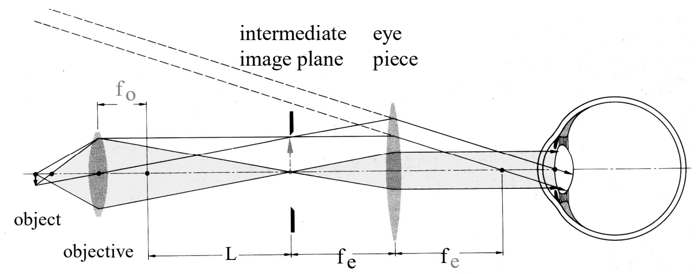
2 Classical optics and microscopy

Classical Microscopy



E. Hecht, Optik (Addison-Wesley 1989)

Ernst Abbe

1840 - 1905

Founder of the theory of microscopic imaging

Zeiss objectives according to Abbe

1600 | 1800

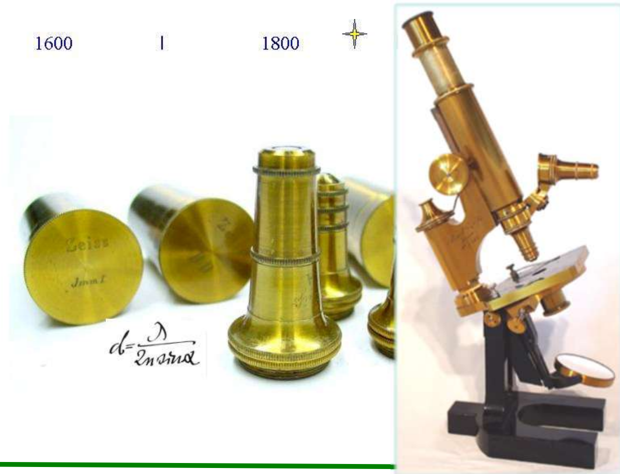


Foto: T. Mappes Zeiss

Zeiss objectives according to Abbe

1600 | 1800 | 2000

29

Zeiss

Modern microscope objectives

Modern microscope

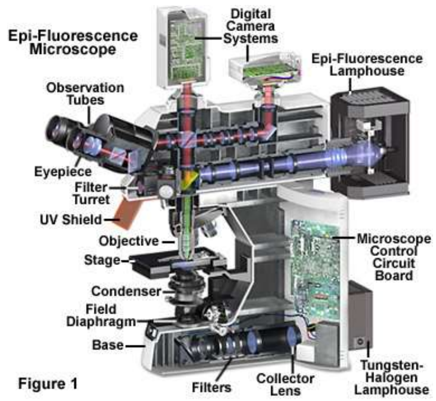
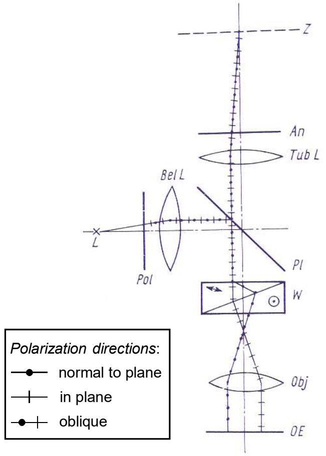


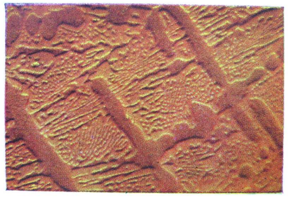
Figure 1

Insect on a water surface

Differential interference contrast (DIC)

Set-up according to Nomarski (reflected-light microscope)



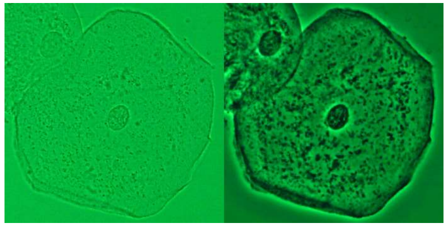


polished metal surface

H. Beyer, Interferenzmikroskopie

Phase Contrast

Unstained epithelial cell



Brightfield **Phase contrast**

http://www.microscopy-uk.org.uk/mag/artmar06/go-phase.html

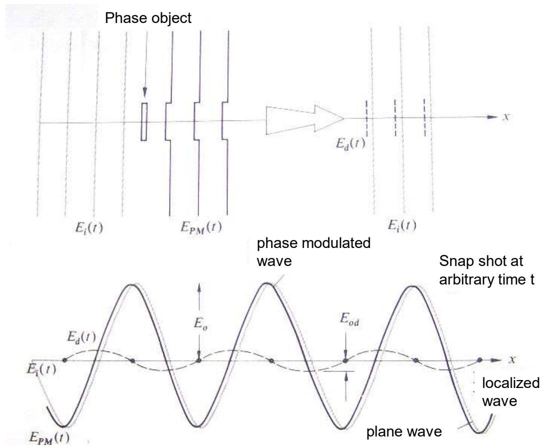
Frits Zernike

Invention of the Phase-Contrast-Microscope (1930)

