Phase Contrast Microscopy

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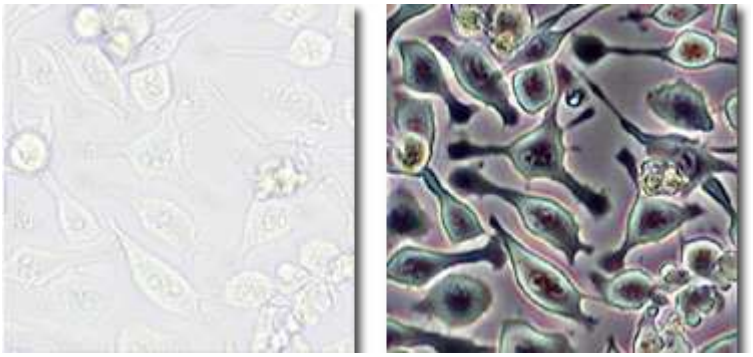
Introduction

- Phase contrast microscopy is an optical microscopy technique that converts phase shifts in light passing through a transparent specimen to brightness changes in the image.
- Phase shifts themselves are invisible, but become visible when shown as brightness variations.
- Phase contrast microscopy is particularly important in biology.
- It reveals many cellular structures that are not visible with a simpler bright field microscope without staining.

Brightfield vs Phase Contrast Microscopy

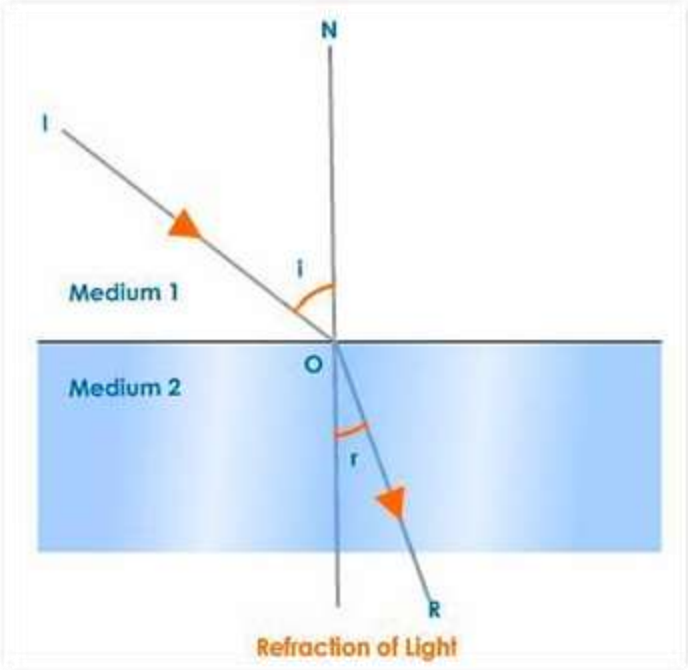
Brightfield

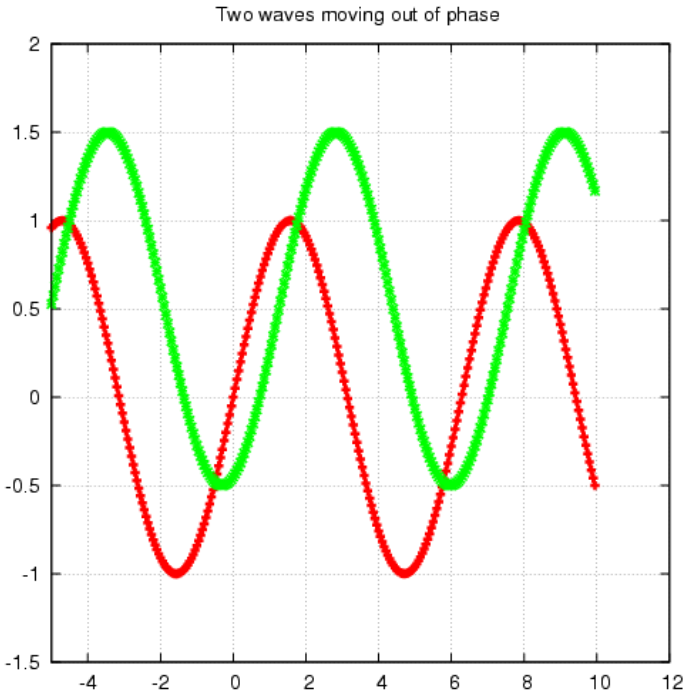


Phase Contrast

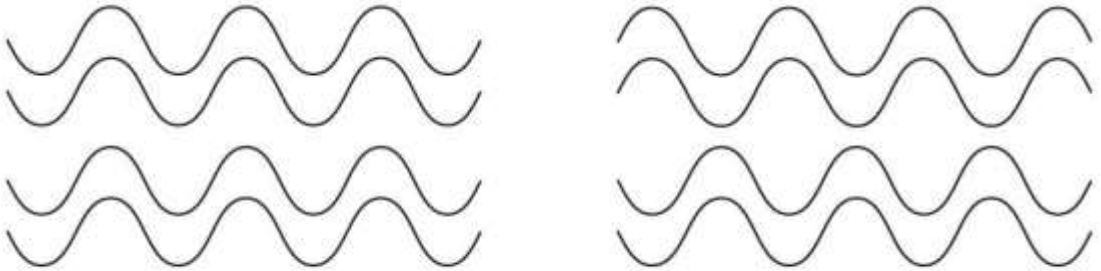
Principle of Phase Contrast Microscopes

- Highly refractive structures bend light to a much greater angle than do structures of low refractive index.
- The same properties that cause the light to bend also delay the passage of light by a quarter of a wavelength or so.

