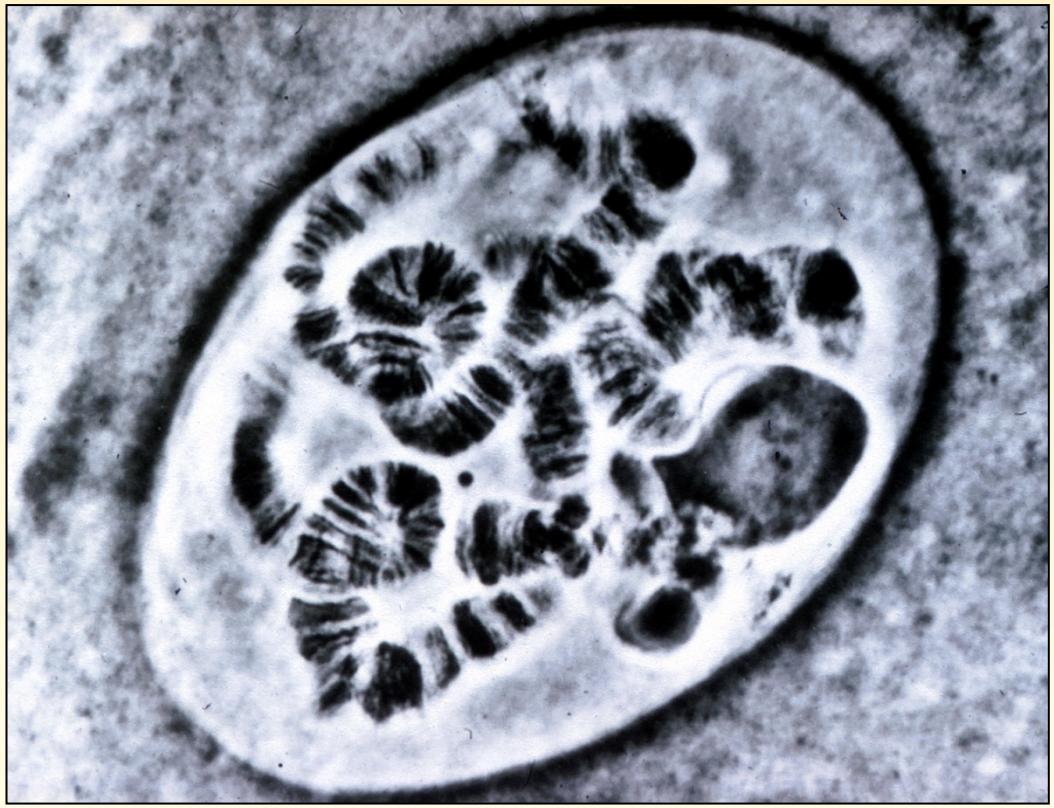
3. Phase Contrast



Chromosomes of *Chironomus*

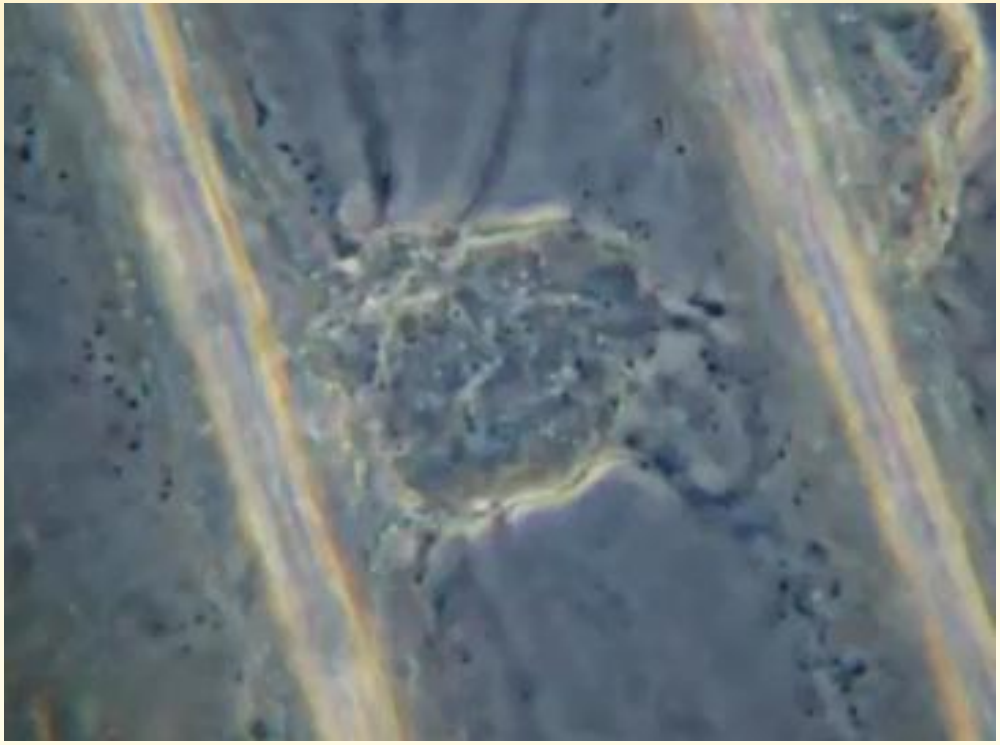
Phase contrast Kurt Michel 1942

3. Phase Contrast

http://www.youtube.com/watch?v=VXbQpRpUDmQ

Advantages:

- Living samples
- no staining required



3. Phase Contrast

Frits Zernike

(1886 – 1966)

