NTNU – Trondheim
Norwegian University of
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Department of physics

Examination paper for TFY 4265 Biophysical microtechniques

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Examination date: 18.12.2013

Examination time (from-to): 15:00-19:00

Permitted examination support material: C

Other information: The problem sets are only given in English. Solutions may be written in either English or Norwegian. The relative weights between the problems are given in parentheses. Express your answers as clearly and succinctly as possible. Short and precise is better than long and unclear. Use legible handwriting and label your answers clearly.

Language: English

Number of pages: 3 (including front page)

Number of pages enclosed: 0

Checked by:

Date Signature

Problem 1. Optical microscopy

a) (8) Make a drawing of the optical system in a light microscope. Including the following parts placed in correct order: Condensor, ocular, objective, light source, collector, sample plane.

b) (5) What is meant by conjugate planes? Indicate where you have conjugate planes in your drawing in problem a).

c) (6) Indicate where in the system you would place these additional components :

- Field aperture
- Condensor aperture
- Phase annulus and phase plate for phase microscopy

d) (6) Imagine you work in a histology lab where you are given a piece of tissue and told that the doctor wants to study the cell nuclei in a microscope. Describe briefly <u>all</u> the steps you need to perform to prepare the sample (there are several ways of doing this – choose one of them).

Problem 2. Advanced optical techniques

a) (7) Explain briefly the principle behind fluorescence correlation spectroscopy (FCS).

b) (9) Figure 1 illustrates a pH-dependent molecular delivery system. Polymer particles (grey circle) contain fluorescent molecules (stars). The polymer has transmembrane proteins (seen in the magnifying glass) in the wall which can open and close depending on pH, releasing the content of the particle. Figure 2 shows FCS measurements of thee free fluorescent molecules in solution (white squares), and particles (prepared with fluorescent molecules inside) with a naturally occurring transmembrane protein at pH 5 and 7 (white and black squares respectively). Figure 3 shows FCS measurements of the particles with a mutant transmembrane protein at pH 5 and 7 (white and black diamonds respectively). <u>Interpret the results, using differences in the FCS curves to say something about how the system works.</u>

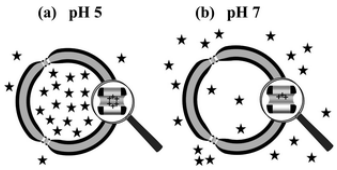

Figure 1

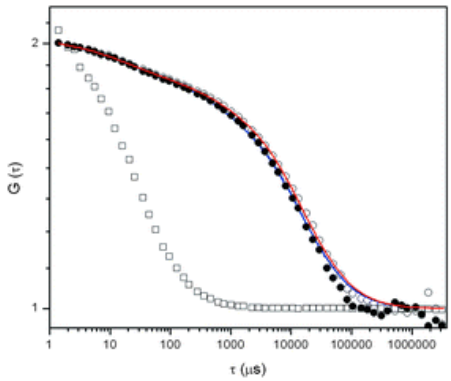

Figure 2

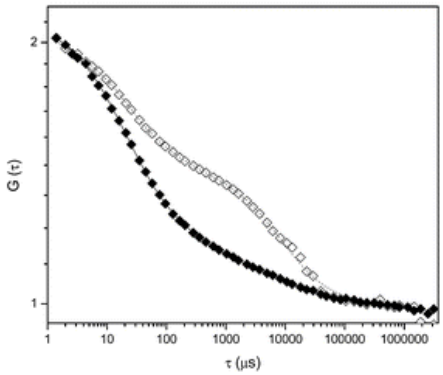

Figure 3

c) (5) What limits the achievable resolution in optical microscopy?

d) (9) Explain how stimulated emission depletion microscopy can be used to overcome this limit.

Problem 3. Force microscopy and spectroscopy

a) (6) Describe what is meant by contact, non-contact and tapping imaging modes in atomic force microscopy.

b) (6) What is the difference between *constant height* and *constant force* imaging?

c) (7) Make a sketch where you use conservation of momentum to illustrate how optical tweezers can achieve lateral trapping of a particle.

d) (9) The output signal from the quadrant photodiode in an optical tweezer setup is a voltage. What calibration measurement(s) do you need to perform to relate a voltage to a force?

Problem 4. Electron microscopy

a) (5) What are secondary electrons and backscattered electrons? Are any of these signals measured in TEM? What about SEM?

b) (5) Why does the image rotate when you change the magnification in a TEM instrument?

c) (7) Explain the steps which are necessary to prepare a sample for TEM. Explain briefly the reason for doing each step.