ENGLISH

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DEPARTMENT OF PHYSICS

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EXAM IN TFY4265/FY8906
Biophysical microtechniques

December 19th, 2012
4 hours

Allowed Aids: C
Specified printed matter: Karl Rottmann, Mathematical formulas. Specified calculator allowed.

General information

There are 10 problems and each problem is worth 10 points for a total of 100 points (i.e. you have on average 24 minutes for each problem).

Problem 1- Optical microscopy

a) Optical microscopy can be severely limited by several aberrations if not corrected for. Explain what is meant by chromatic and spherical aberrations. Under which circumstances are these effects significant and what can be done to reduce them?

b) What are the advantages of nonlinear optical microscopy (multiphoton microscopy)? What extra components are needed to upgrade a standard confocal microscope to a nonlinear optical microscope? Describe the following interactions and include the appropriate Jablonski diagrams:

- Two-photon fluorescence
- Second harmonic generation
- Stimulated Raman scattering.

c) What is *fluorescence resonance energy transfer* (FRET)? What are its uses? What are the spectral (emission and absorptions) requirements for the fluorophores used in FRET?

d) Explain what is meant by the diffraction limit in optical microscopy. How does Stimulated emission depletion (STED) microscopy overcome this limit?

Problem 2- Optical Tweezers

Explain how light can be used to trap a particle in three dimensions. Sketch a setup for optical tweezers that is used to measure forces, explaining what the necessary components are used for.

Problem 3- Atomic force microscopy

a) What are the main components necessary to realize an atomic force microscope? What determines the resolution in atomic force microscopy?

b) What characterizes contact mode vs. tapping mode? How are forces typically measured (differently) in the two modes?

c) In dynamic force spectroscopy, a typical measurement is to find the most likely unbinding force F^* of some interaction and plot this against the loading rate r_f. Typically we plot $F^*(\ln r_\mathrm{f})$. Show how the width x_β and height E_b of an energy barrier can be found from this plot. You may have use of the following relations (Assume that t_D is a known constant. It should be evident what the other variables are from the context.):

$$
\begin{aligned}
F^* &= F_\beta \ln \frac{r_\mathrm{f}}{r_0} \\
\frac{1}{t_0} &= \frac{1}{t_\mathrm{D}} \exp\left(-\frac{E_b}{k_\mathrm{B}T}\right) \\
r_0 &= F_\beta / t_0 \\
F_\beta &= \frac{k_\mathrm{B}T}{x_\beta}
\end{aligned}
$$

Problem 4- Electron microscopy

a) Describe the possible interactions and emitted particles (photons are also particles) when high energy electrons strike a sample in electron microscopy. Which of these signals are typically utilized in SEM and TEM?

b) Describe the briefly the main steps necessary to prepare a biological sample for TEM. EM is typically performed in vacuum. How is it possible to perform electron microscopy under physiological conditions (environmental TEM)?