- Dutch physicist
- discovered principle of phase contrast 1932
- not manufactured until 1941 by Zeiss
- won nobel prize in physics 1953 for his work

http://de.wikipedia.org

3. Phase Contrast

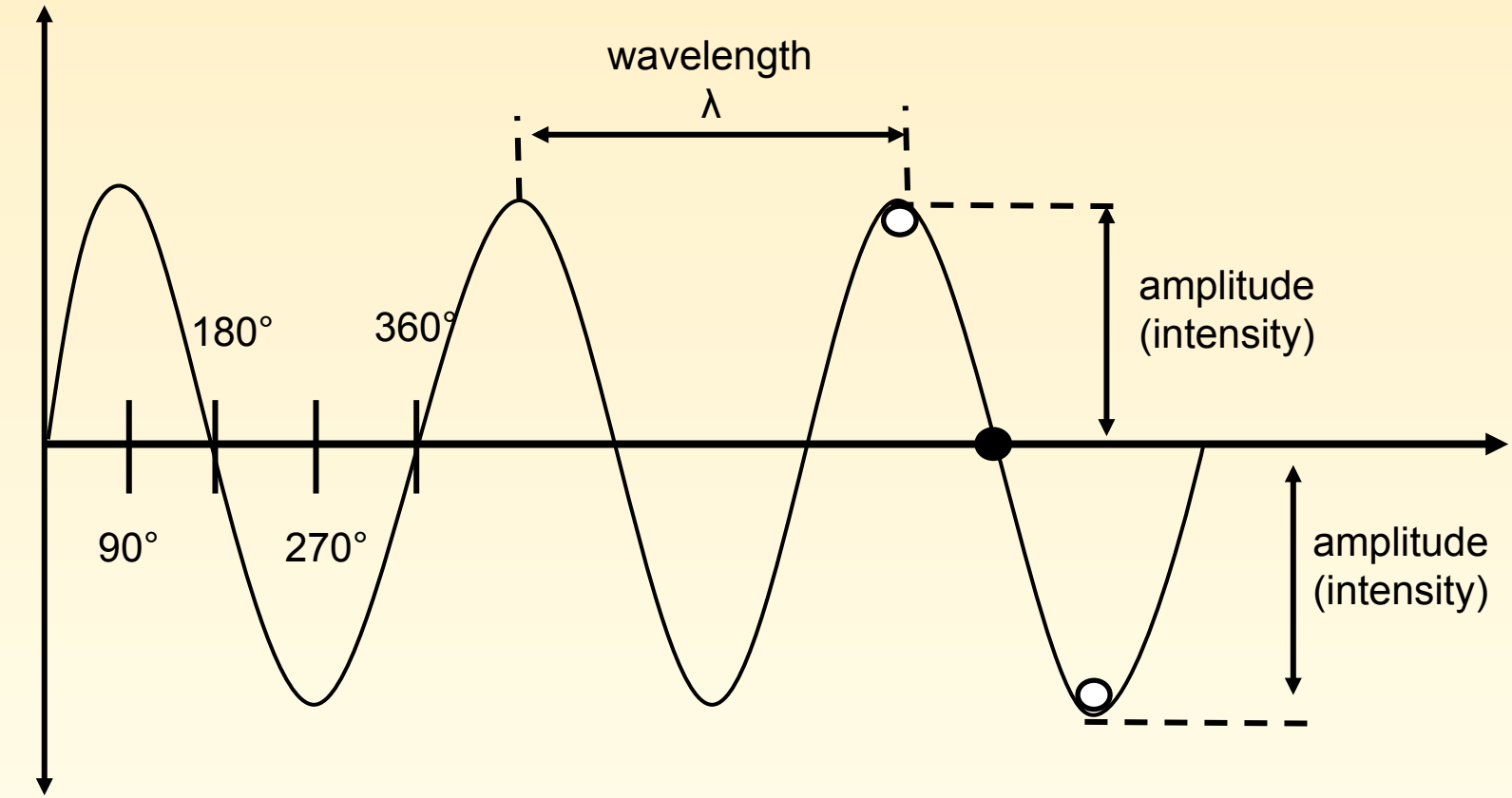
In transmitted brightfield illumination contrast is usually generated through differential absorption of light by the sample.

Transparent / unstained samples do not absorb light (or very little).

The effect of phase contrast illumination is

as if we stained the object with a dye
which stains each point with an intensity
proportional to the product of ist **thickness** and **refractive index.**

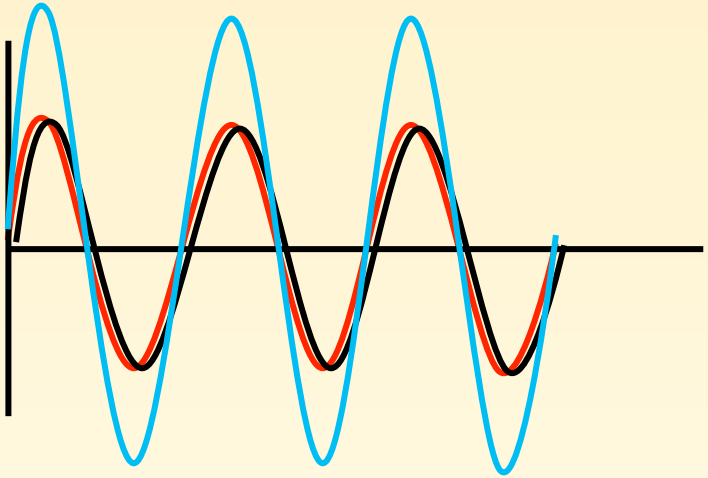
3. Phase Contrast



phase: the positioning of the peaks and troughs at a given time point.

3. Phase Contrast

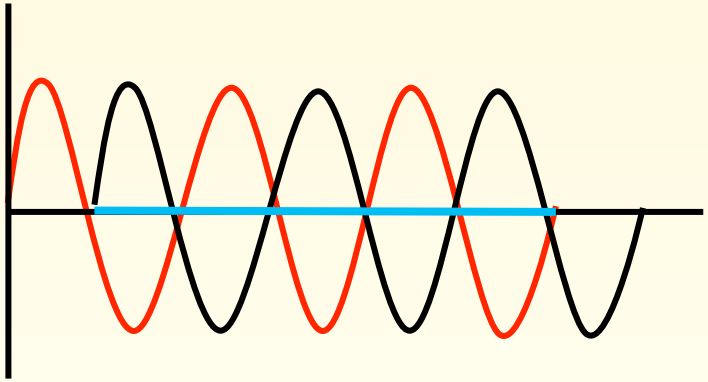
Waves can interfere (adding together): amplitude of the resulting wave depends on the phase relation of the interfering waves



constructive interference -

peaks correspond

(phase difference of 0°or 360°)



