Biophysics I

Methods of Biophysics

Literature: • no general text book
- literature will be given to each topic
- ~~sp~~ script will be put into WWW

Structure:
- Methods of Biophysics (Biophysics I)
- Cell Biophysics (Biophysics II)
- Molecular Biophysics (Biophysics III)
- Biophysics seminar
- Biophysics lab class

Requirements:
- successful talk in seminar
- successful participation in all experiments of the lab class

Research:
- student: Hilfskraft, Diplom/Master, PhD
- Gaub/Rädler/Selle, Kreß, Parak, Lösche Nachfolge, Arnold, Schmiedel, and many others

Sylabus:

1. Light microscopy
   (basics, phase contrast, fluorescence, caged fluorophores, FRAP, GFP, quantum dots, single molecule, SPT, total internal reflection, multiphoton/confocal microscopy, 4π, deconvolution, reflection interference contrast)
2. Electron microscopy (scanning, transmission, 3-dim cryo)
3. Spectroscopy (absorption, fluorescence, Raman, FTIR, FCS)
4. Ligh scattering (protein size, multiple, OCT)
5. Optical traps (optical tweezers, optical scalpells, optical stretcher, cell rheology, optical cell guidance, magnetical tweezers)
6. Scanning probe microscopy (STM, SFM, SNOM, single molecule pulling, cell mechanics)
7. Acustical Microscopy
8. Membrane Model systems (Langmuir monolayers, vesicles, supported bilayers, DSC)
9. X-ray crystallography
10. Magnetic resonance (NMR, EPR)
11. Microfluidics and flowcytometry
12. Recombinant DNA, biochips and microarrays
13. Cell culture

I. Light Microscopy

1. Basics

Lit.: • Bergmann, Schäfer, Optics, chapt. 1.92.2
• H. Robenek, Mikroskopie in Forschung und Praxis, chapt 1.1 + 1.2 + 1.3

most important tool in biology!

history: 1673 Leeuwenhoek first microscope
1684 Huygens dual lens ~~ocular~~ eye piece
1860 Spencer microscope objective
1890–1905 E. Abbe fundamental understanding
1953 Zernicke Nobel price for phase contrast microscopy

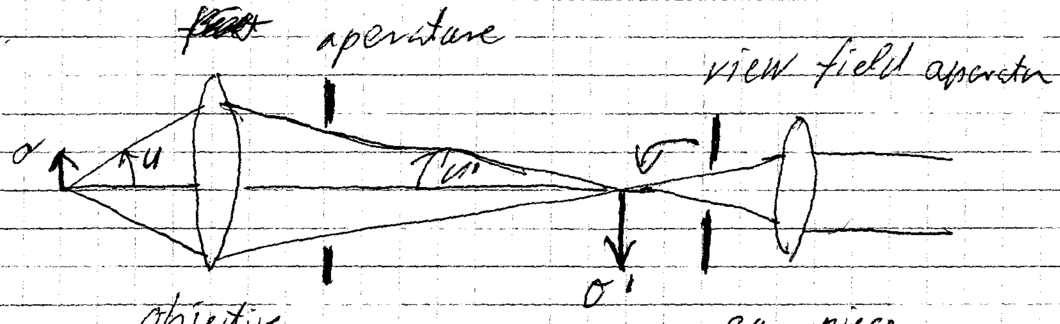
basic principles and setup:

2 components:

(i) objective ⇒ generates from a object of size L a virtual enlarged picture of size L' $L < L'$

(ii) eye piece ⇒ further enhancement of the virtual picture and generation of a picture in infinity to be visible by the eye

focal length of the objective and the eye piece: f_{ob} f_{ok}



magnification objective: $\beta_{ob} = \frac{l'}{l} = \frac{n \sin u}{n' \sin u'} = -\frac{x'_{ob}}{f_{ob}}$
(10x — 100x)

$-x'_{ob}$ tubus length $= F'_{ob} F_{ok}$

magnification eyepiece: $\Gamma_{ok} = \frac{250}{f_{ok}}$
(5x — 20x)

⇒ total magnification: $\Gamma_M = -\beta_{ob} \Gamma_{ok} = -\frac{250}{f_M}$
(≤ 2000)

250 nm object ⇒ 0.5 mm picture

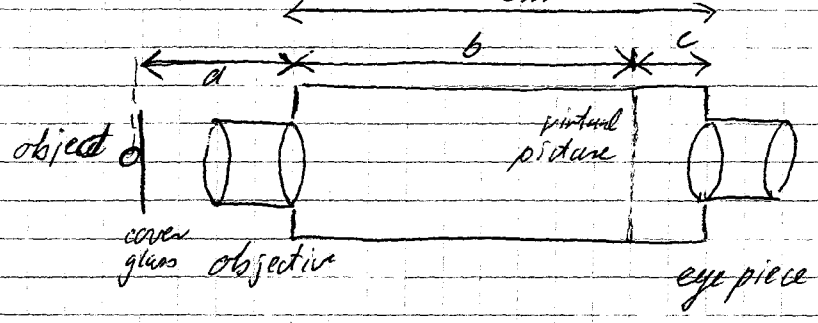
to center optical axis aperature (AP) and a view field aperature

position of the aperature: $x'_{AP} = \frac{f_{ok}^2}{x'_{ob}}$

aperature diameter:

