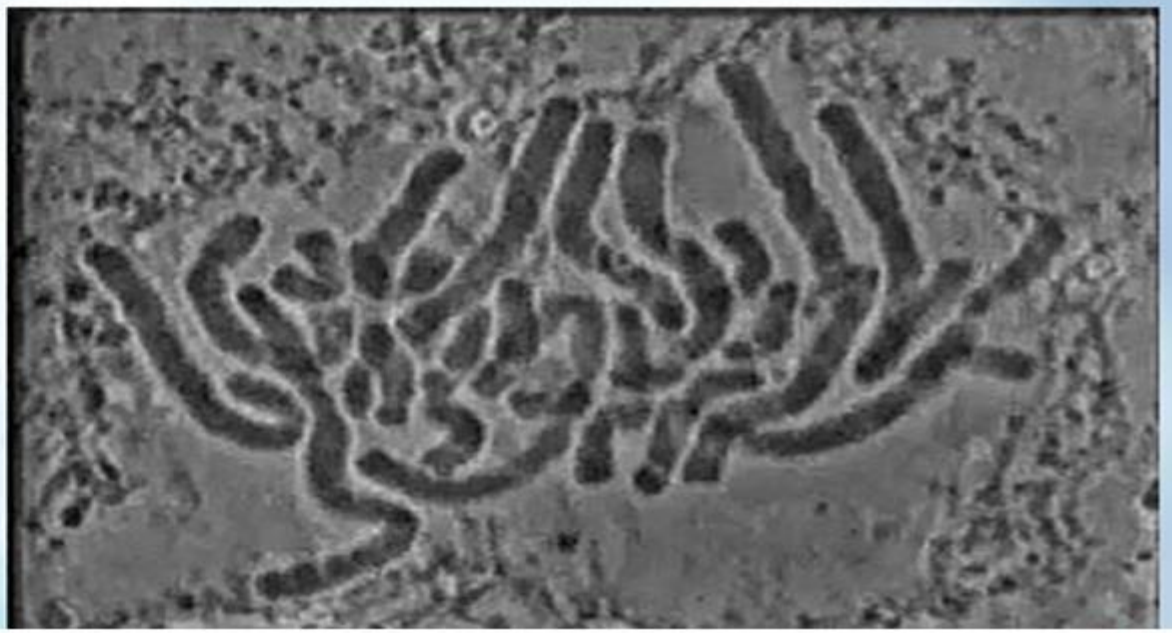
PHASE CONTRAST MICROSCOPE

Optical Components & Working Principle

Learning Objectives and outcomes

- Phase Contrast Microscope (PCM):
  - Working Principle
  - Parts of Phase Contrast Microscope
    - Sub-state annular diaphragm
    - Phase Plate
  - Applications
  - Advantages
  - Limitations



Working of an ordinary microscope:

- In an ordinary microscopy, the object is viewed due to differences in colour intensities of the specimen.
- To create colour intensities, the specimen is stained with suitable dyes.
- Contrast (as the image below) is obtained when the light rays pass through a stained specimen(because different stains absorb different amounts of light).