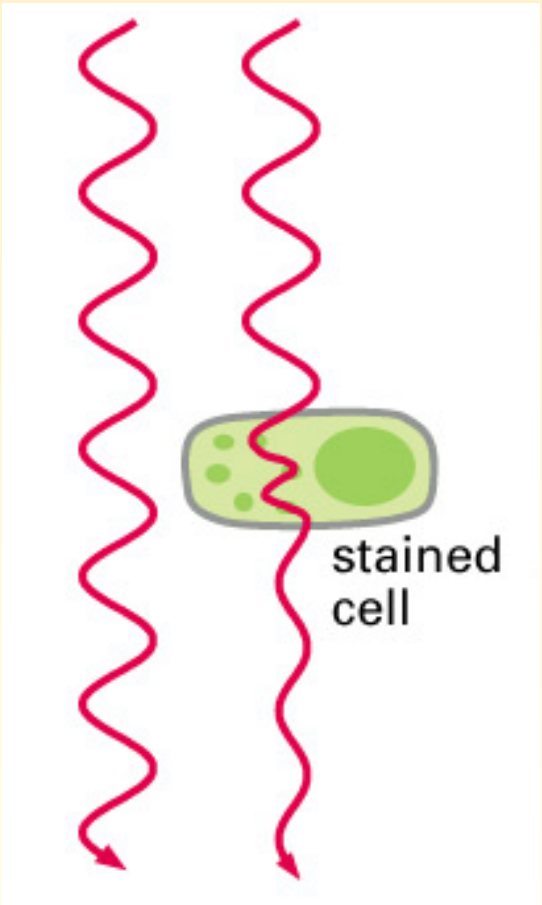
destructive interference –

peaks and troughs

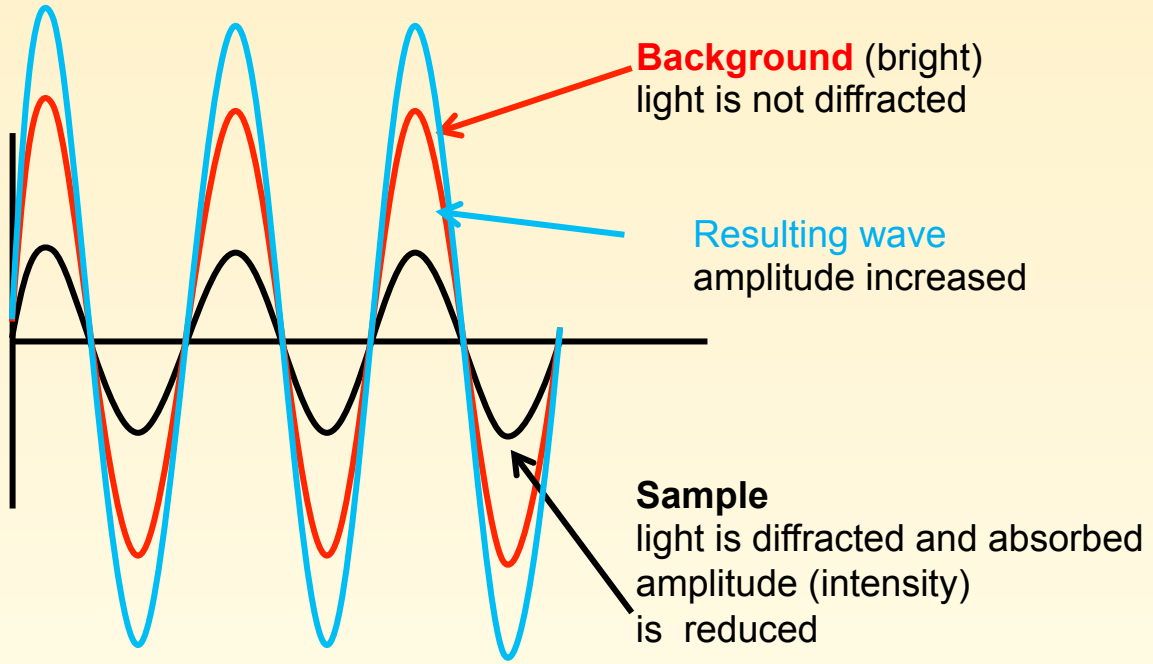
(phase difference of 180°)

