Phase Contrast Microscopy

(Lit.: Bergmann Schaefer, Optik, p. 415)

F. Zernike 1932

→ Problem: biological objects such as cells are nearly transparent
⇒ barely visible in normal light microscope
⇒ requires staining of cells, stains harmful

→ interaction of light with matter:
- amplitude modulation, object absorbs light
- phase modulation, no absorption

→ object and image are related by a Fourier-transformation:

$$E(x,y) \sim \mathrm{Re}\, e^{-\frac{2\pi i l}{\lambda}} \iint F(\xi,\zeta)\, e^{2\pi i \omega t} \cdot e^{-\frac{2\pi i}{\lambda f}(x\xi + y\zeta)}\, d\xi\, d\zeta$$

($E(x,y)$: image)

f: focal length of lense

l: distance image object

$\mathrm{Re}\, F(\xi,\zeta)\, e^{-2\pi i \omega t}$: electrical field in the image plane

in case of amplitude modulation:
- $F(\xi,\zeta)$: real ⇒ amplitude modulation
- $e^{-2\pi i \omega t}$: constant phase
- spatially varying absorption

phase modulation: $F(\xi,\eta) = e^{-i\nu^2(\xi,\eta)}$

cells are thin objects ⇒ weak phase modulation

$\nu^2 \ll 1 \Rightarrow F(\xi,\eta) \approx 1 - i\nu^2(\xi,\eta)$

$-i = e^{-i\pi/2}$

$$\Rightarrow E_P(x,y) \sim Re\, e^{-\frac{2\pi i l}{\lambda}} \iint e^{2\pi i \omega t} e^{-\frac{2\pi i}{\lambda f}(x\xi+y\eta)} d\xi d\eta$$

unscattered wave

$$+ Re\, e^{-\frac{2\pi i l}{\lambda} - \frac{i\pi}{2}} \iint \nu^2(\xi,\eta) e^{2\pi i \omega t} e^{-\frac{2\pi i}{\lambda f}(x\xi+y\eta)} d\xi d\eta$$

scattered wave

amplitude modulation: thin sample, weak absorption

$F(\xi,\eta) = e^{-k(\xi,\eta)} \approx 1 - k$

$-1 = e^{-i\pi}$

$$E_A(x,y) \sim Re\, e^{-\frac{2\pi i l}{\lambda}} \iint e^{2\pi i \omega t} e^{-\frac{2\pi i}{\lambda f}(x\xi+y\eta)} d\xi d\eta$$

unscattered wave

$$+ Re\, e^{-\frac{2\pi i l}{\lambda} - i\pi} \iint k(\xi,\eta) e^{2\pi i \omega t} e^{-\frac{2\pi i}{\lambda f}(x\xi+y\eta)} d\xi d\eta$$

⇒ • picture is an interference phenomena between scattered and unscattered wave

• difference between phase and amplitude modulation is a phase factor of $e^{-i\pi/2}$!

phase contrast microscopy:
converting ~~an amplitude~~ a phase object into an amplitude object!

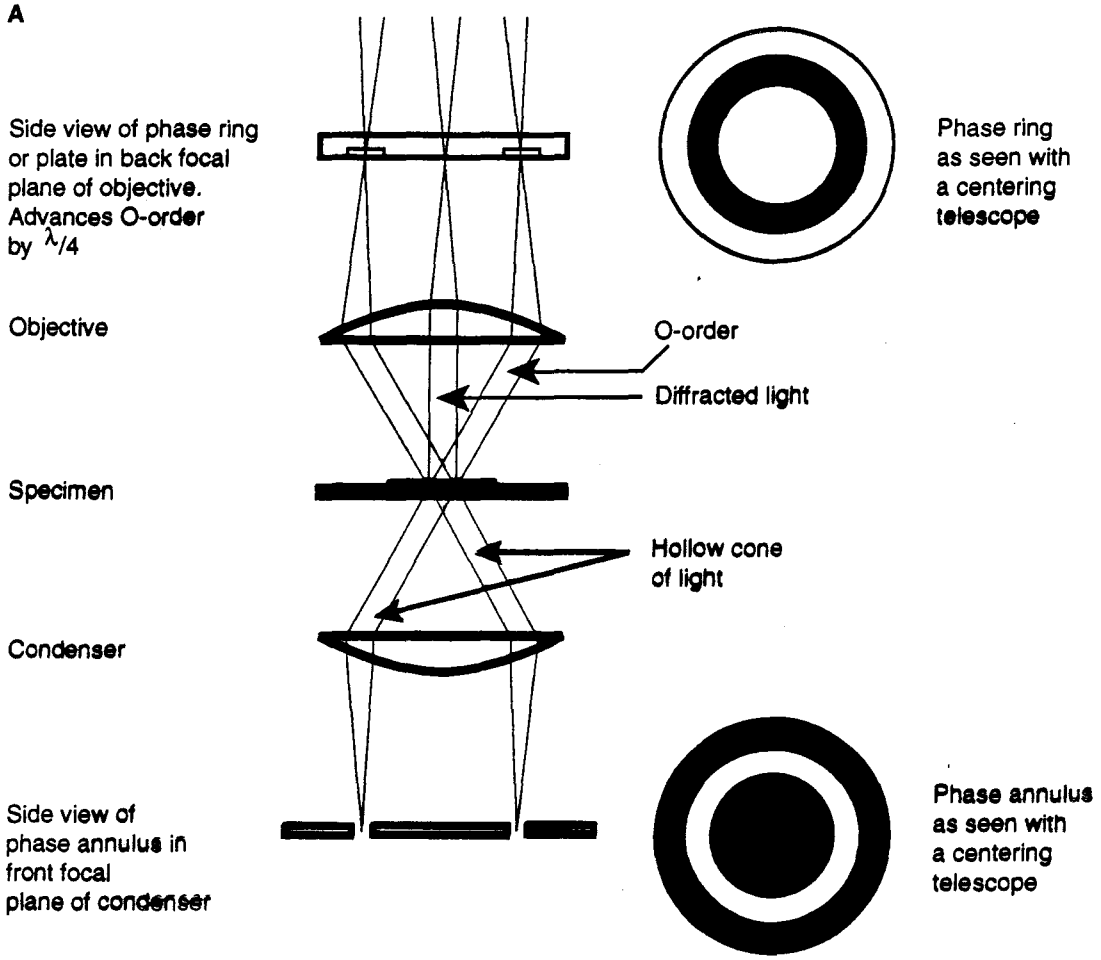
⇒ phase structure become visible if an additional phase difference of $\pi/2$ is added to the scattered wave