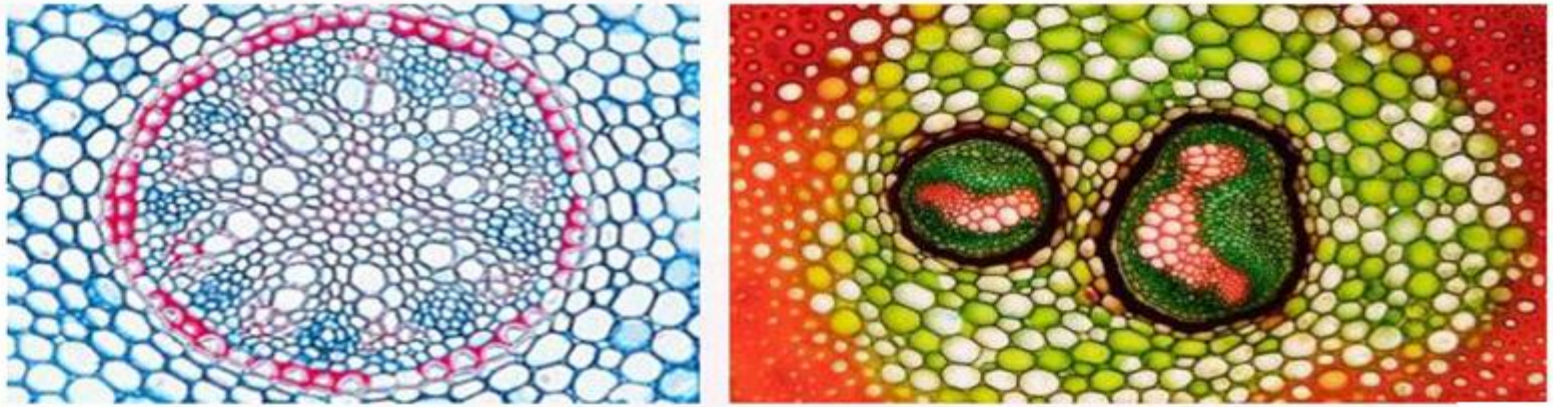
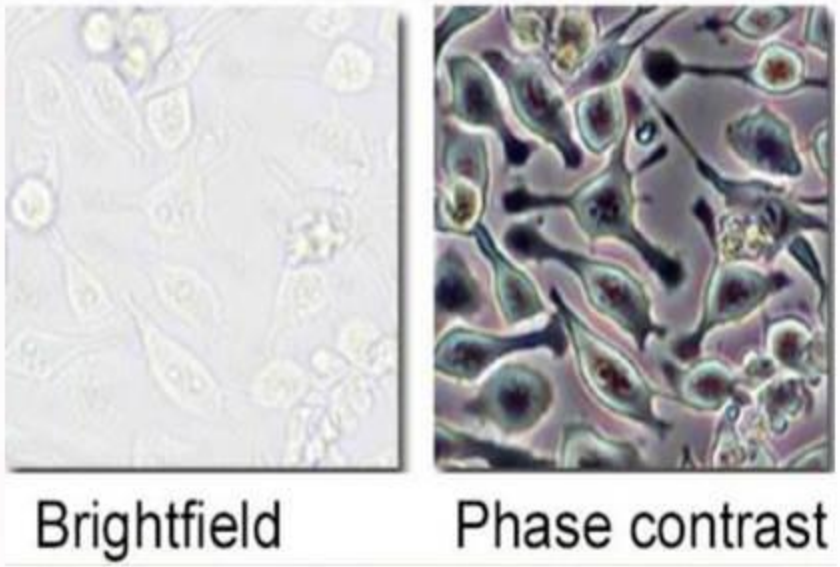
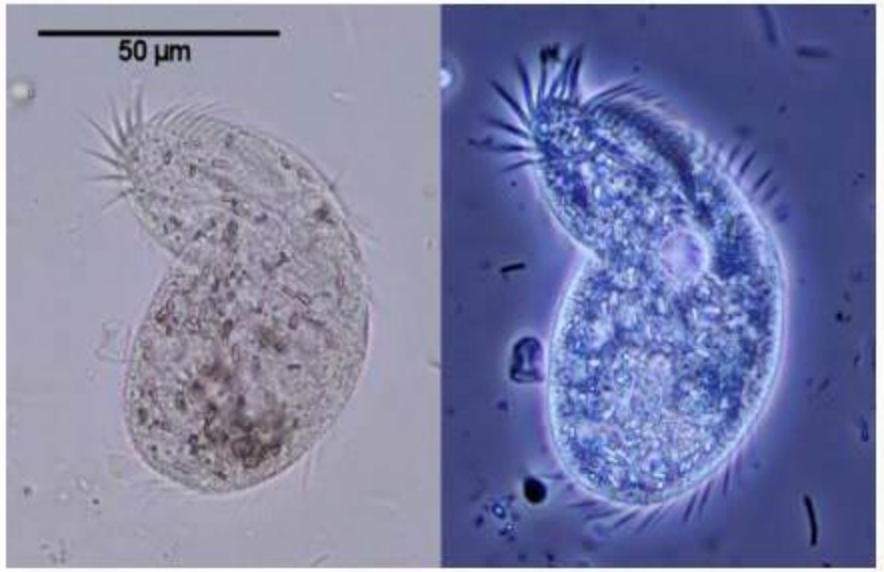


Image formation in Ordinary Compound Microscope

Why Phase Contrast Microscope?