http://www.mikroskopie.de/pfad/bildentstehung/drei.html

3. Phase Contrast



Amplitude objects



Changes in amplitude result in differences in image contrast

Fig.: 9-7 Molecular Biology of the Cell, 4t Edition

3. Phase Contrast

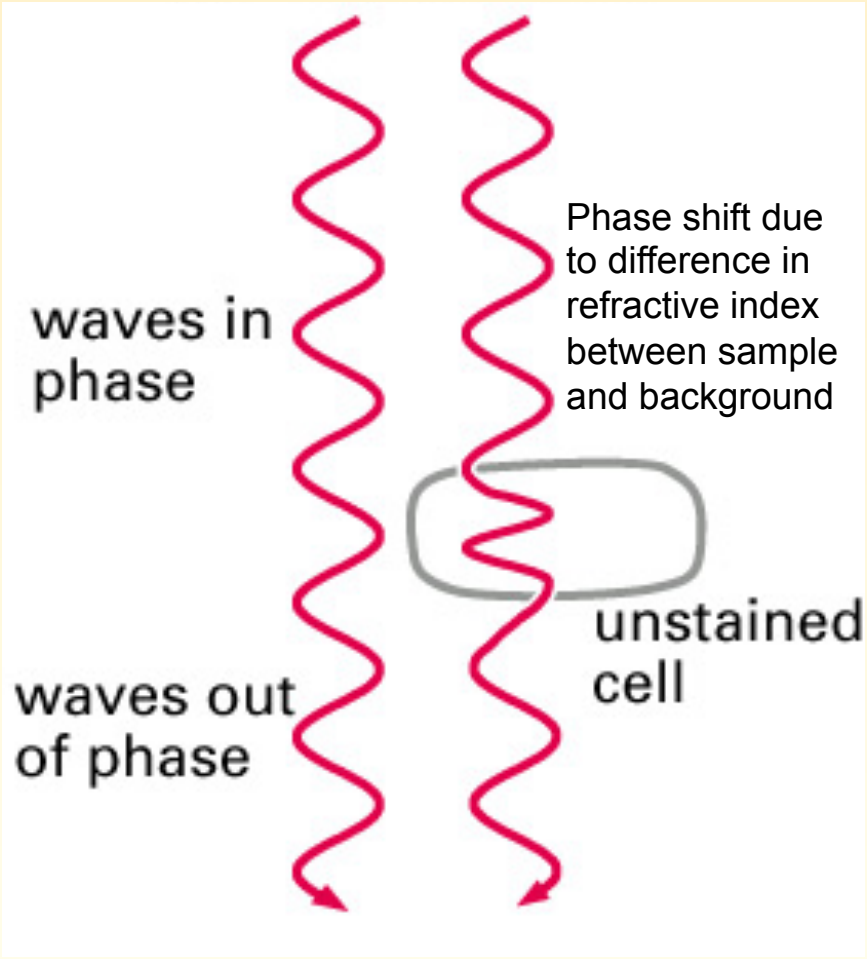
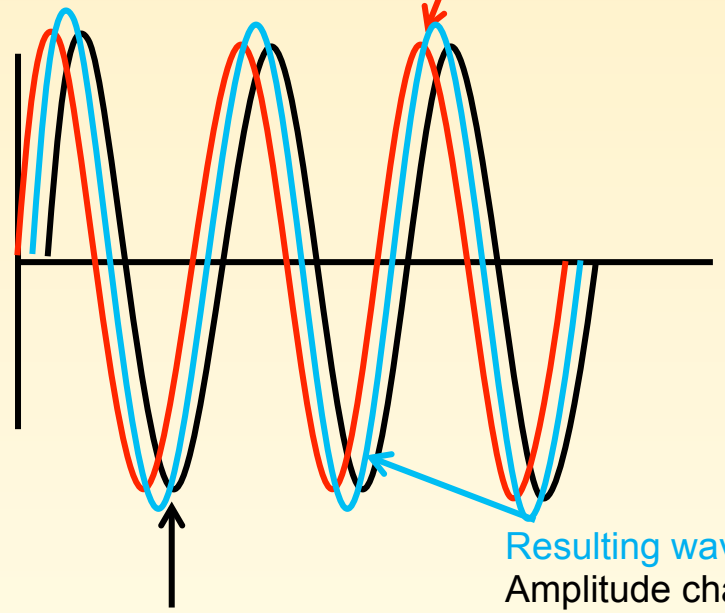


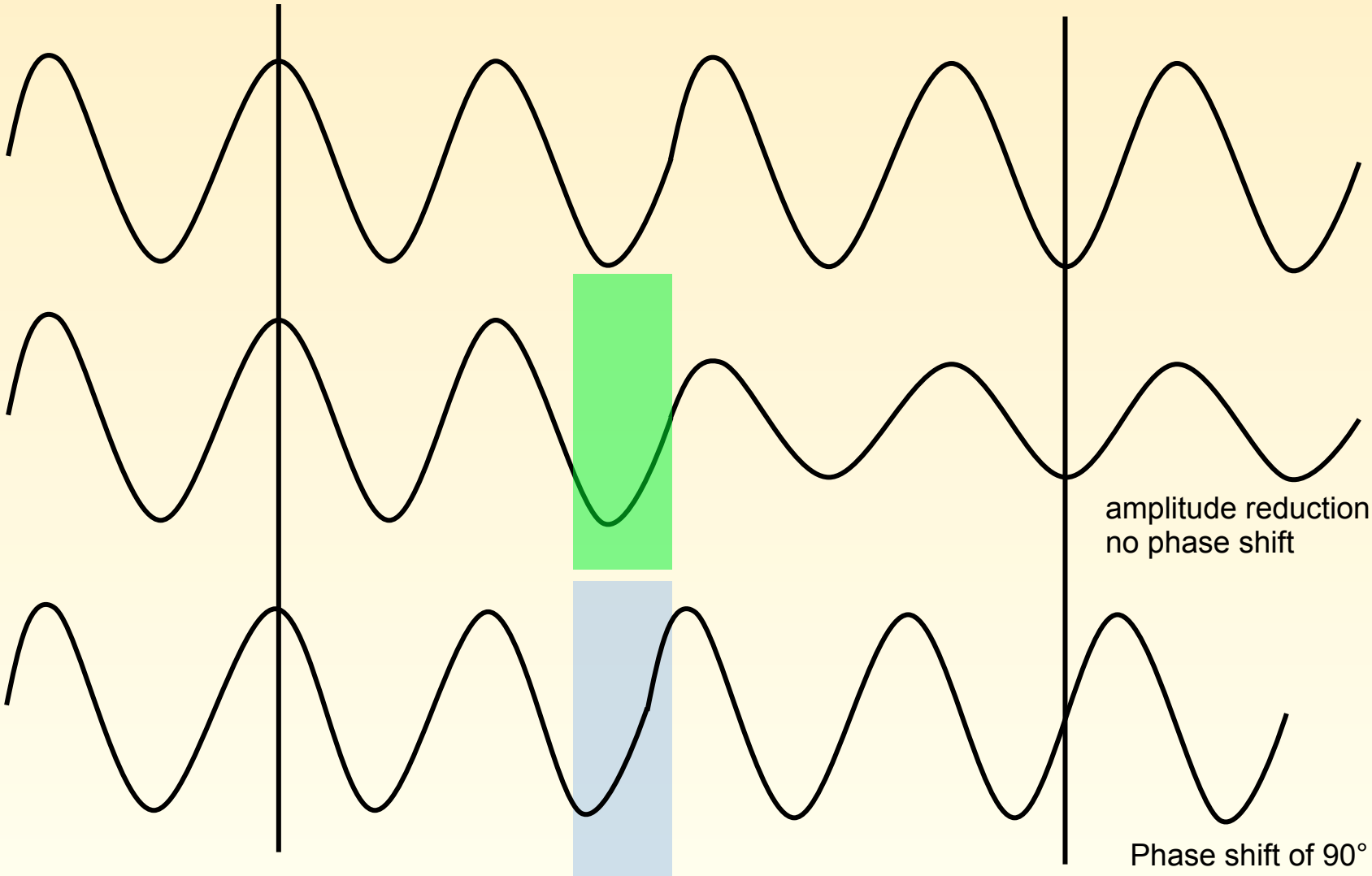
Fig.: 9-7 Molecular Biology of the Cell, 4t Edition

