye level.

The ***base*** gives ***support*** and ***weight*** to the microscope for keeping it in a position.

Method of Viewing

The chosen ***transparent specimen*** is mounted on a ***glass slide*** using a suitable medium.

A slide containing the specimen is placed on the ***microscope stage*** and clipped with the ***stage clips.***

The ***lenses*** are brought to a correct position by adjusting the ***coarse*** and ***fine adjustments.***

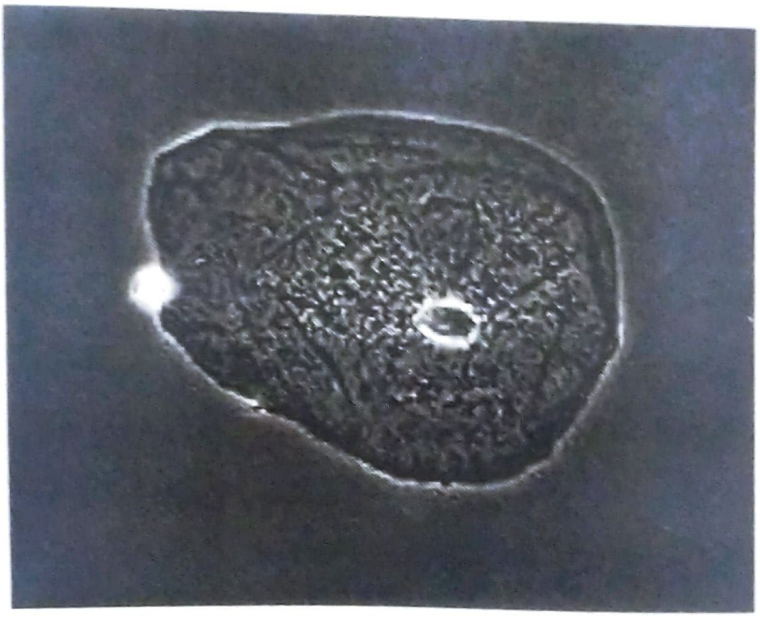

Fig.12.8: Image of cheek epithelial cell under phase contrast microscope.

Light rays passing through the specimen and annular phase plate give ***direct image*** of the specimen. The image is given by diffracted waves and a part of surrounding waves. The S-waves and D-waves are brought to the image plane by the ***eye-piece.***

Phase contrast microscope gives dark image on a grey- coloured background.

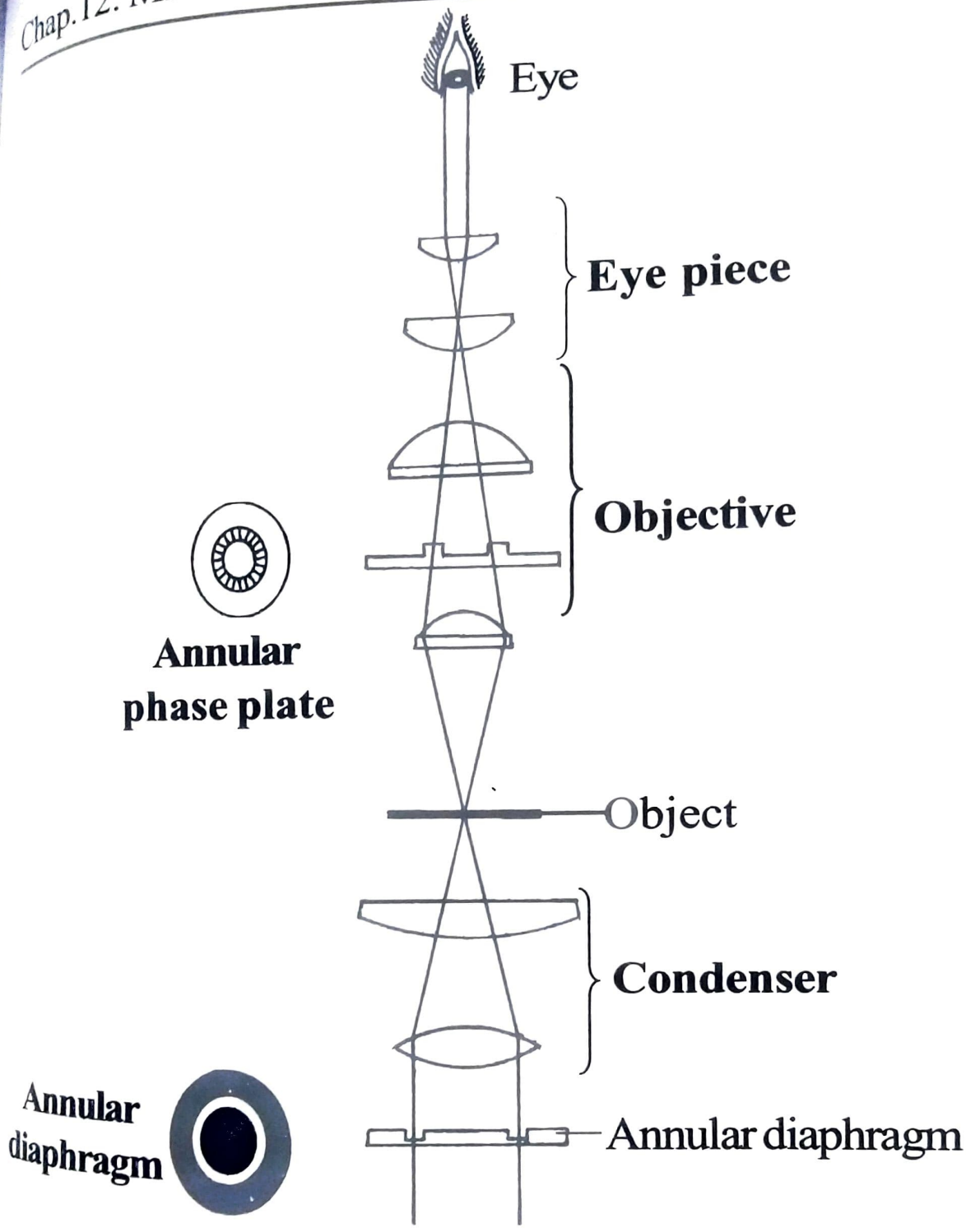

Fig.12.9: Phase contrast microscope.

Uses of Phase Contrast Microscope

1. Phase contrast microscope is used to view the cellular parts of ***protozoa, bacteria*** and others that cannot be seen without staining.
2. It is employed to observe cells in the culture without staining.

3. It is used to examine water and other fluids for the presence of minute transparent microbes (bacteria and flagellates).

4. It is a must to study physiological processes such as ***phagocytosis, pinocytosis, cell divisions***, etc. in cells.

5. It is used to study the behaviour of chromosomes during ***mitosis*** and ***meiosis.***

6. It is best to study living cells without fixation and staining.