⇒ this can be achieved ~~by~~ with a phase plate in the focal plane which add another $\lambda/4$ in optical path length to the scattered wave

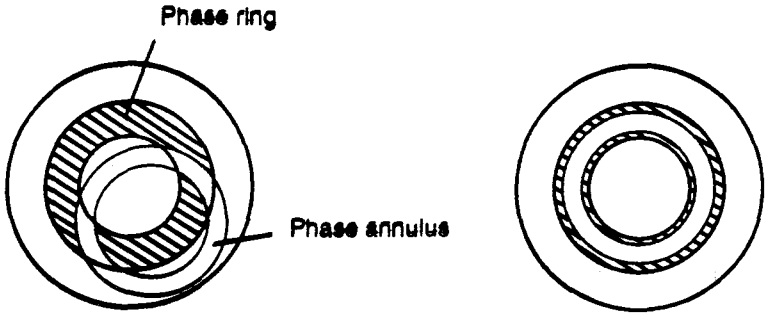
the according optical setup is shown in the transparency!

Note: The phase ring is also slightly opaque to reduce the intensity of the unscattered wave!

A


Side view of phase ring or plate in back focal plane of objective. Advances O-order by λ/4
Objective
Specimen
Condenser
Side view of phase annulus in front focal plane of condenser
O-order
Diffracted light
Hollow cone of light
Phase ring as seen with a centering telescope
Phase annulus as seen with a centering telescope

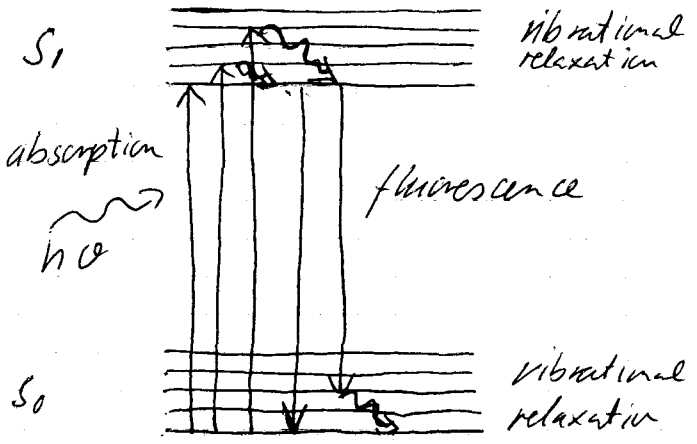
B


Phase ring
Phase annulus

a b

Fluorescence microscopy

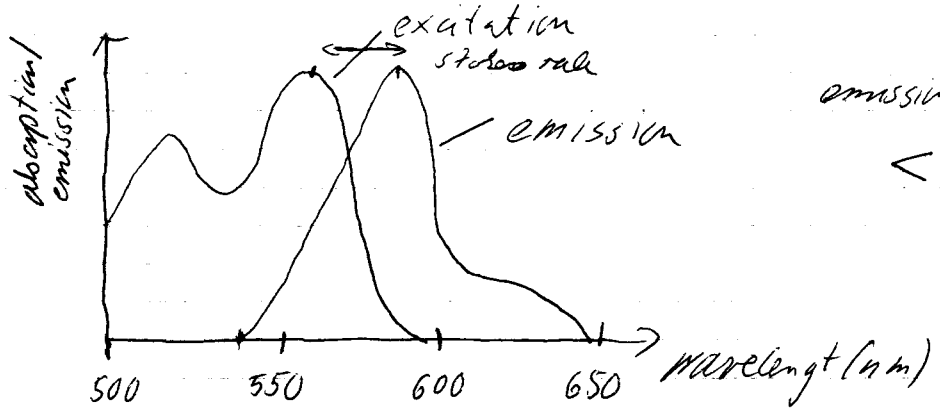
1) Fluorescence:



Lit.:

- H. Rosenek, Mikroskopie in Forschung und Praxis, C.I.T. Verlag
- D. L. Spector, R. D. Goldmann, L. A. Leinwand, Cells - A laboratory manual, Light Microscopy and cell Structure, Cold Spring Harbour Press

Excitation and emission spectrum of the fluorophore Alexa Fluor 555



emission intensity < excitation intensity

idea: to stick fluorophore of a certain colour on a specific cellular structure or protein