

PHASE CONTRAST MICROSCOPY

**Observing Unstained Objects Through a
Microscope**

Phase Contrast Microscope

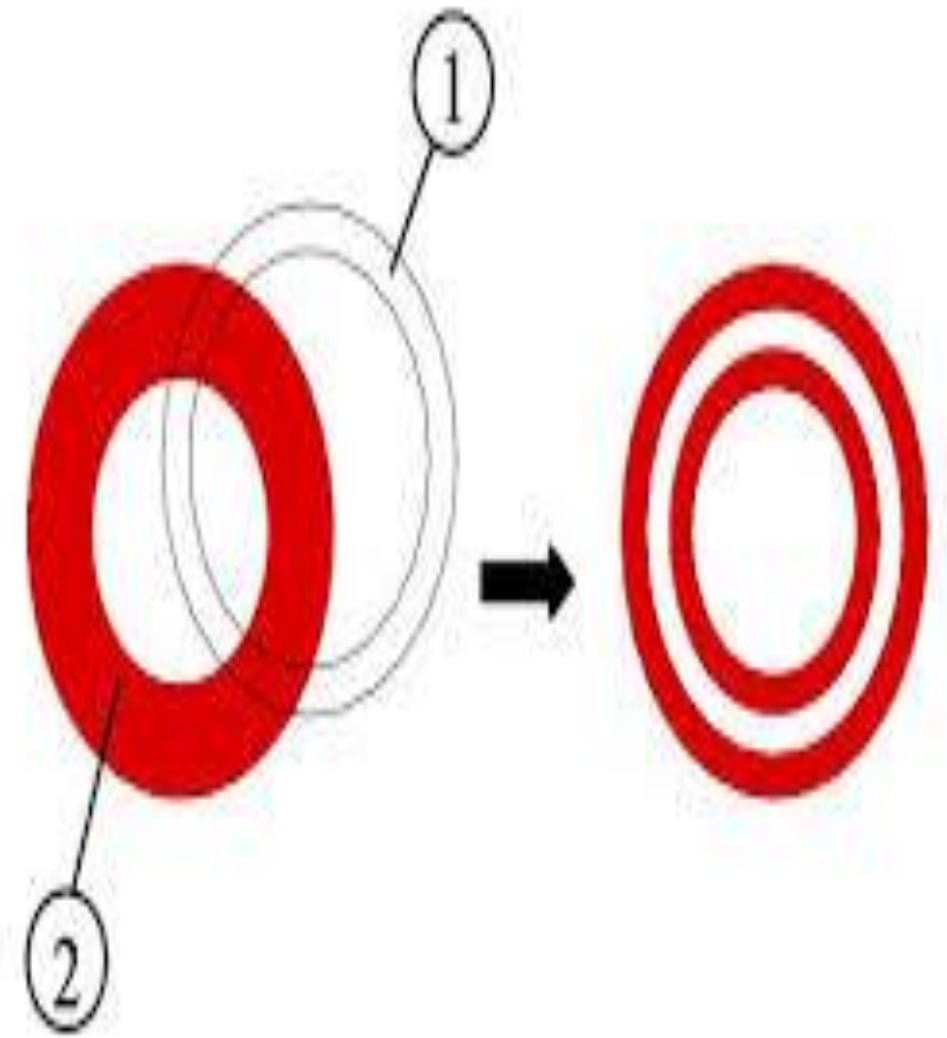
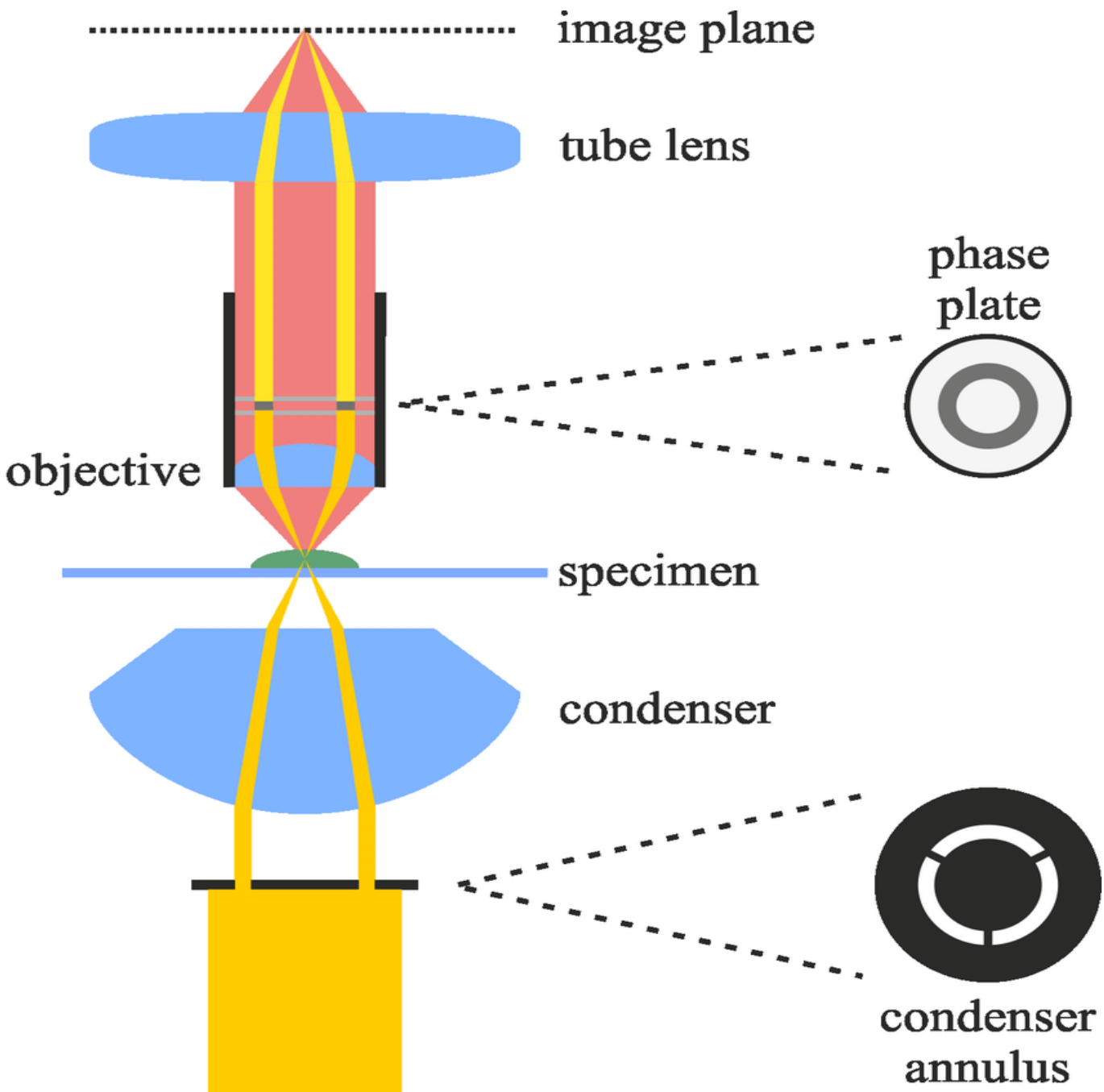
- This is a type of optical microscope is developed as small light deviations know as phase shifts occur during light penetration into the unstained specimen
- These phase shifts are converted into the image to mean, when light passes through the opaque specimen, the phase shifts brighten the specimen forming an illuminated (bright) image in the background
- The phase-contrast microscope produces high contrast images when using a transparent specimen more so those of microbial cultures, thin tissue fragments, cell tissues, and subcellular particles