- Phase contrast microscope is used to visualize **unstained** cells
- Enable the visualization of **living cells** and life events (live cell imaging)
- Most of the stains or staining procedures will **Kill** the cells.





Bright Field **Phase Contrast**

History

- Developed by **Zernike** in early 1930s.
- Won Nobel Prize in Physics -1953.

The Nobel Prize in Physics 1953
Frits Zernike

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The Nobel Prize in Physics 1953

Frits Zernike
Prize share: 1/1

The Nobel Prize in Physics 1953 was awarded to Frits Zernike *"for his demonstration of the phase contrast method, especially for his invention of the phase contrast microscope".*

Working Principle

- 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***.
- It is the translation of **invisible phase shifts** into **visible differences** of **intensities**.
- Phase changes are not detectable to human eye whereas the brightness or light intensity can be detected by the human eyes.

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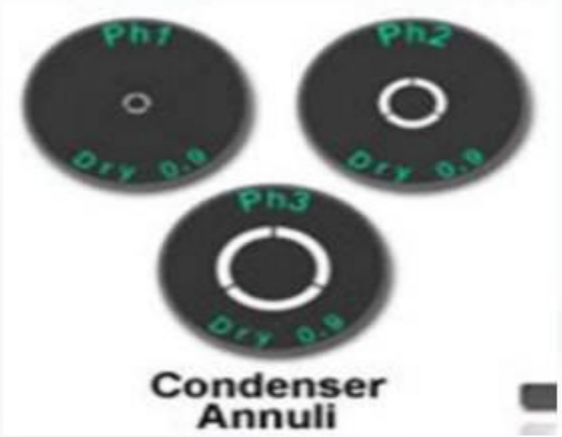
Components of a Phase Contrast Microscope

- PCM is similar to an ordinary compound microscope.
- Possess – light source, condenser, objective and ocular lenses.
- Differ from the normal microscope in having **TWO** additional components:

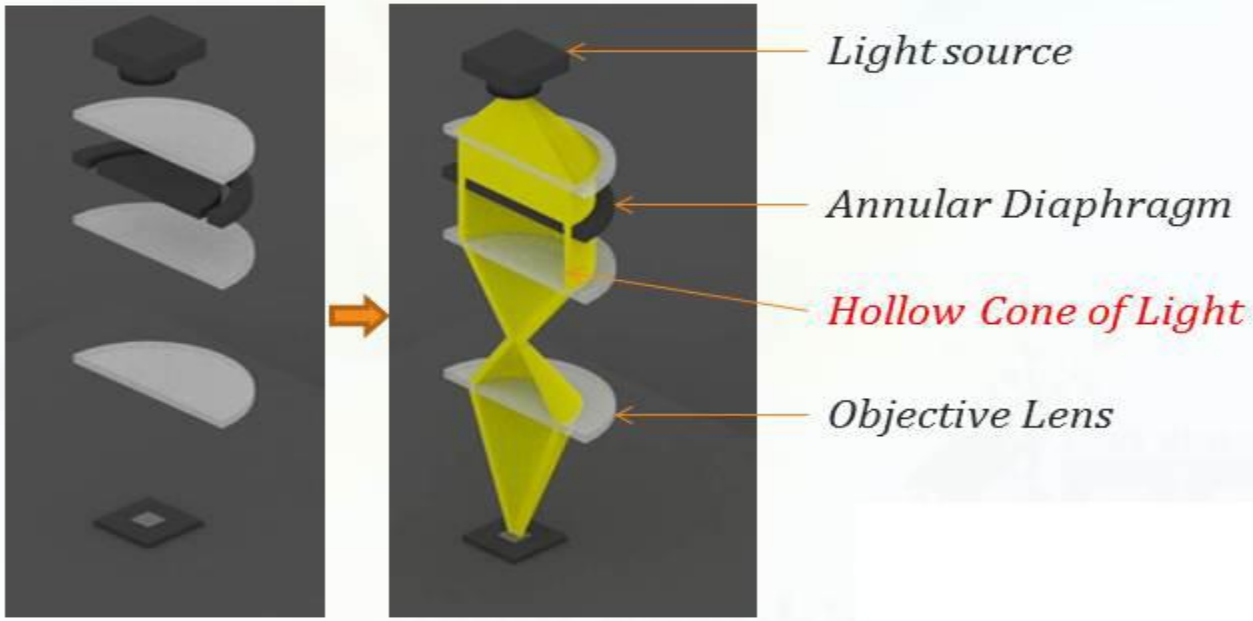
1. **Sub-stage Annular Diaphragm**
2. **Phase Plate**