Phase object



Changes in phase alone do NOT result in differences in image contrast

3. Phase Contrast



3. Phase Contrast – Phase shift

Example: Cell in medium

Calculate optical path difference (OPD) **Δ**:

Δ = (refractive index medium 2-refractive index medium 1) • thickness of object

Δ = (n2-n1) • t

Δ = (1.36-1.335) • 5µm = 0.125µm = 125nm

Calculate phase shift **δ**:

δ = 2π • Δ / λ

δ = 360° • 125nm / 500nm = 90° (=λ/4)

This is difference in phase of a wave passing through a cell against a wave passing next to a cell

3. Phase Contrast

Problem:

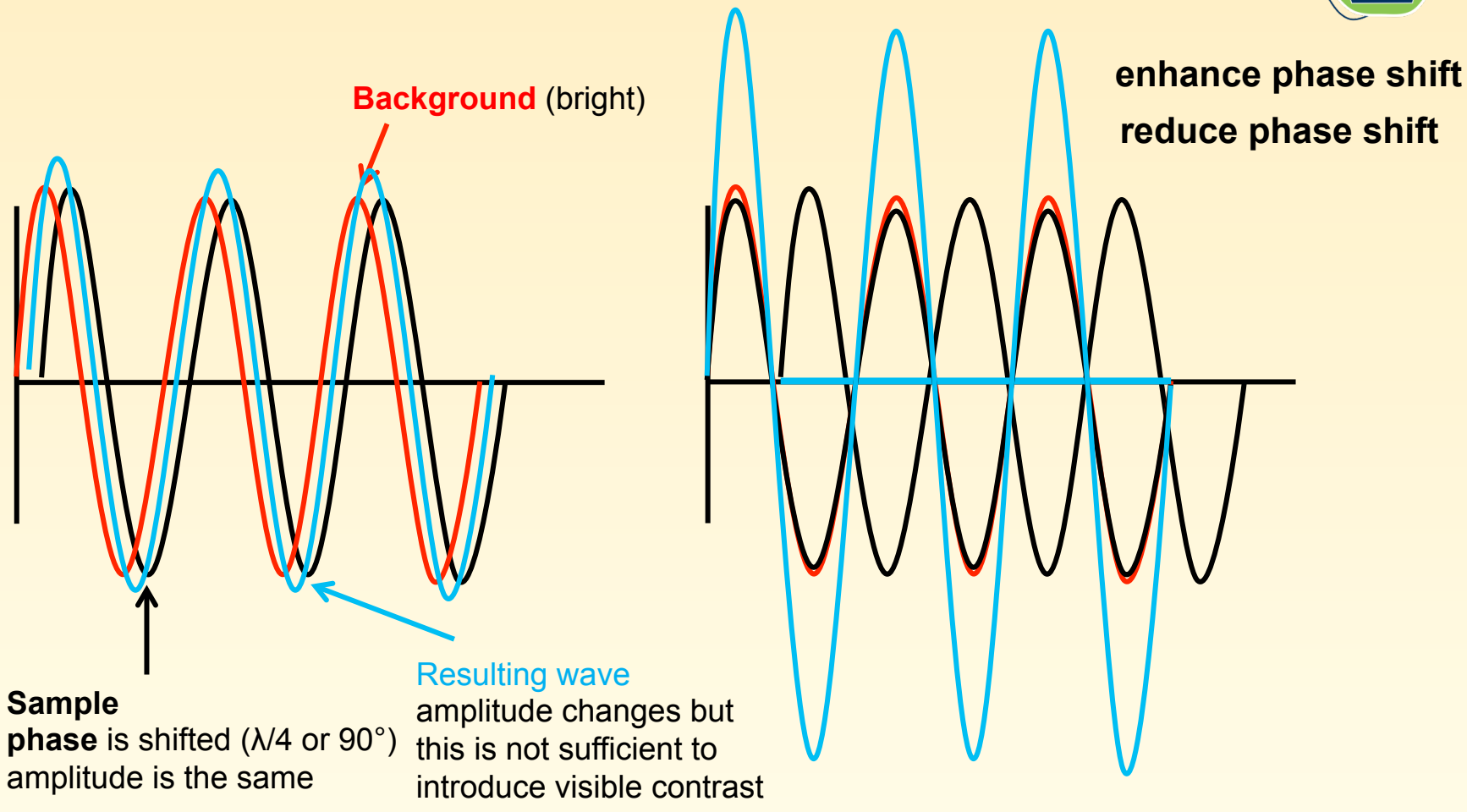
The human eye cannot detect **phase shifts**