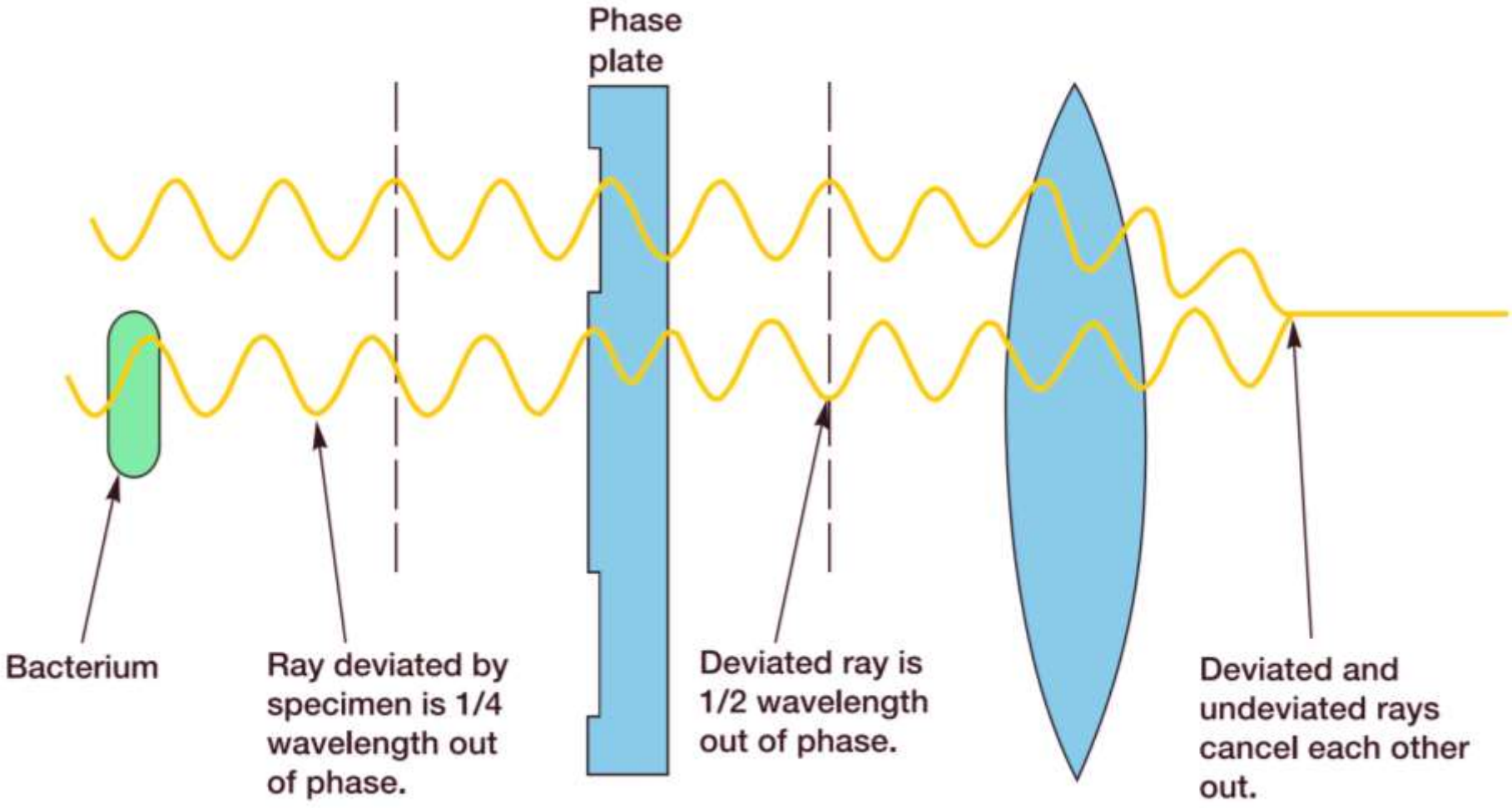




- In a light microscope in bright field mode, light from highly refractive structures bends farther away from the center of the lens than light from less refractive structures and arrives about a quarter of a wavelength out of phase.
- Light from most objects passes through the center of the lens as well as to the periphery.



- Now if the light from an object to the edges of the objective lens is retarded a half wavelength and the light to the center is not retarded at all, then the light rays are out of phase by a half wavelength.
- They cancel each other when the objective lens brings the image into focus.
- A reduction in brightness of the object is observed.
- The degree of reduction in brightness depends on the refractive index of the object.