(1). Sub-stage Annular Diaphragm

- Located below the sub-stage condenser
- Helps to create a **narrow**, **hollow cone** or ring of light to illuminate the object.



Annular Diaphragm



Formation of Hollow Cone of Light

(2). Phase Plate (Diffraction Plate or Phase Retardation Plate)

- Located at the **back** focal plane of the **objective** lens.
- **Phase retarding** components are coated on this plate.
- A transparent **glass disc** with one or few **channels**
- Channel is coated with material that can absorb light, but **<u>cannot</u>** retard it
- Other portions (other than channel) coated with light retarding materials (such as *Magnesium fluoride*)

Phase Plate

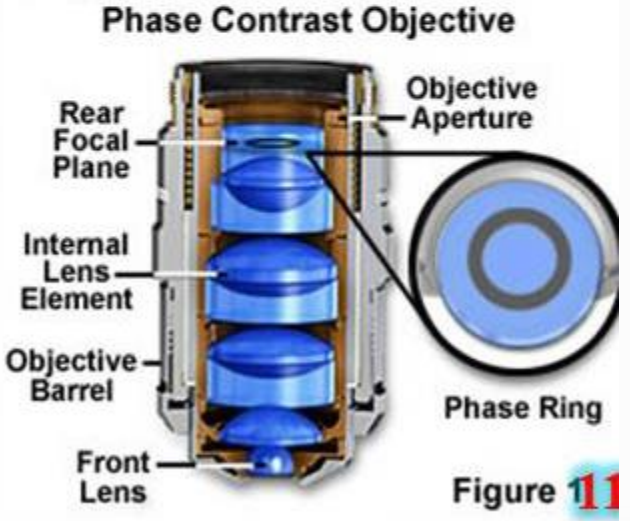


Figure 11

PHASE CONTRAST MICROSCOPE

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Phase Plate

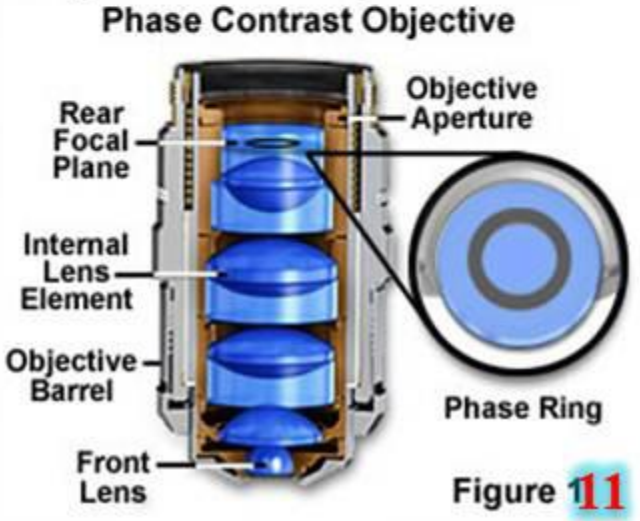


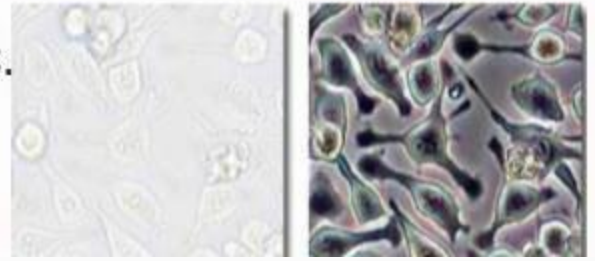
Figure 11

Working of Phase Contrast Microscope

- Unstained cells cannot create contrast under a normal microscope
- When the light pass through the cell, it encounter regions in the cells with different **refractive indexes** and **thickness**.
- When light rays pass through an area of **high refractive index** it is **deviated** from its normal path.
- Such a light ray experience a **phase change** or phase **retardation**.
- Light rays pass though the area of **less refractive** index remain **undeviated** (no phase change).

Working of Phase Contrast Microscope

- The difference in the **phase** between the **retarded** and **un-retarded** light rays is about ¼ of original weave length (i.e., $\lambda/4$).
- Human eyes are **NOT** able to detect his minute changes in the phase of light.
- Thus, such a small phase change **do not** create any contrast.
- The Phase Contrast Microscope has special devices (**Annular Diaphragm and Phase Plate**), which convert this minute phase change into **amplitude change** or **brightness change** so that a **contrast difference** can be created.
- This contrast difference can be **detected** by our eyes.



Working of Phase Contrast Microscope

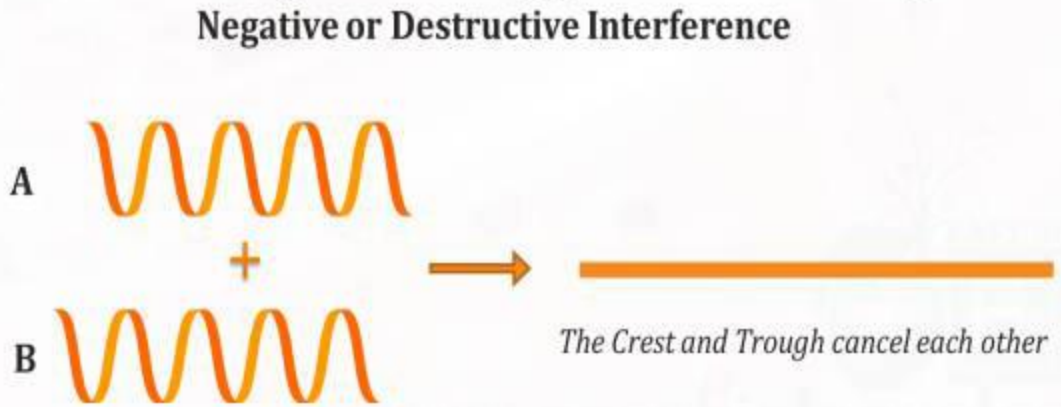
- In PCM, the diffracted waves have to be separated from the direct waves.
- This is achieved by the **sub-stage annular diaphragm**
- Annular diaphragm illuminate the specimen with a hollow cone of light.
- Some rays (direct rays) pass through the thinner region of the specimen and do not undergo any retardation and they directly enter into the objective lens.
- While, the rays passing through the denser region of the specimen get regarded and they run with a delayed phase than the undeviated rays.
- The retardation is about ¼ of the λ of the incident light.

