

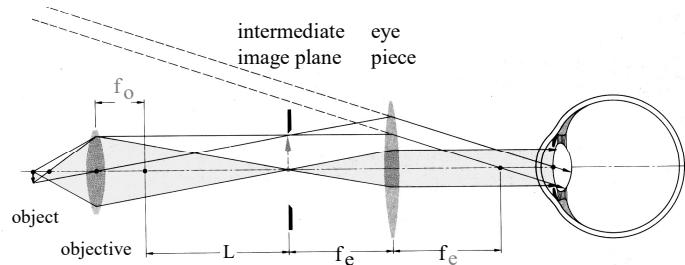
2 Classical optics and microscopy

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)

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Classical Microscopy



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E. Hecht, Optik (Addison-Wesley 1989)

Ernst Abbe



Founder of the theory of
microscopic imaging

1840 - 1905

Nanooptics 12/3

Zeiss objectives according to Abbe



Nanooptics 12/4

Foto: T. Mappes

Zeiss



Zeiss objectives according to Abbe

1600 | 1800 ⚡ | 2000



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Zeiss



Modern microscope objectives



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Modern microscope

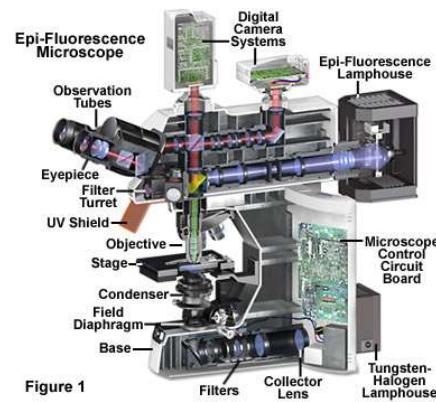


Figure 1

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Insect on a water surface



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KIT Differential interference contrast (DIC)

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

Polarization directions:

- normal to plane
- in plane
- oblique

Nano optics 12/9 H. Beyer, Interferenzmikroskopie

KIT Phase Contrast

Unstained epithelial cell

Brightfield Phase contrast

Nano optics 12/10 <http://www.microscopy-uk.org.uk/mag/artmar06/go-phase.html>

KIT Frits Zernike

Invention of the Phase-Contrast-Microscope (1930)

Nobel prize 1953

1888 - 1966

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KIT Principle of Phase contrast Imaging

Phase object

phase modulated wave

Snap shot at arbitrary time t

plane wave

localized wave

Nano optics 12/12



Principle of Phase contrast Imaging

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

Phase-modulated wave at $x = 0$:

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

If Φ is very small, then

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

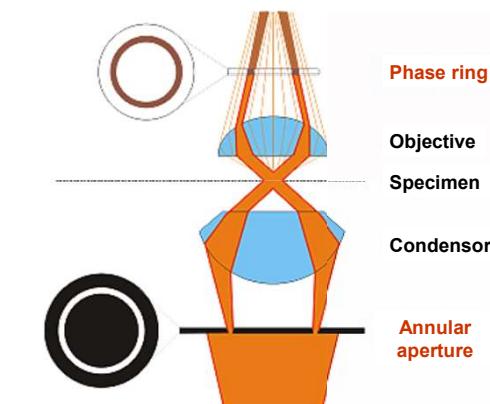
Basic idea: Change the phase of E_i after transmission by $\frac{\pi}{2}$

$$\begin{aligned} \sin \omega t &\rightarrow \cos \omega t \\ E_{PM} &\rightarrow E_{AM} \end{aligned} \quad \left. \begin{array}{l} \text{Amplitude-modulated wave} \\ E_{AM}(y, z, t) = (1 + \Phi(y, z)) \cdot E_0 \cos \omega t \end{array} \right\}$$

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Phase contrast microscope

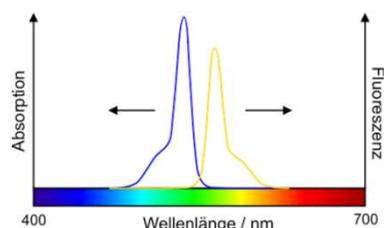


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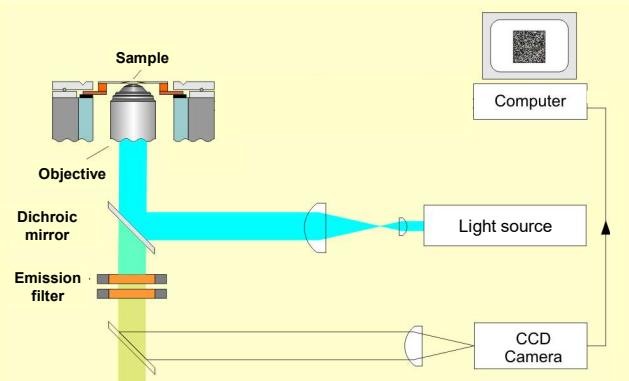
Absorption & Emission of Molecules



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Fluorescence microscopy



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