Microscopy
Problem Set 3

May 10, 2019

Please hand in the solutions of this problem set next lecture on May 17th of 2019.

3 Phase contrast

a) Which statement is correct – please give a brief reason?

(i) Objects without amplitude contrast can still cause a phase contrast.

(ii) Amplitude contrast is only employed in bright-field microscopy while dark-field microscopy employs phase contrast.

(iii) Phase contrast microscopy transfers phase into amplitude contrasts.

(iv) Phase contrast results from phase delays.

(v) By employing a sampling and reference beam of different polarization at a spatial separation of less than the usual resolving power of the microscope, DIC microscopy improves the spatial resolution of the microscope to below 200 nm.

b) The Institute of Biochemistry bought a used phase contrast microscope. Its detection sensitivity requires phase differences of $\lambda/3$ between neighbouring objects in the sample to be visualized.

(i) How thick has a cell layer (refractive index 1.36) to be to be visible when using a cell medium with refractive index 1.335 and light with wavelength 630, 510 or 420 nm?

(ii) What phase plate has to be used to see the cells as bright spots?

(iii) One user wants to visualize a cell layer of 2 µm thickness. How has the refractive index of the cell medium to be adapted to visualize the cells?

(iv) How can the nucleus (refractive index 1.45, height 1 µm) within a single cell be visualized?

4 Atomic Force Microscopy (AFM)

a) Draw a basic scheme of the AFM and explain **with your own words** the working principle.

b) **Working modes.** Explain, illustrate and give an example each of the aspects presented in the lecture:

(i) Imaging
- Imaging (height)
- Spectroscopy

(ii) Sample-Probe interaction
- Contact
- Non-contact
- Tapping

(iii) Distance control
- Constant height
- Constant force mode

c) Name and explain the requirements and characteristics a sample should fullfill for AFM.

d) Draw a typical Force-Distance curve for a tip approaching and withdrawing the polyethylene surface. What happens with the force-distance curve if the tip would be functionalized with a hydrophobic chemical?

e) Explain and illustrate one tip artifact