Working of Phase Contrast Microscope

- Both the retarded and unretarded light have to pass through the phase plate kept on the back focal plane of the objective.
- The phase plate is designed and posited in such a way that the retarded light rays will pass through the area of phase plate where light retarding materials are coated.
- When the ¼ (or λ/4) retarded light is passed through the plate, it is further retarded by ¼ (or λ/4)
- Thus the final change or retardation will be:
  - ¼λ + ¼λ = ½λ retardation of phase (or λ/4 + λ/4 = λ/2)

Working of Phase Contrast Microscope

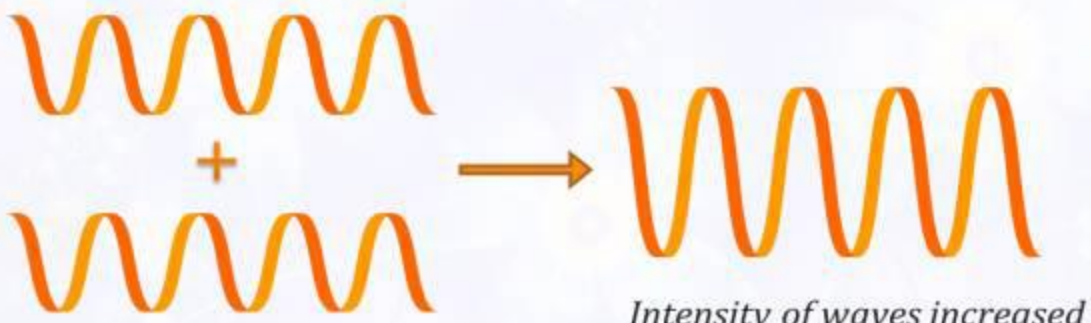
- The un-retarded rays will pass through the channels of the phase plate and their phase is not altered by the phase plate.
- When the **unretarded** and **½λ** (or λ/2) retarded light are recombined (at the focal point) a **negative** or **destructive interference** is created because the crest and trough cancel each other.
- With the destructive interference, the specimen appears darker against a bright background.



Working of Phase Contrast Microscope

- On the other hand, if the un-deviated light rays are passed through the phase regarding material, the two rays will be in same phase and the result is the **positive** or **constructive** interference.
- In constructive interference, the specimen become **brighter** against a dark background.