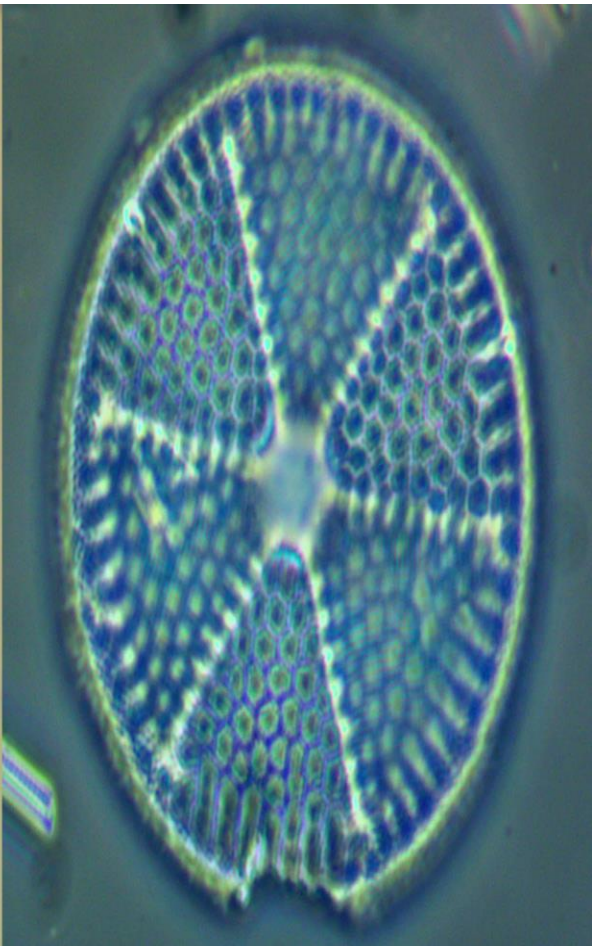
Principle

- **The principle behind the working of the phase-contrast microscope is the use of an optical method to transform a specimen into an amplitude image, that's viewed by the eyepiece of the microscope**
- **This facilitates to view unstained cells, the morphology of the cell and the natural cells, in high contrast and efficient clarity**
- **The shifts that occur during light penetration, become converted to changes in amplitude which causes the image contrast**
- **Coupled with contrast-enhancing elements such as fluorescence, they produce better visuals of the specimens' image**

Parts of the Phase Contrast Microscope: The instrumentation of the Phase Contrast Microscope is based on its light pathways from receiving the source of light to the visualization of the image

- **Light source (Mercury arc lamp)**
- **Collective lens**
- **Aperture**
- **Condenser**
- **Condenser annular**
- **Specimen**
- **Objective**
- **Phase plate**
- **Deflected light**
- **Phase ring**





The functioning of the Phase Contrast microscope:

- The change caused by the deviated scattered (Deflected) light and the un-deviated light that reaches the specimen which is absorbed, create at a certain wavelength, producing color**
- The difference created by the scattered light and that of the absorbed light is known as amplitude variations**
- These amplitude variations are sensitive to allowing visualization by photographic equipment like the Phase Contrast Microscope**
- The Condenser of the phase-contrast microscope has an opaque disk that is known as an annular ring, with a transparent ring that produces a cone of light, that passes through a specimen**
- Due to light variations some light bend at the specimen, caused by variations in light density, forming an image at the objective lens**
- The un-deviated light will strike the phase ring on the phase plate and the deviated light will miss the phase ring passing through the phase plate directly, this forms an image**

Applications of Phase-Contrast Microscope:

- **Determine morphologies of living cells such as plant and animal cells**
- **Studying microbial motility and structures of locomotion**
- **To detect certain microbial elements such as the bacterial endospores**

Phase Contrast Microscopy:

- ❖ Useful to examine live specimens**
- ❖ Doesn't require fixing or staining , which usually kill and/or distort microorganisms**
- ❖ Permits detailed examination of internal structures**
- ❖ Special objective lenses and condenser with ring shaped diaphragm accentuate small differences in refractive indexes of internal structures**
- ❖ Image: Direct rays and reflected light rays come together, forming an image with many shades of gray to black**