Nobel prize 1953

1888 - 1966

Principle of Phase contrast Imaging



Principle of Phase contrast Imaging

Incident wave: $E_i = E_0 \sin \omega t$

Phase-modulated wave at $x = 0$:

$$E_{PM}(r,t)\Big|_{x=0} = E_0 \sin\big(\omega t + \Phi(y,z)\big) \quad = \quad E_0 \sin \omega t \cdot \cos \Phi + E_0 \cos \omega t \cdot \sin \Phi$$

If Φ is very small, then

$$E_{PM}(y,z,t) \cong \underbrace{E_0 \sin \omega t}_{E_i} + \underbrace{E_0 \cos \omega t \cdot \Phi(y,z)}_{E_d}$$

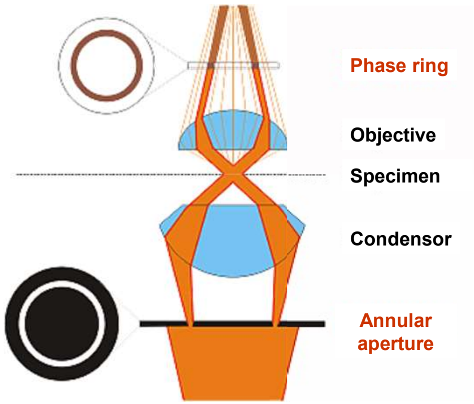
Basic idea: Change the phase of E_i after transmission by $\frac{\pi}{2}$

$$\left.\begin{array}{l} \sin \omega t \;\rightarrow\; \cos \omega t \\ E_{PM} \;\rightarrow\; E_{AM} \end{array}\right\}$$

Amplitude-modulated wave

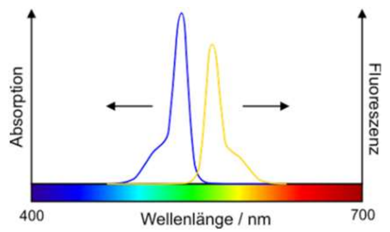
$$E_{AM}(y,z,t) = \big(1 + \Phi(y,z)\big) \cdot E_0 \cos \omega t$$

Phase contrast microscope

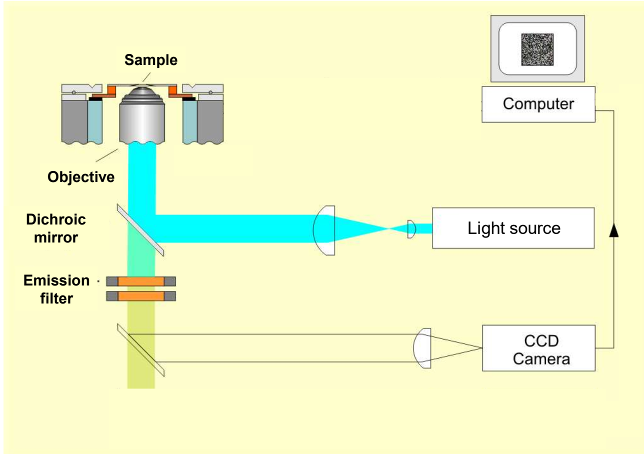


www.mikroskopie.de

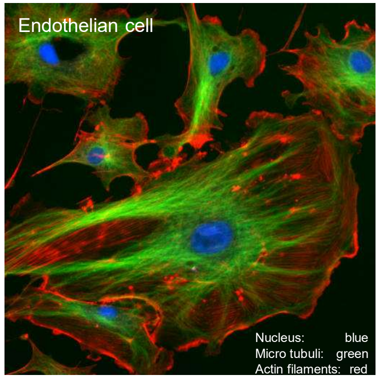
Absorption & Emission of Molecules



Fluorescence microscopy

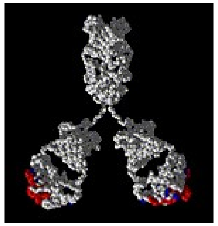


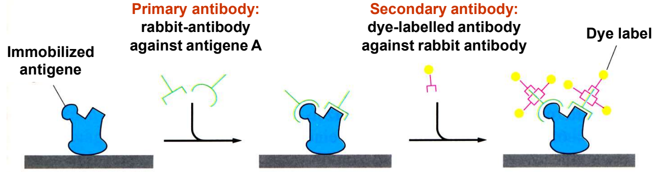
Example



Wikipedia

Specific Labelling through antibodies





Alberts et al., Molekularbiologie der Zelle, VCH-Verlag