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2 Classical optics and microscopy

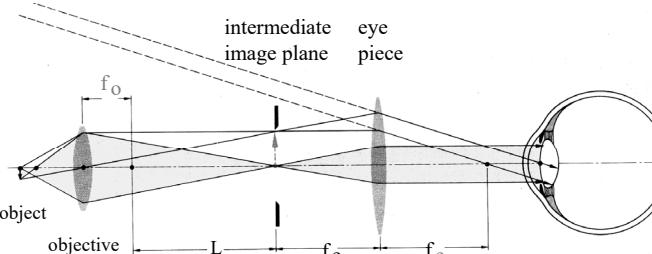
2.2 Methodology

- 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



The diagram illustrates the optical path in a microscope. Light rays from an object (represented by a small grey circle) pass through an objective lens, forming a real, inverted image at the intermediate image plane. This image then passes through an eyepiece lens, which creates a second, virtual, upright image at the eye piece. The distance between the objective lens and the intermediate image plane is labeled f_o . The distance between the intermediate image plane and the eyepiece lens is labeled L . The focal length of the eyepiece lens is labeled f_e .

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E. Hecht, Optik (Addison-Wesley 1989)

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Zeiss objectives according to Abbe

1600 | 1800 | 2000



$d = \frac{\lambda}{2 \sin \alpha}$

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Zeiss

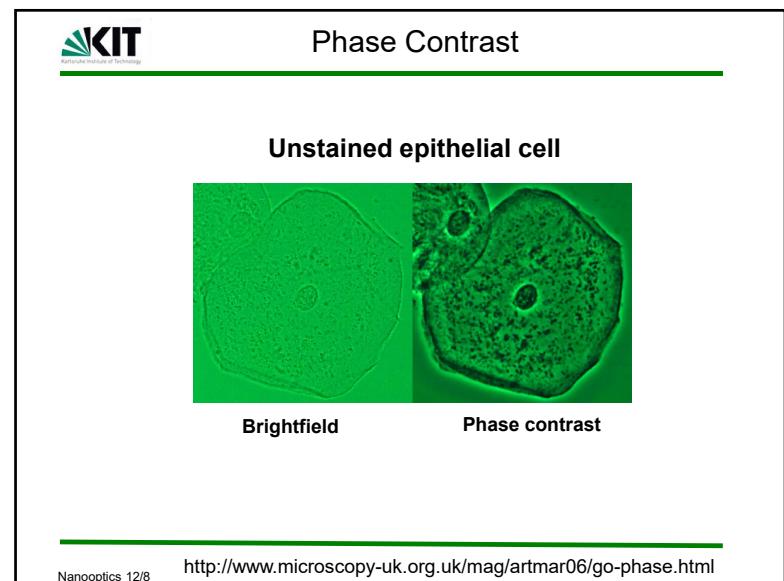
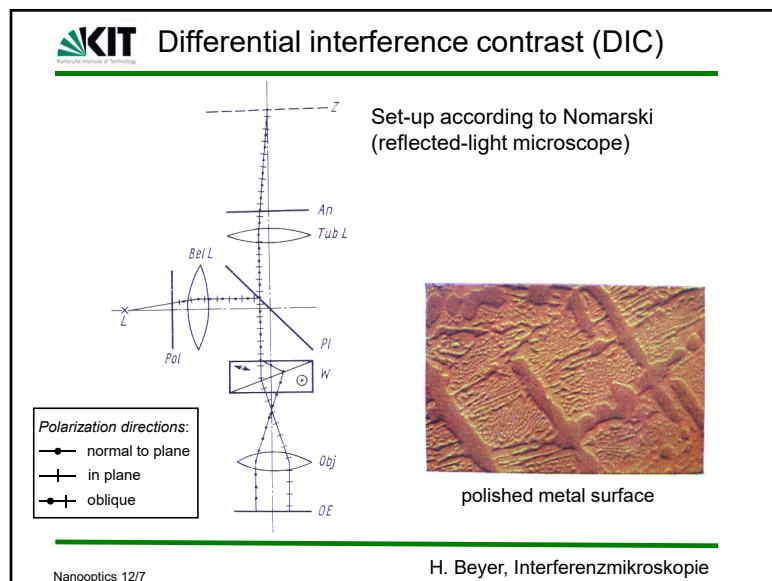
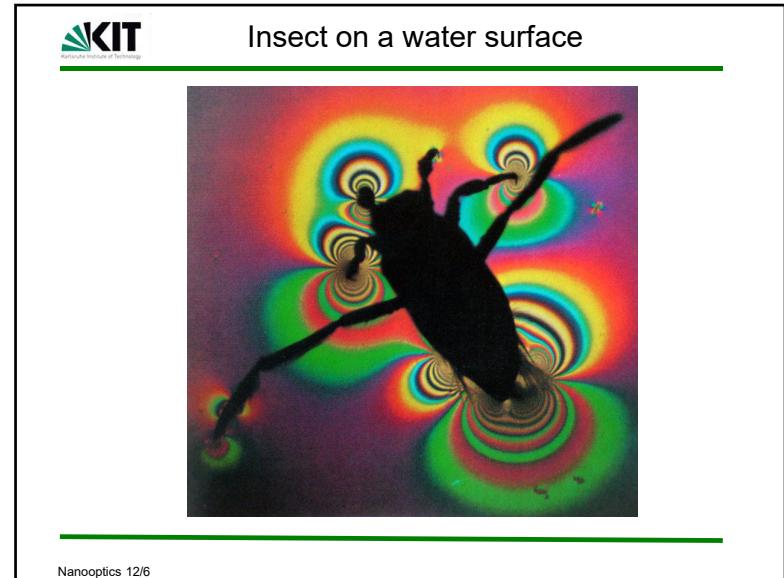
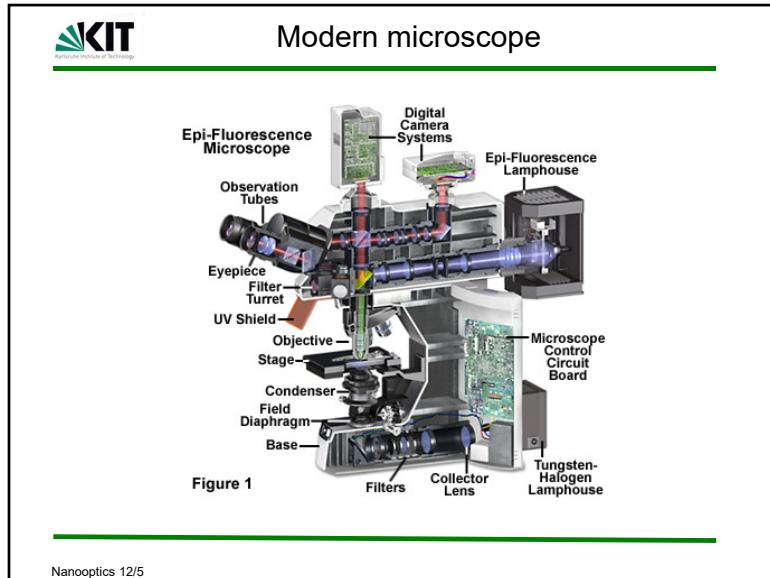
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Modern microscope objectives



