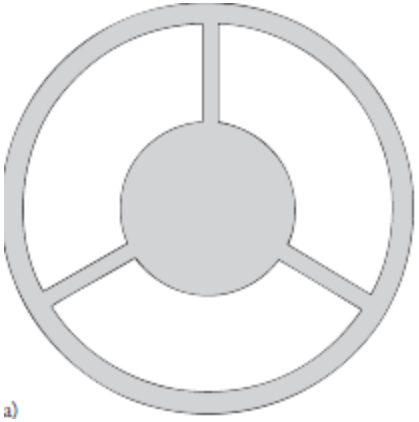
# Unit 1: part 2
# Microscopy

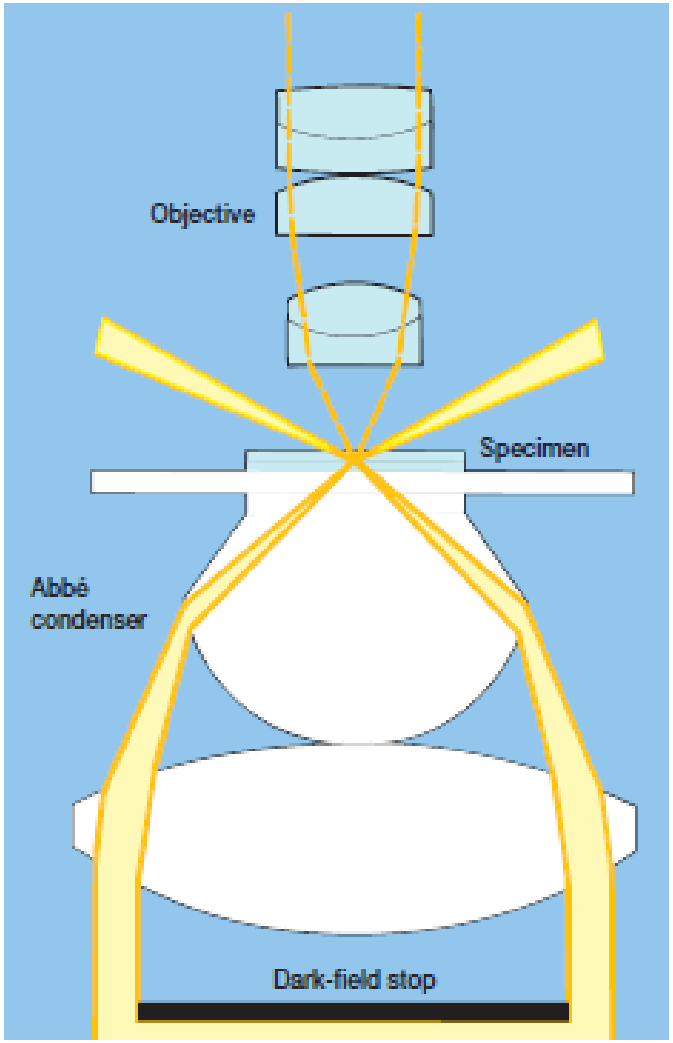
**Instrumentation and Biotechniques,**
**DSE-7, Sem VI**

# Dark field Microscopy

- The field surrounding a specimen appears black, while the object itself is brightly illuminated.
- Background is dark, so this type of microscopy is called **dark-field microscopy**
- used to identify bacteria like the thin and distinctively shaped *Treponema pallidum, the causative agent of syphilis*
- Living, unstained cells and organisms can be observed by simply changing the way in which they are illuminated.
- A hollow cone of light is focused on the specimen in such a way that unreflected and unrefracted rays do not enter the objective. Only light that has been reflected or refracted by the specimen forms an image



a)



$$d_{\text{microscope}} = \frac{\lambda}{(\text{NA}_{\text{objective}} + \text{NA}_{\text{condenser}})}$$

# Limitations

- Un-pigmented live cells are not clearly visible in the bright field microscope.
- No or little difference in refractive index
- Phase contrast microscope makes them visible