$$\phi_{AP} = 500 \frac{N_A}{\Gamma_M}$$

$N_A = n_1 \sin u_1$, numerical aperature



in real microscopes: objectives and eye pieces are exchangeable

⇒ standards

$a = 37\,mm$ / $45\,mm$

$b = 150\,mm$

$c = 10\,mm$

mechanical tubus length: $t_m = 160\,mm$

note: • can be longer if prisms are used to change the beam pathway

• can have arbitary length when microscope is infinity corrected

optical resolution:

due to the back aperture a point source emitting a radial electromagnetic wave is in its image broadened due to diffraction

point spread function:

$$P(x,y) = A(x,y)\, e^{ik\,W(x,y)}$$

$A(x,y)$: amplitude of the light wave

$e^{ik\,W(x,y)}$: modulated by optical aberrations

⇒ image is generated by Fraunhofer diffraction i.e. Fourier trafo

$$F(l_x, l_y) = c \iint P(x,y)\, e^{-ik(x l_x + y l_y)/r}\, dx\, dy$$

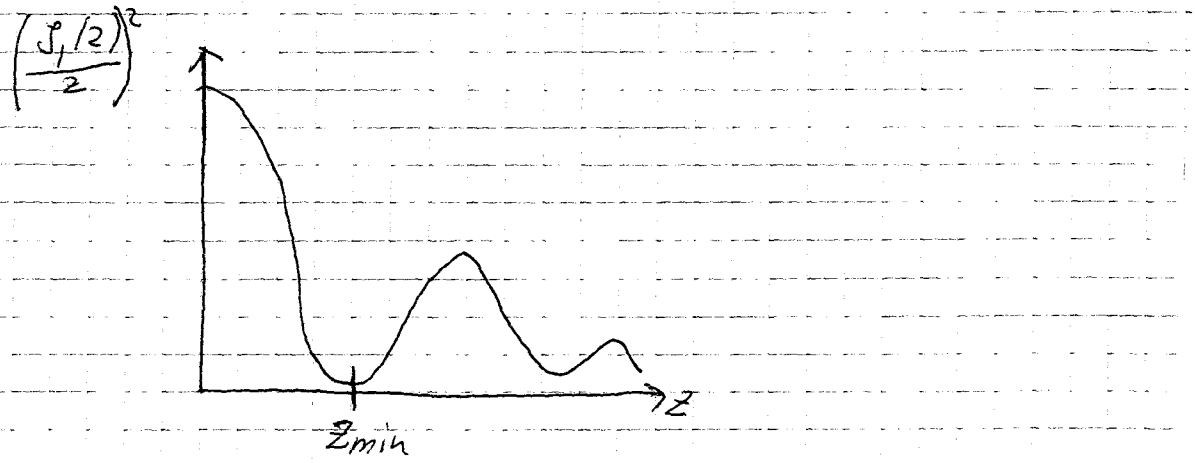
⇒ image intensity: $B(l_x, l_y) = F(l_y, l_y)\, F^*(l_x, l_y)$

without optical aberrations image is a Airy-disc:

$$B(l_x, l_y) \overset{\text{radial symmetric}}{=} \left(\frac{J_1(z)}{z}\right)$$

J_1: 1. order Bessel function

$$z = \frac{2\pi}{\lambda} n' l' \sin u'_{max}$$



minimal distance of objects which can be resolved are determined by z_{min}:

$$\ell'_{min} = \frac{0.61\lambda}{n' \sin u'_{max}} \approx \frac{\lambda}{2 N_A'}$$

$$\ell'_{min} N_A' = \ell_{min} N_A = \ell_{min} n_1 \sin u_{max}$$

$$\Rightarrow \ell_{min} = \frac{\lambda}{2 N_A} = \frac{\lambda}{2 n_1 \sin u_{max}} \qquad \text{Abbé 1870}$$

$\lambda = 450\,nm$, $N_A \leq 1.3 - 1.4$

$$\Rightarrow \boxed{\ell_{min} \geq 200\,nm} \quad \text{optical resolution}$$

maximal magnification:

minimal structure eye can resolve easily

$$\frac{\ell'_{min}}{f_{ok}} = 6 \cdot 10^{-4} = 2'$$

$$\ell'_{min} = \beta_{ob} \ell_{min} = \beta_{ob} N_A \frac{\lambda}{2} \qquad \Gamma_{ok} = \frac{250}{f_{ok}}$$

$$\Gamma_m = -\beta_{ob} \Gamma_{ok} = 0.3 \frac{N_A}{\lambda}$$

$$\Gamma_m = 600\, N_A \quad \text{for } \lambda = 0.5\,\mu m$$

reasonable maximal magnification:

$$\Gamma_m = 500 - 1000 \times N_A$$

more magnification only costs light!

Numerical aperature:

air objective:

$n = 1 \quad u_{max} \leq 72° \Rightarrow \sin u_{max} \leq 0.95$

$\Rightarrow N_A \leq 0.95$

water or oil immersion objective

water $n = 1.333$ oil $n = 1.515$

$\Rightarrow N_A \leq 1.4$

Cover glasses: $d = 0.17\,mm \quad n = 1.515$

Microscope objektives:

achromate: corrected for blue and red wavelength only

apochromate: corrected for visible wave length

plan apochromate: not only corrected in the axis of the lense!

Köhler illumination:

light source is imaged to infinity

using a collector and condenser lense

variable illumination aperature, controls light intensity