The image shows four modern Nikon Plan Fluor microscope objectives arranged in a row. From left to right, they are labeled: "Nikon Plan Fluor 100X/0.5-1.3 DIC L", "Nikon Plan Fluor 60X/0.85", "Nikon Plan Fluor 40X/0.65 DIC L", and "Nikon Plan Fluor 10X/0.30 DIC L". Each objective has its specific magnification, numerical aperture, and type (DIC L) printed on its side.

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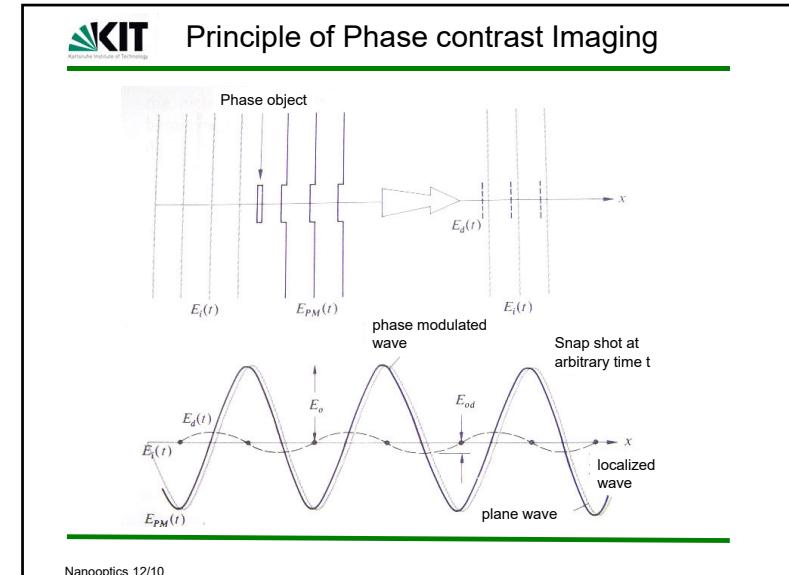
Frits Zernike

