2.3 Microscopy: Exercises

Lecture 4&5: Contrasting & Fluorescence methods

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Phase contrast

1. Which statement is correct – please give a brief reason?
   a) Objects without amplitude contrast can still cause a phase contrast.
   b) Amplitude contrast is only employed in bright-field microscopy while dark-field microscopy employs phase contrast.
   c) Phase contrast microscopy transfers phase into amplitude contrasts.
   d) Phase contrast results from phase delays.
   e) 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.

2. The Institute of Biochemistry I bought a used phase contrast microscope. Its detection sensitivity requires phase differences of $>\lambda/3$ between neighboring objects in the sample to be visualized.
   a) 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?
   b) What phase plate has to be used to see the cells as bright spots?
   c) One user wants to visualize a cell layer of 2µm thickness. How has the refractive index of the cell medium to be adapted to visualize the cells?
   d) How can the nucleus (refractive index 1.45, height 1µm) within a single cell be visualized?

Fluorescence

3. What is the most important property of fluorescence and why?
4. What laser intensity I (in W/cm^2) of $\lambda = 532$ nm has to be employed to excite a fluorophore with an absorption cross-section $\varepsilon = 10^5$ L mol^{-1} cm^{-1} with a rate $k = 10^9$ s^{-1} (*hint: first calculate molecular absorption cross-section σ and the energy of a photon*)?
5. The energy difference to the first excited vibronic state of the electronic ground state of a fluorophore is $750cm^{-1}$. How much has the fluorophore have to be cooled down to reduce the population of the first excited vibronic state to a minimum (<0.01)?
6. A fluorophore has a lifetime of $\tau = 4.0$ns. An interaction introduces an additional de-excitation process with rate constant $k = 10^8$ s^{-1}. What is the change in quantum yield (before 1) and lifetime?
7. An additive reduces the probability of photobleaching of a fluorophore by a factor of 3 and quenches the fluorescence by a factor of 4. Is there a win in total number of photons?
8. Two fluorophores have a lifetime of $\tau = 4.0$ns and 1.0ns. What is their respective change in anisotropy r when the hydrodynamic radius R_{rot} of the investigated (spherical) molecule (rotating in a solvent with viscosity $\eta = 1$ c_P, $r_0 = 0.2$) changes from $r_m = 1$nm to 3nm (*hint: rotational diffusion coefficient*)? In which case is it possible to monitor the rotational decay r(t)?