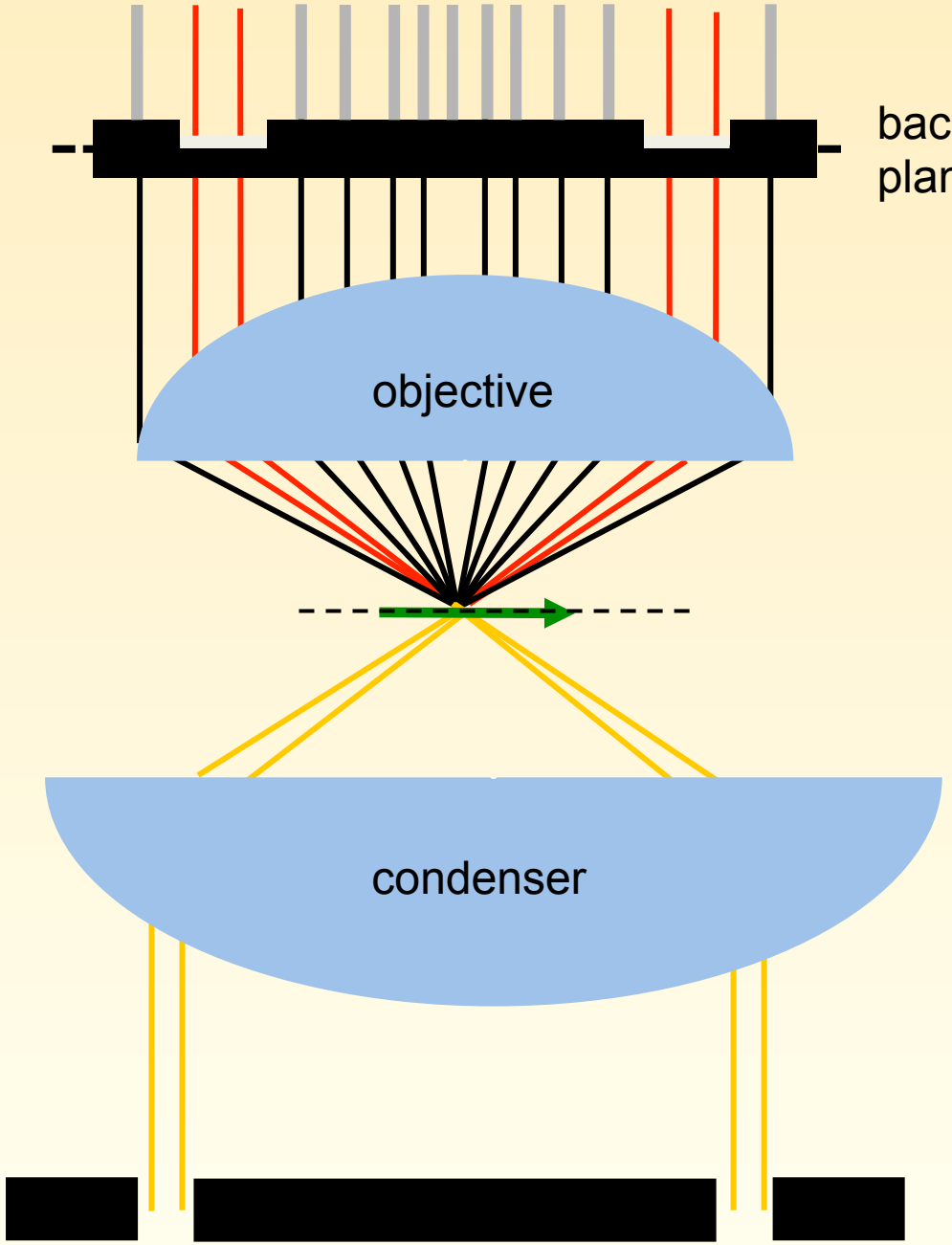
But — information **is** present in light beams from specimen and in image
How do we see this?

Phase contrast technique
transforms phase shift into amplitude differences that are observable by the human eye.

The change in phase produced by the difference in refractive index between specimen and surroundings can be as small as 1/20th of a wavelength and still produce phase contrast.

3. Phase Contrast





direct light is partially absorbed to reduce brightness

back focal plane

Phase plate **retards** scattered light another ¼λ, providing ½λ phase difference