Invention of the Phase-Contrast-Microscope (1930)

Nobel prize 1953

1888 - 1966

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Principle of Phase contrast Imaging

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

Phase-modulated wave at $x = 0$:

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

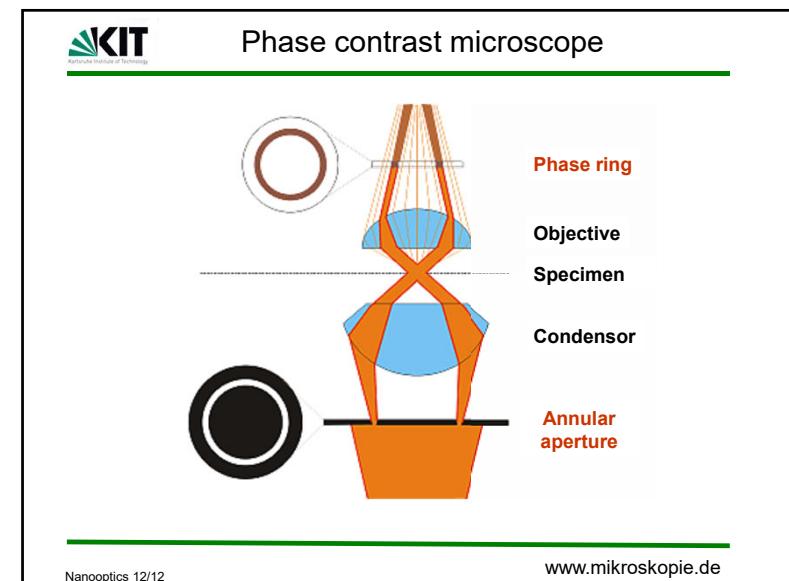
If Φ is very small, then

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

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

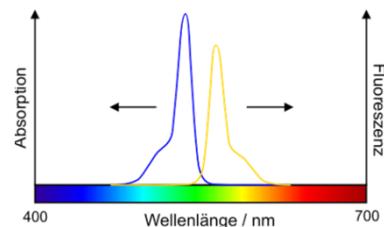
$$\begin{aligned} \sin \omega t &\rightarrow \cos \omega t \\ E_{PM} &\rightarrow E_{AM} \end{aligned} \quad \left. \begin{array}{l} \text{Amplitude-modulated wave} \\ E_{AM}(y, z, t) = (1 + \Phi(y, z)) \cdot E_0 \cos \omega t \end{array} \right\}$$

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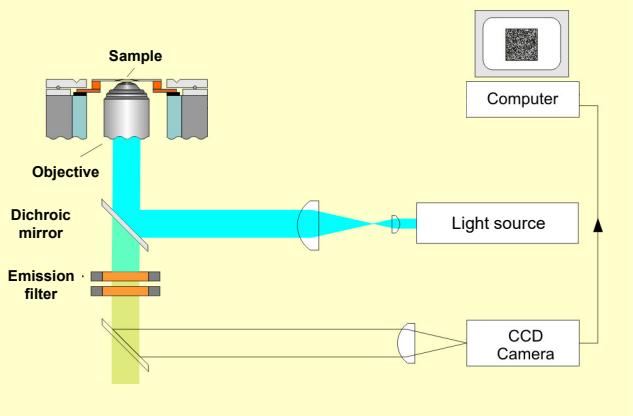
Absorption & Emission of Molecules



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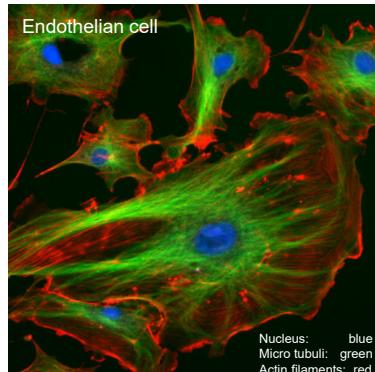
Fluorescence microscopy



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Example



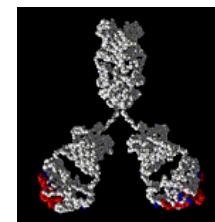
Nucleus: blue
Micro tubuli: green
Actin filaments: red

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Wikipedia



Specific Labelling through antibodies



Immobilized Primary antibody:
rabbit-antibody
against antigene A

Secondary antibody:
dye-labelled antibody
against rabbit antibody

Dye label

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