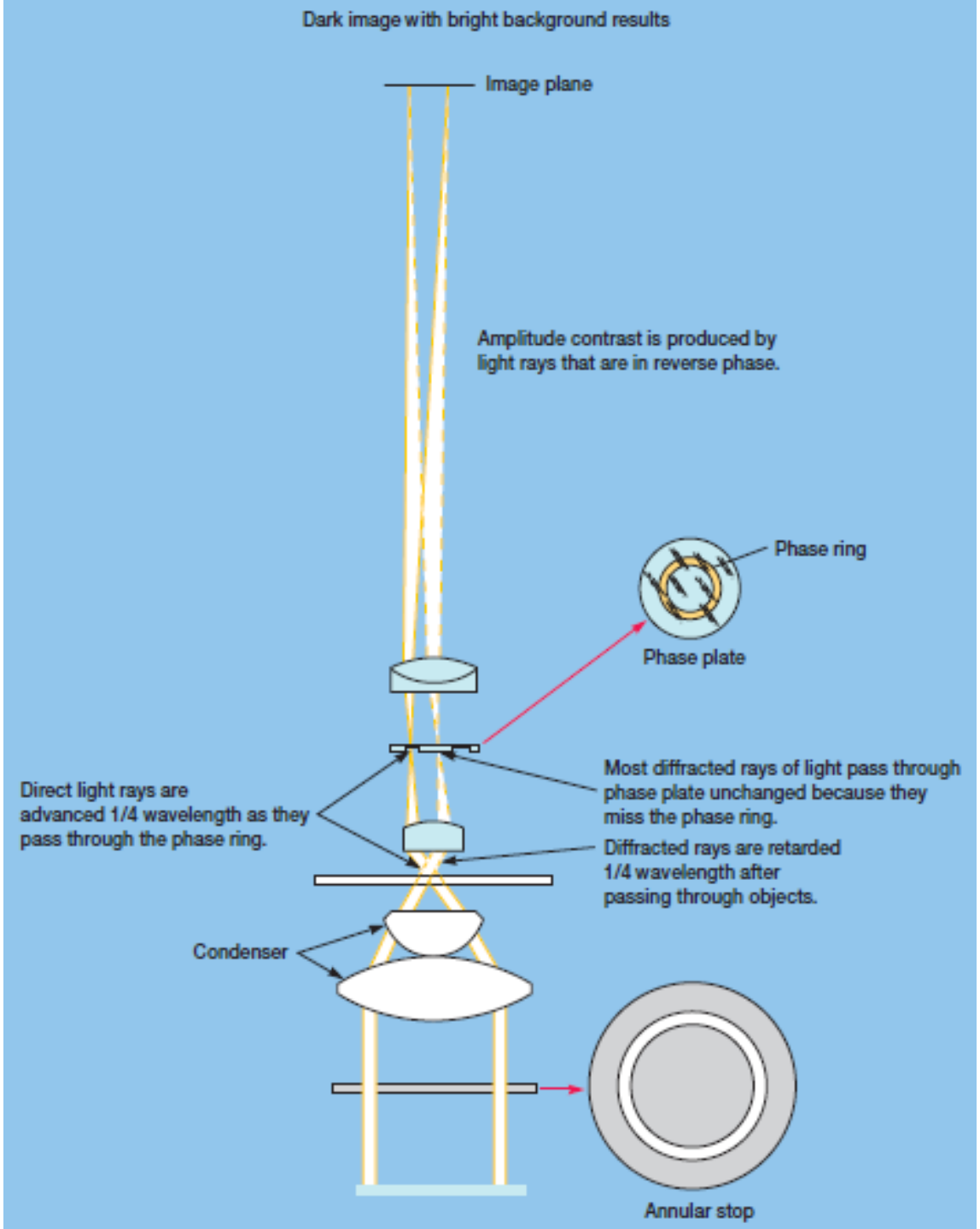
# Phase contrast microscopy

- Can you differentiate easily :
- If similar objects made of clear glass, ice, cellophane, or plastic were immersed in the same container of water
- an observer would have difficulty telling them apart because they have similar optical properties.
- Internal components of a live, unstained cell also lack contrast and can be difficult to distinguish. But cell structures do differ slightly in density, enough that they can alter the light that passes through them in subtle ways.

# Phase contrast microscopy

- The **phase-contrast microscope** has been constructed to take advantage of this characteristic.

- This microscope contains devices that transform the subtle changes in light waves passing through the specimen into differences in light intensity. For example, denser cell parts such as organelles alter the pathway of light more than less dense regions (the cytoplasm).
- Light patterns coming from these regions will vary in contrast.
- The amount of internal detail visible by this method is greater than by either bright-field or dark-field methods.


