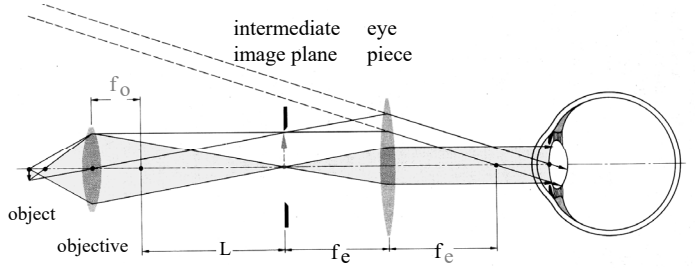
2 Classical optics and microscopy

2.2 *Methodology*

2.2.1 Conventional wide-field optical microscopy
2.2.2 Interference contrast microscopy
2.2.3 Phase contrast microscopy
2.2.4 Fluorescence microscopy
2.2.5 Confocal light scanning microscopy (CLSM)
2.2.6 Total internal reflection microscopy (TIRF)

Classical Microscopy



E. Hecht, Optik (Addison-Wesley 1989)

Zeiss objectives according to Abbe

Zeiss

Modern microscope objectives

Modern microscope

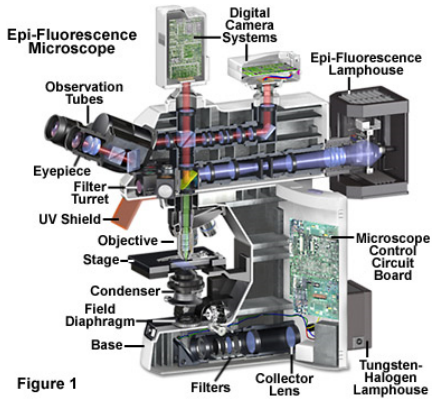
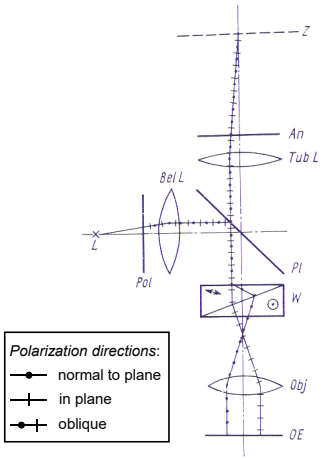


Figure 1

Insect on a water surface

Differential interference contrast (DIC)



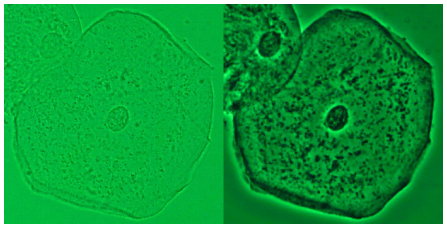
Set-up according to Nomarski (reflected-light microscope)

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

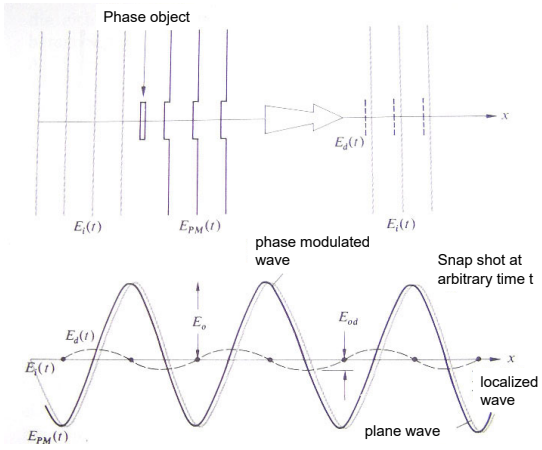
Frits Zernike

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 $x = 0$:

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

If Φ is very small, then

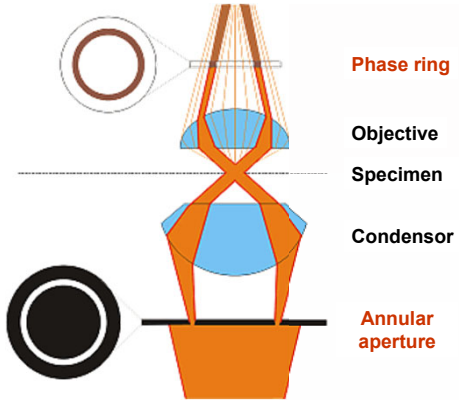
$$E_{PM}(y,z,t) \cong \underbrace{E_0 \sin \omega t}_{E_i} + \underbrace{E_0 \cos \omega t \cdot \Phi(y,z)}_{E_d}$$

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

$$\left.\begin{array}{rcl} \sin \omega t & \rightarrow & \cos \omega t \\ E_{PM} & \rightarrow & E_{AM} \end{array}\right\} \quad \textbf{Amplitude-modulated wave}$$

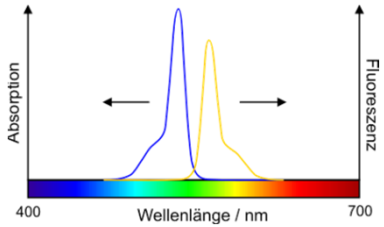
$$E_{AM}(y,z,t) = \big(1 + \Phi(y,z)\big) \cdot E_0 \cos \omega t$$

Phase contrast microscope

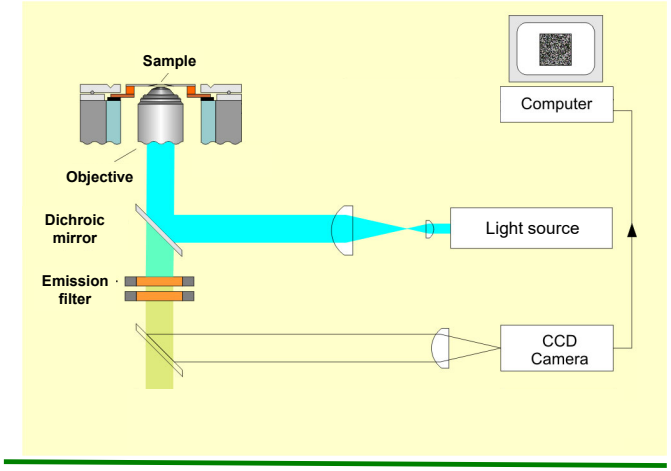


www.mikroskopie.de

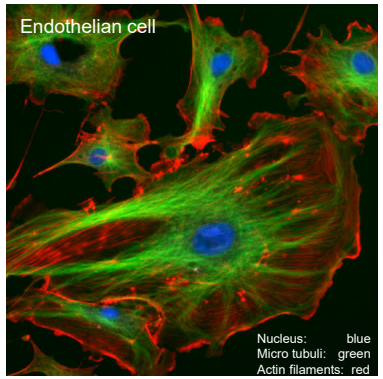
Absorption & Emission of Molecules



Fluorescence microscopy

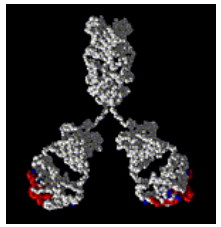


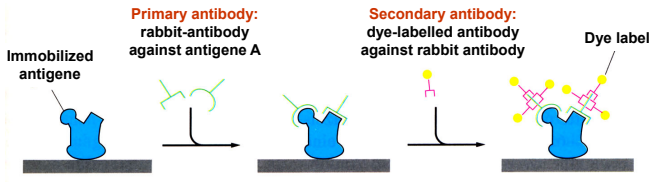
Example



Wikipedia

Specific Labelling through antibodies





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