Microscopy
Problem Set 4

May 27, 2019

Please hand in the solutions of this problem set next lecture on May 31st of 2019.

5 Image formation in ray optics

a) Draw a 4f system with lenses of diameter d focal lengths f_1 and f_2. Calculate the magnification and the aperture angle α.

b) Draw the chief and marginal ray coming from an off axis point in the object plane. Explain with your own words how the image is formed in this case.

c) Name the optical effects that cannot be described with ray optics.

6 Image formation in wave optics

a) With the Huygens principle, explain interference and diffraction.

b) Explain and illustrate the following experiments and therefore the concepts of coherence:

(i) Michelson Interferometer, Temporal coherence.

(ii) Young's double slit experiment, Spatial coherence. Draw the obtained pattern (dependent of d, the distance between slits) and explain the origin of the maxima and minima.

7 Fourier transformations

a) Read the paper attached and explain how an object can be described as a superposition of sine or cosine functions.

b) With the previous concept, explain what is a Fourier transform.

c) With he previous concept, describe the process of image formation shown in the animation "Towards the image: Constructing from waves" (Lecture 4).

8 Fluorescence

a) What is the most important property of fluorescence and why?

b) 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 $\epsilon =$ with a rate $k =$? (Hint: $k = \sigma \times$ number of photons, with molecular absorption cross-section σ for a single photon).

c) The energy difference to the first excited vibronic state of the electronic ground state of a fluorophore is $\Delta E = 750\,\text{cm}^{-1}$. 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)? (Hint: The relative population of higher vibronic states is given by the Boltzmann distribution).

d) A fluorophore has a lifetime of $\tau = 4.0\,$ns. An interaction introduces an additional de-excitation process with rate constant $k =$. What is the change in quantum yield (before 1) and lifetime?

e) (i) Two fluorophores have a lifetime of $\tau = 4.0\,$ns and $1.0\,$ns. What is their respective change in anisotropy r when the hydrodynamic radius R_{rot} of the investigated (spherical) molecule (rotating at a temperature $T = 25\,\text{C}^{\text{o}}$) in a solvent with viscosity $\eta = 1\,$cP, $r_0 = 0.2$) changes from $r_m = 1\,$nm to $3\,$nm? (Hint: $1/r = 1/r_0(1+\tau/\tau_c)$ with rotational correlation time $\tau_c = 1(6D_r)$ and rotational diffusion coefficient $D_r = k_B T/(8\pi\eta R_{rot}^3)$).

(ii) In which case is it possible to monitor the rotational decay $r(t) = r_0 \exp(-t/\tau_c)$? (Hint: The total fluorescence intensity decays with the lifetime).

f) (i) How long does it take to image a $30\,\mu\text{m} \times 100\,\mu\text{m} \times 100\,\mu\text{m}$ large area with widefield (observation spot diameter $> 100\,\mu$m, camera frame rate 100 Hz, axial step size $1.5\,\mu$m), confocal (focal spot size $0.3\,\mu$m laterally and $1\,\mu$m axially, scan step size $0.2\,\mu$m laterally and $0.5\,\mu$m axially, scan dwell time $1\,\mu$s) and lightsheet (sheet size $> 100\,\mu$m (lateral) $\times > 100\,\mu$m (lateral) $\times > 3\,\mu$m (axial), with sheet scan step size $1.5\,\mu$m and camera frame rate 100 Hz).

(ii) Which method will give the best contrast and why?