Dark image with bright background results
Image plane
Amplitude contrast is produced by light rays that are in reverse phase.
Phase ring
Phase plate
Most diffracted rays of light pass through phase plate unchanged because they miss the phase ring.
Direct light rays are advanced 1/4 wavelength as they pass through the phase ring.
Diffracted rays are retarded 1/4 wavelength after passing through objects.
Condenser
Annular stop

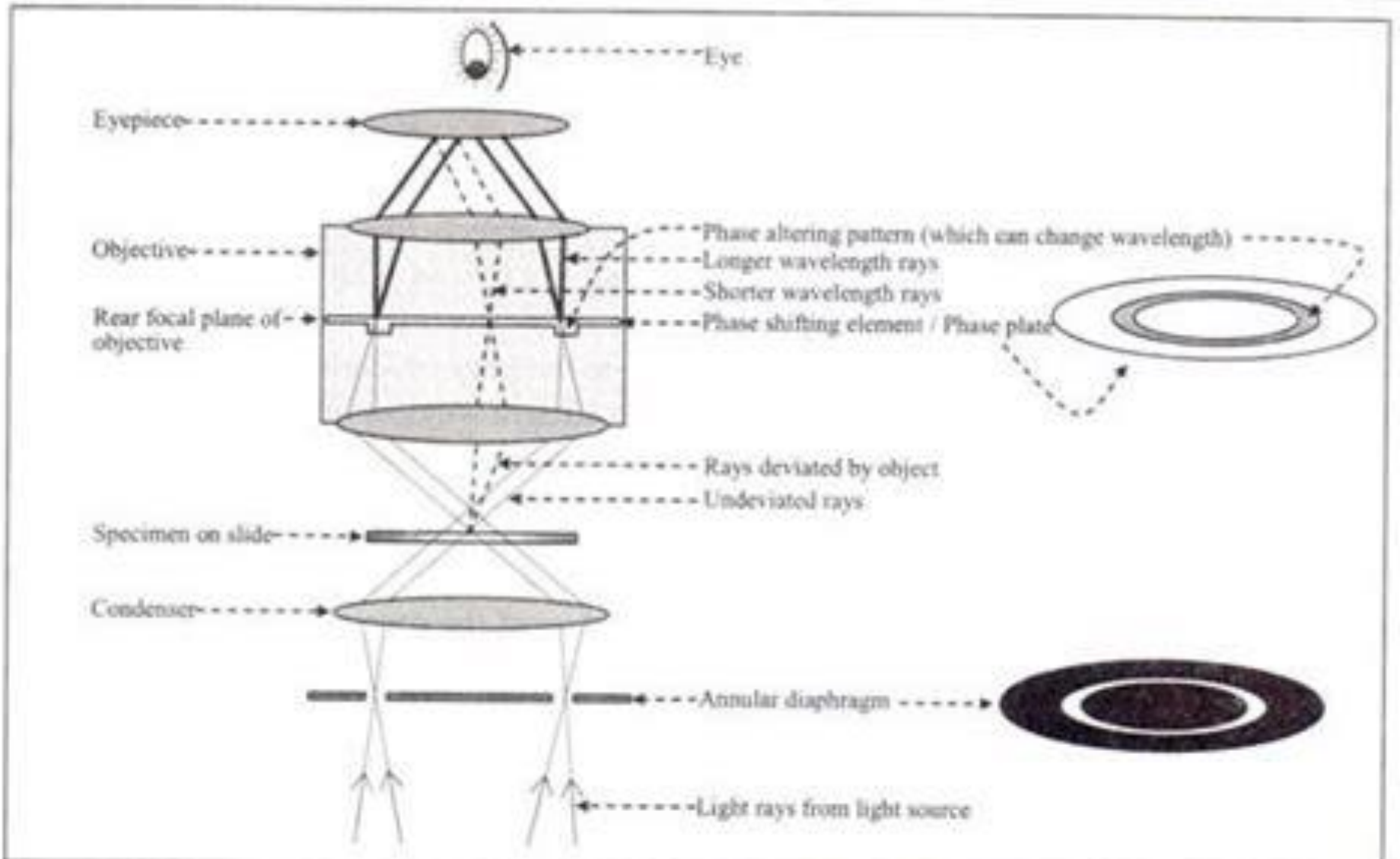
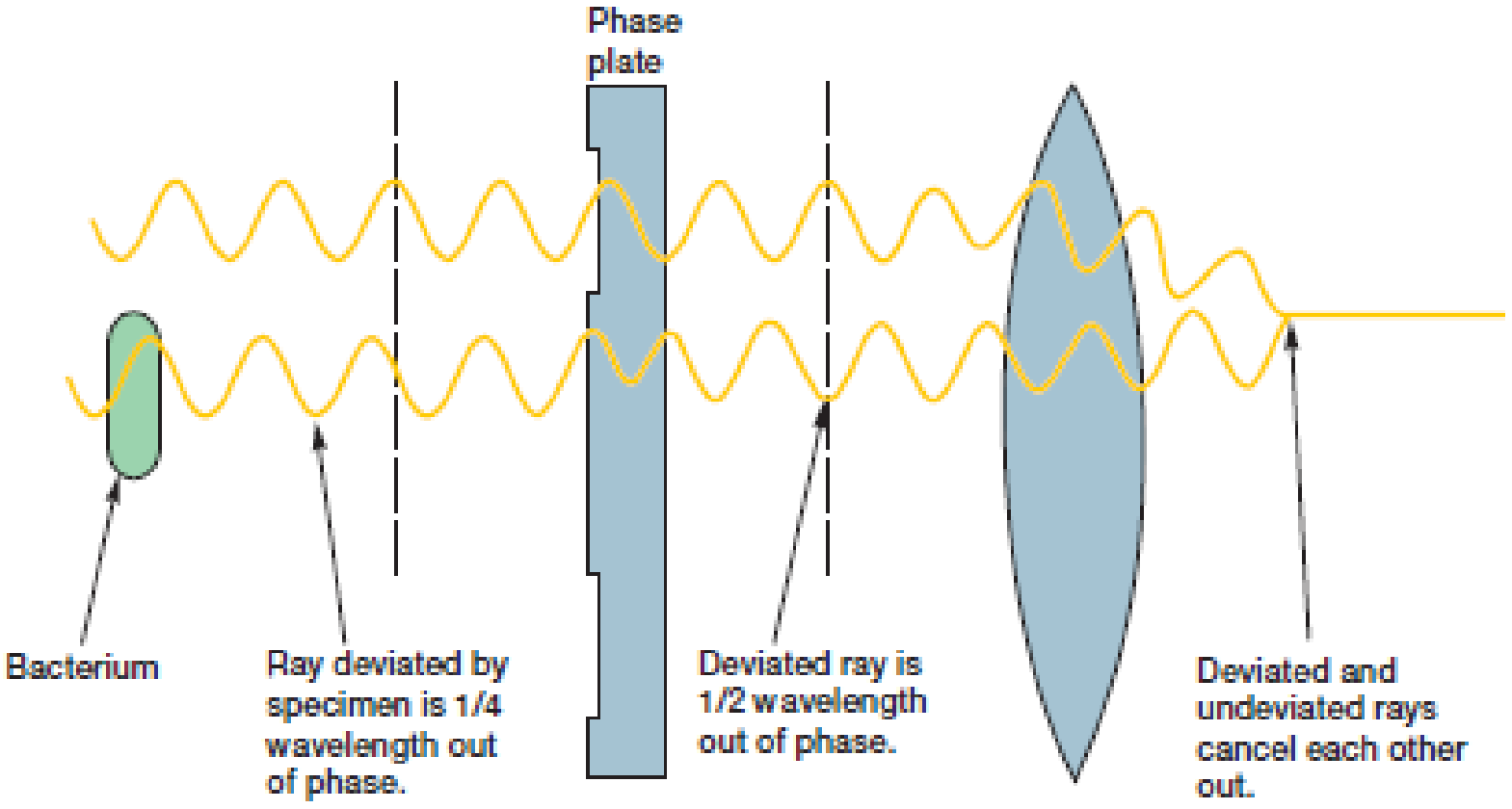