Specimen diffracts light and **retards** it a little - about ¼ λ

Illuminating annulus in front focal plane of condenser

3. Phase Contrast

Phase plate in the objective

Glass

Partially absorbing material

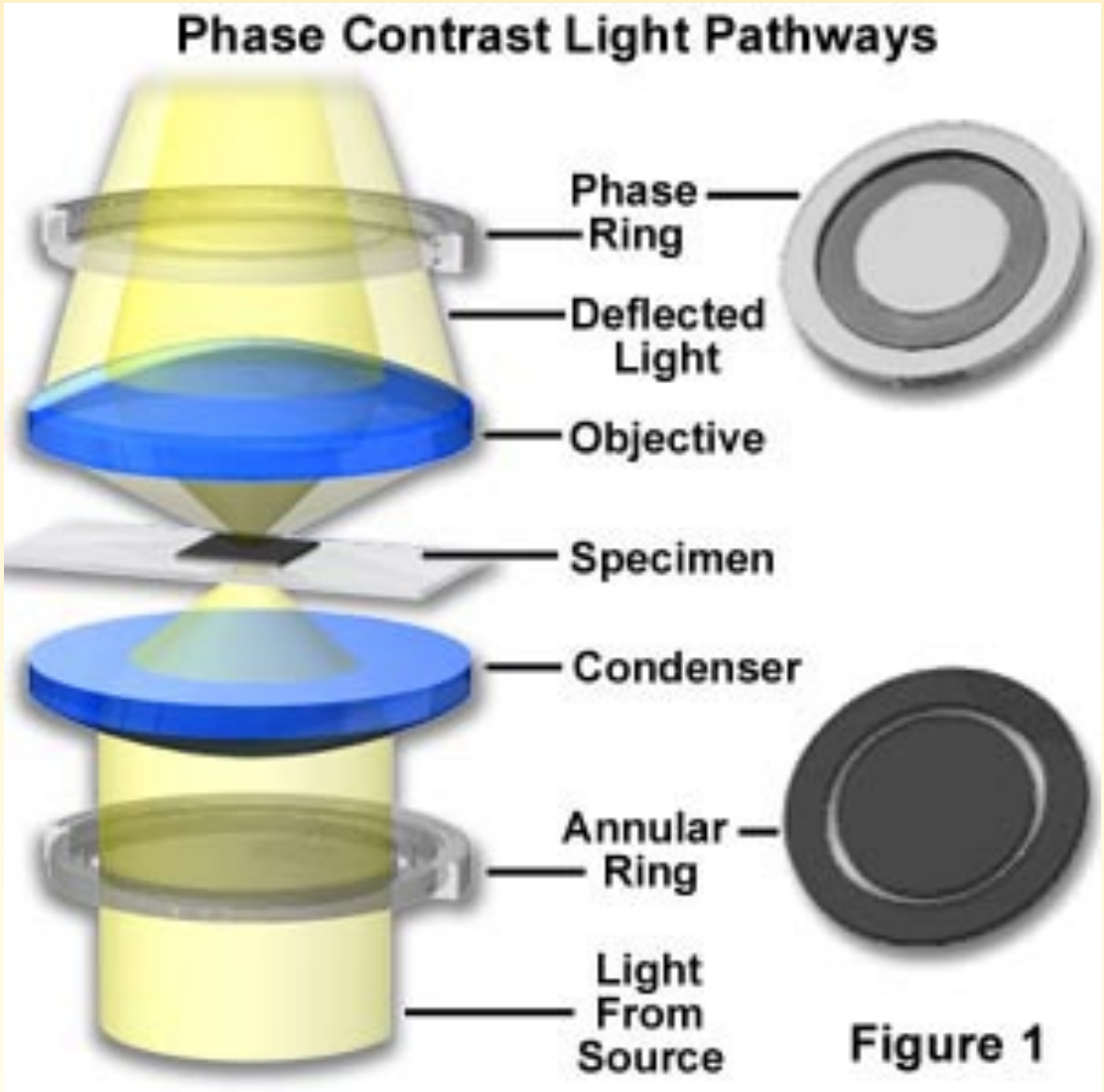
Phase retarding material

direct light is partially absorbed to reduce brightness

diffracted light is retarded

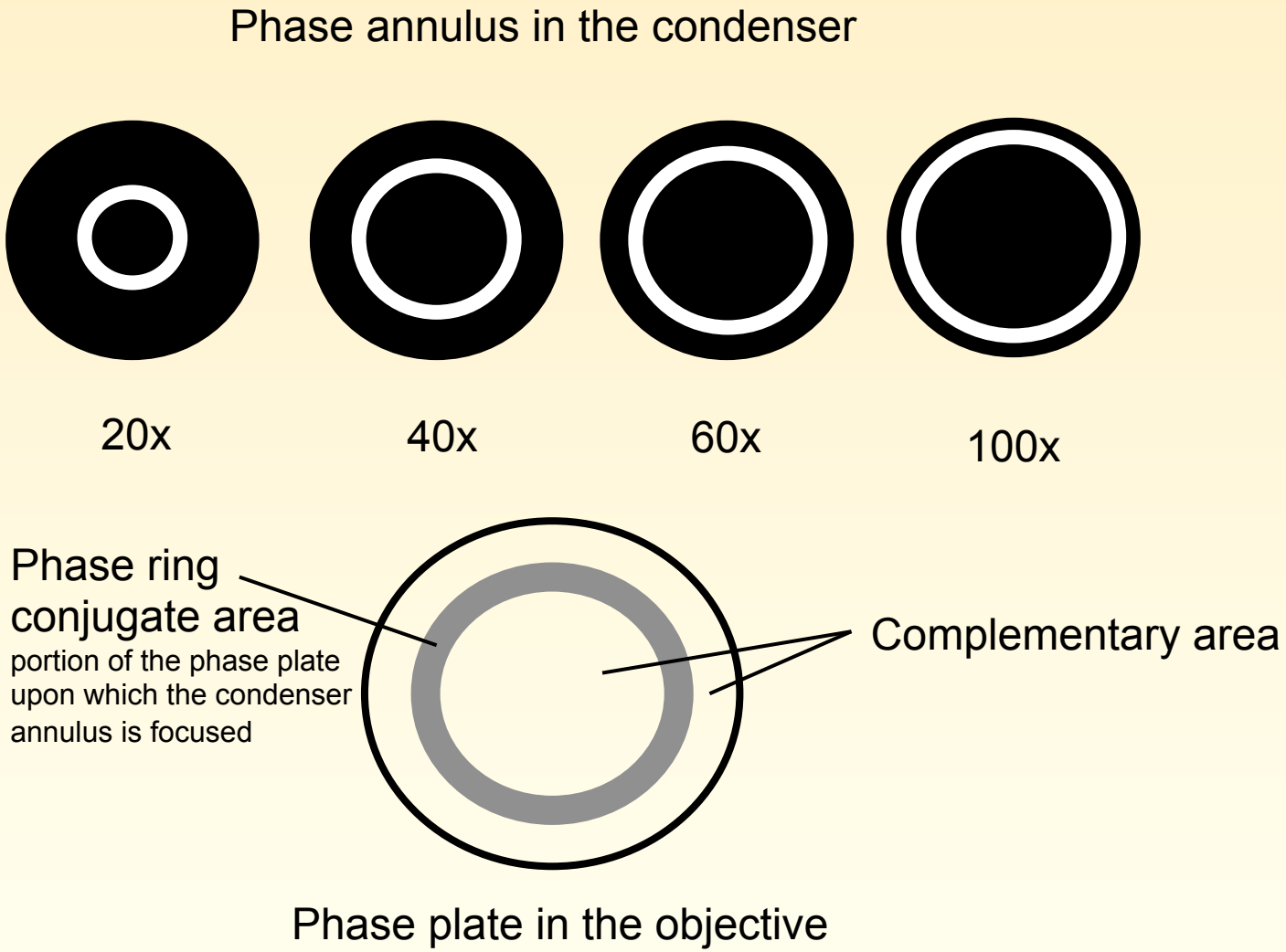
modified Theory & Appl. Light Microscopy