Positive or Constructive Interference

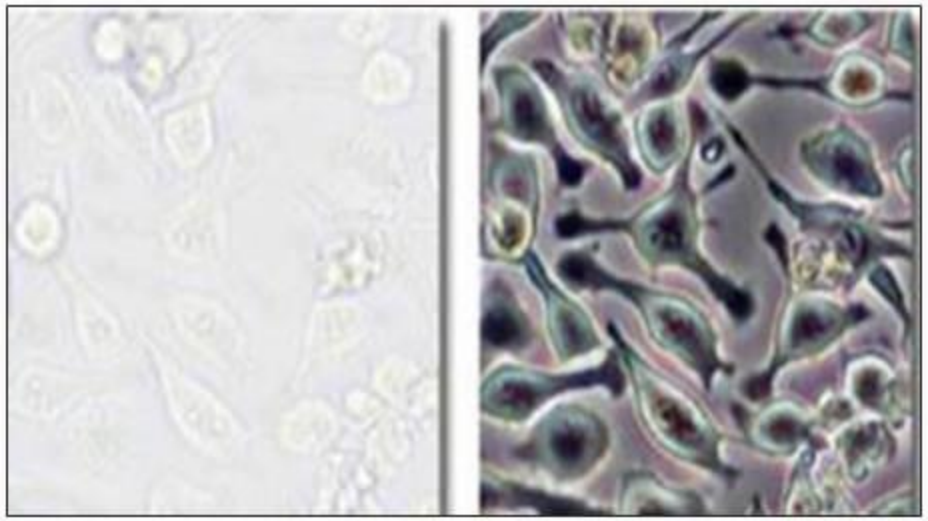


Both the rays are in same phase

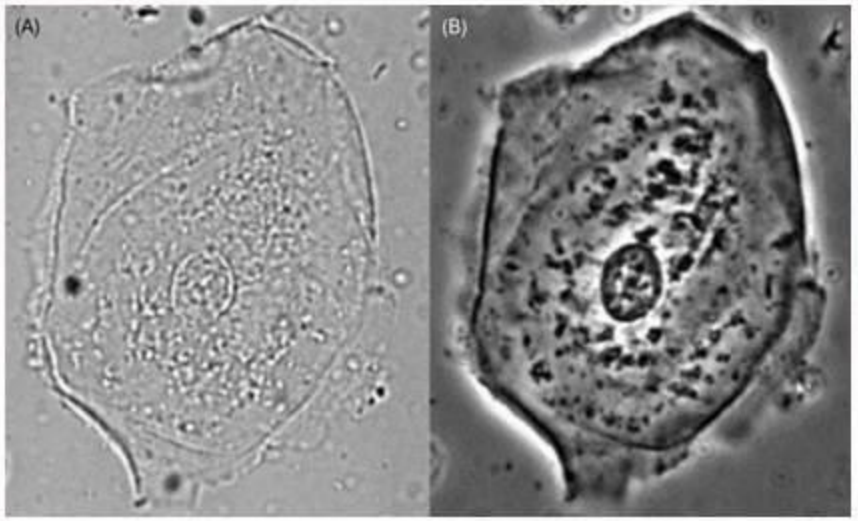
Intensity of waves increased