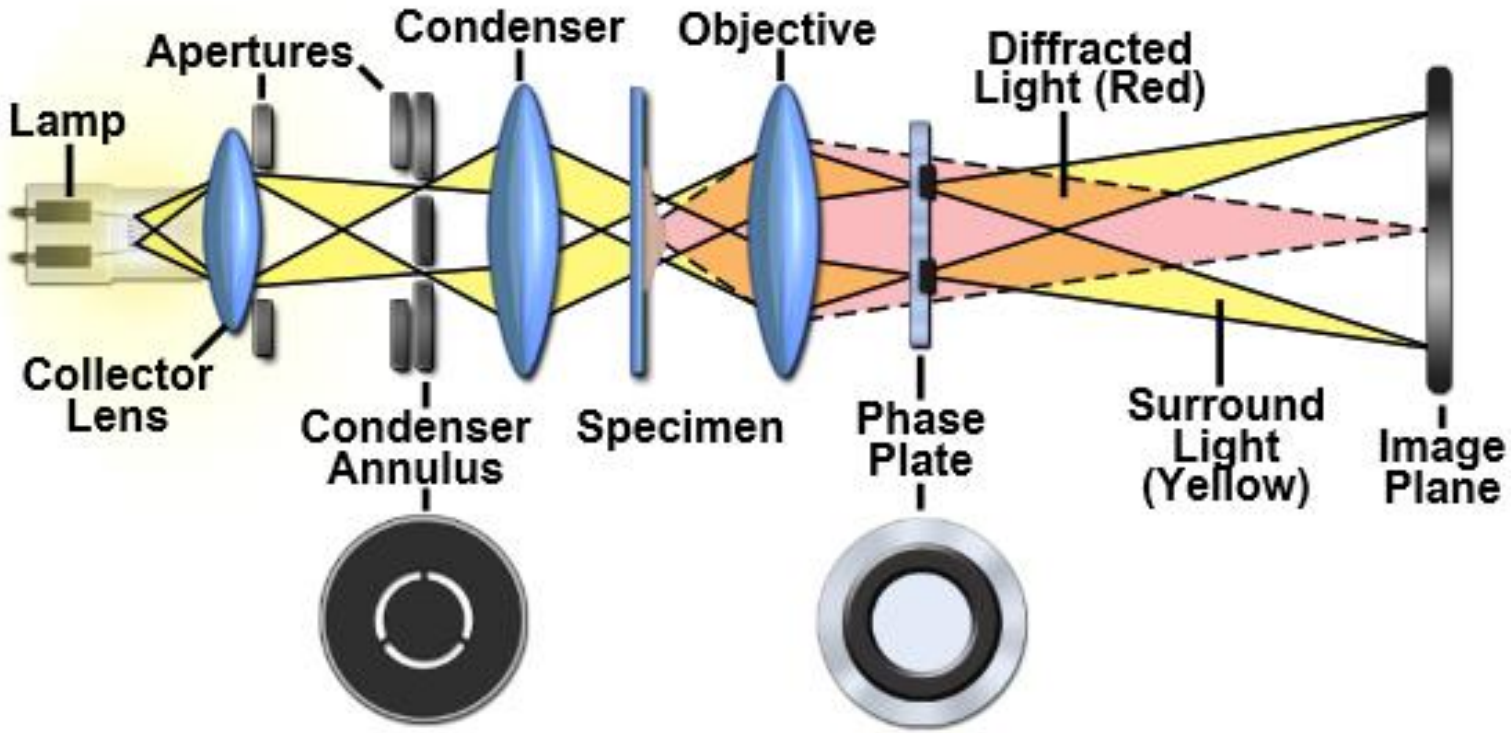

**Figure 4.12: Ray diagram of a phase-contrast microscope**

# Working of a phase contrast microscope

- condenser of a phase-contrast microscope has an annular stop, an opaque disk with a thin transparent ring, which produces a hollow cone of light.
- As this cone passes through a cell, some light rays are bent due to variations in density and refractive index within the specimen and are retarded by about 1/4 wavelength. The deviated light is focused to form an image of the object.
- Undeviated light rays strike a phase ring in the phase plate, a special optical disk located in the objective, while the deviated rays miss the ring and pass through the rest of the plate.
- If the phase ring is constructed in such a way that the undeviated light passing through it is advanced by 1/4 wavelength, the deviated and undeviated waves will be about 1/2 wavelength out of phase and will cancel each other when they come together to form an image.
- The background, formed by undeviated light, is bright, while the unstained object appears dark and well-defined. This type of microscopy is called **dark-phase-contrast microscopy**


Phase plate
Bacterium
Ray deviated by specimen is 1/4 wavelength out of phase.
Deviated ray is 1/2 wavelength out of phase.
Deviated and undeviated rays cancel each other out.

# Phase Contrast Microscope : Optical Path



# Applications

- It is most useful for observing intracellular structures such as:
- bacterial spores,
- granules,
- inclusion bodies that contain poly--hydroxybutyrate, polymetaphosphate,sulfur, or other substances
- locomotor structures of eucaryotic cells,
- to study bacterial motility,