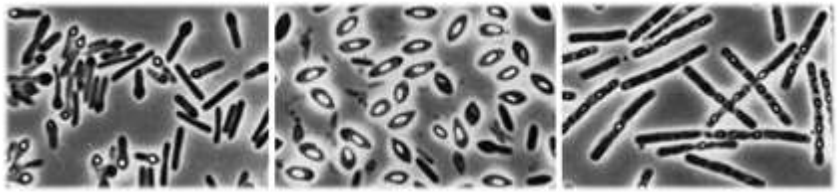


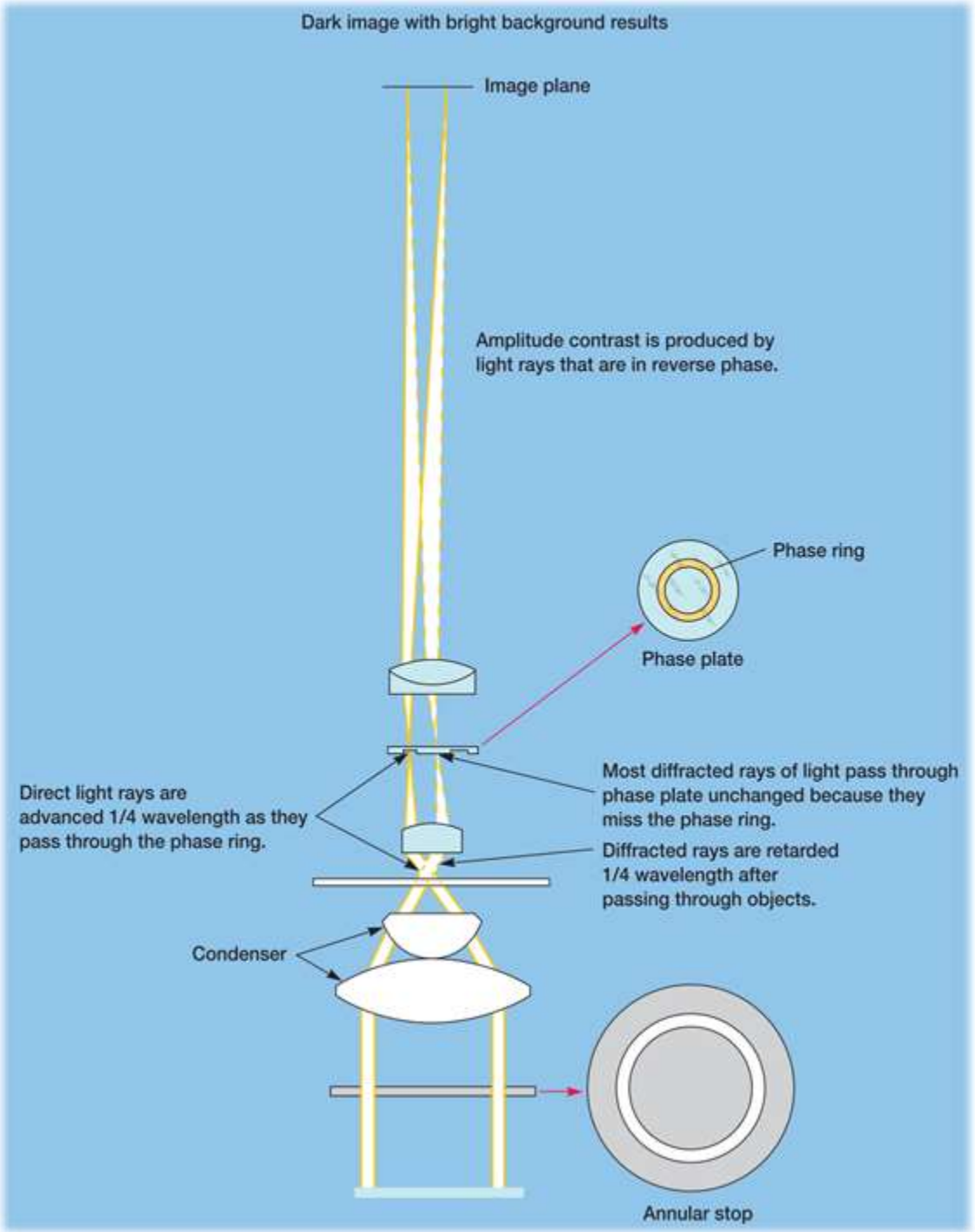
The Production of Contrast in Phase Microscopy

Working of a phase-contrast microscope

- The 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 ¼ 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 ¼ wavelength, the deviated and undeviated waves will be about ½ 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.

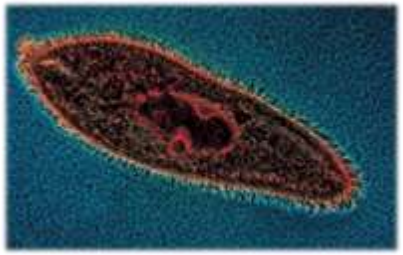


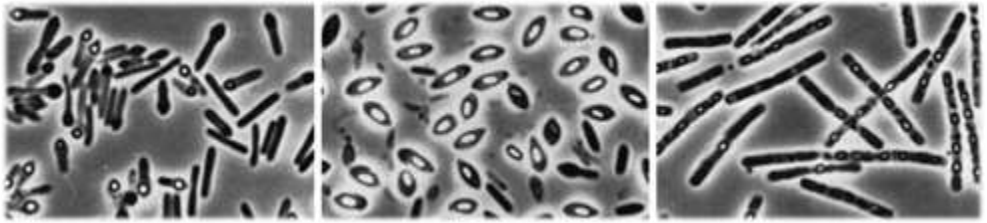


The optics of a dark-phase contrast microscope

Applications of Phase Contrast Microscopes

- Phase-contrast microscopy is especially useful for studying microbial motility, determining the shape of living cells, and detecting bacterial components such as endospores and inclusion bodies that contain poly-hydroxybutyrate, polymetaphosphate, sulfur, or other substances.
- These are clearly visible because they have refractive indexes markedly different from that of water.
- Phase contrast microscopes also are widely used in studying eukaryotic cells.





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