3. Phase Contrast – Set up



Theory & Appl. Light Microscopy

3. Phase Contrast



Adjustment – as seen in BFP

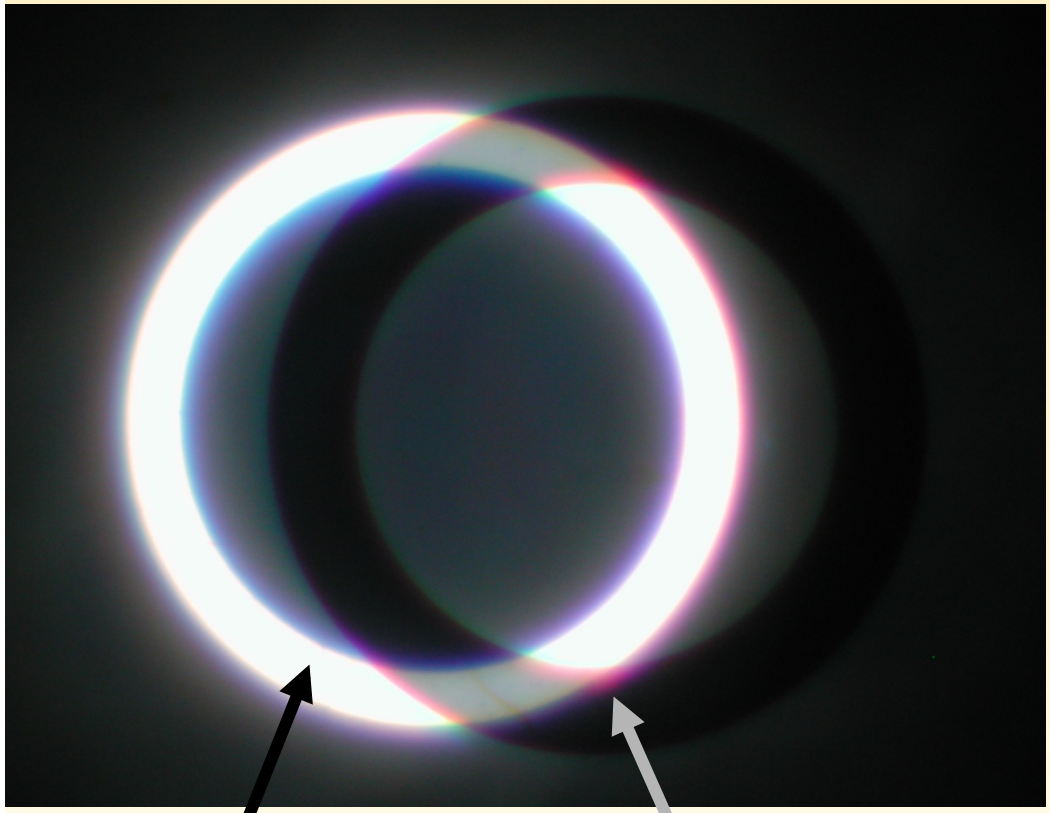
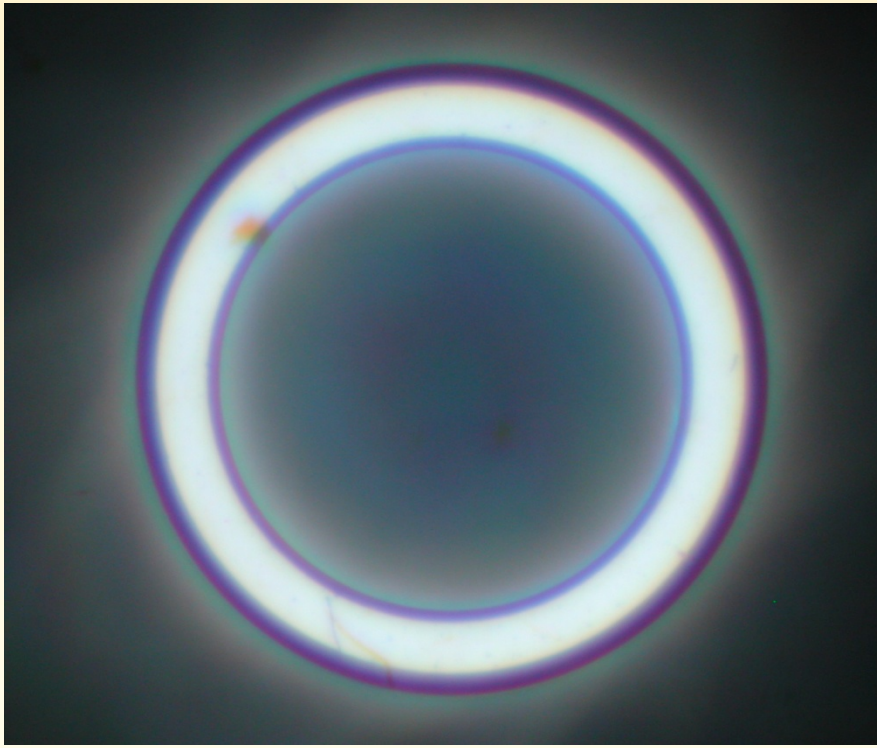


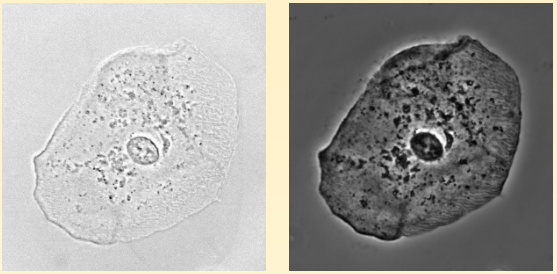
Image of annulus

Phase ring

Illuminating annulus adjusted so that its image coincides with phase ring



3. Phase Contrast – Pros/Cons



- equipment not expensive and easy to set up
- no staining required
- live cells
- use for **qualitative,** not quantitative evaluation of specimens
  Intensity differences in image not uniquely related to index of refraction differences of specimen
- not suitable in combination with Fluorescence microscopy because phase contrast objectives „eat“ light
- artifacts
  Phase halo — optical artifact most prominent at boundaries of sharp differences in refractive index

Practical Phase Contrast

- **Setup:**
  - Köhler the microscope (use another microscope than before)
  - adjust illuminating annulus in first focal plane of the condenser, so that its image falls on the phase ring in the back focal plane of the objective
    - To observe this:
    - remove eyepiece
    - use Telescope, or Bertrand lens

- **Objective:**
  - Several phase contrast objectives and appropriate annuli in the condenser (objectives are not suited for fluorescence)

- **Specimen:**
  - cheek cells, diatomes
  - good for: thin cell layers, cell division