Working of Phase Contrast Microscope

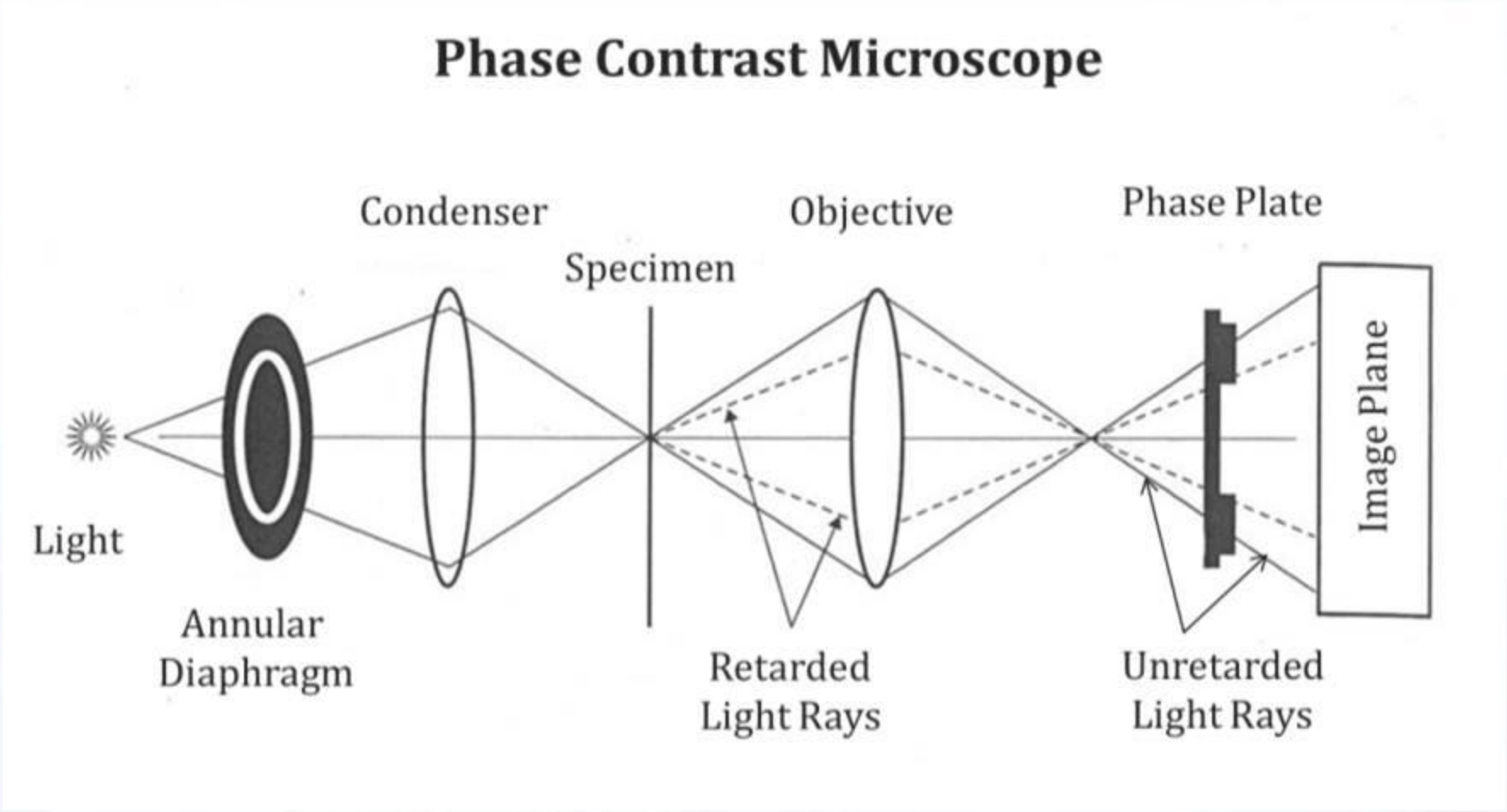
- A combination of destructive and constructive interference create high **contrast** in the final image.



Bright Field ***Phase Contrast***



Bright Field ***Phase Contrast***



The Components and the Optical Path of Phase Contrast Microscope

Applications of Phase Contrast Microscope

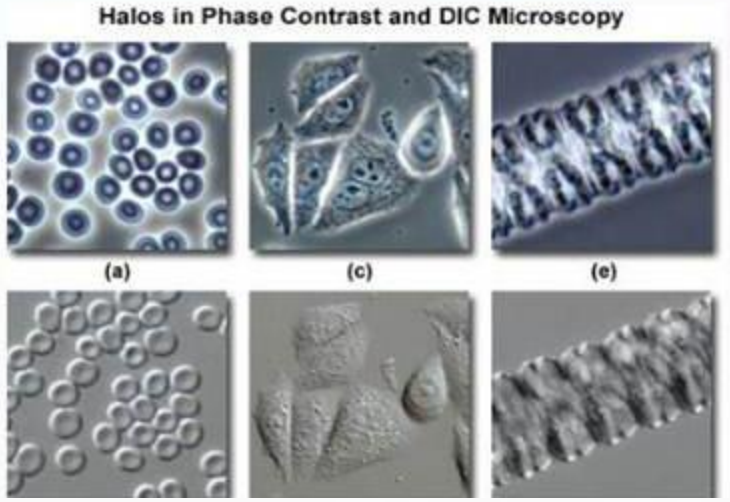
- Magnification and resolution PCM is similar to an ordinary microscope. Still, PCM have many applications in biological sciences, such as:
  - *Enable visualization of **living** cells.*
  - *Enable visualization of **unstained** cells.*
  - *Can be used to view various **cell organelles**.*
  - *Helps to study **cellular events** such as cell division, phagocytosis, cyclosis etc.*
  - *Visualize all types of **cellular movements** (chromosomal & flagellar).*
  - *Enable the study of **cytoskeleton dynamics**.*
  - *Enable the study of **membrane permeability** (phagocytosis).*
  - *Extensively used to observe living cells in **tissue culture** to monitor their growth*

Advantages of Phase Contrast Microscope

- Provide the clear image of **unstained** cells.
- Avoid damages of the cells due to chemical preparation and staining.
- Provide **high contrast** images highlighting the fine details of the cells.
- The optical construction is relatively **simple**.
- A compound microscope can be **elevated to PCM** with minor additions.
- Enable **prolonged observation** of living cells.
- **Live cell imaging** possible.
- Affordable **cost**.

Disadvantages / Limitations of Phase Contrast Microscope

- Main limitations are:
  - Produce a bright **halo** around the images.
  - The formation of halo is due to the partial or incomplete separation of direct and deviated rays.
  - **Only** useful for **viewing individual cells** or thin layer of cells



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Thank You...