NTNU

Department of physics

Examination paper for TFY4265 Biophysical micromethods

Academic contact during examination: Magnus Lilledahl

Phone: 73591873/92851014

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Instructions

Each subquestions carries equal weight. Answer as concisely and precisely as possible. You may answer in English or Norwegian.

Problem 1: Optical microscopy

(a) Name three types of microscopy where the polarization of the light is important. Explain why and how the polarization is important for the function of the type of microscopy. The importance of the polarization can either be in the interaction with the sample or to somehow modify the light illuminating the sample.

(b) Draw a sketch which illustrates the image formation in a compound microscope using geometrical optics. Include the intermediate image plane and indicate whether the images are real or virtual.

(c) Two essential components in a phase contrast microscope are the *condensor annulus* and the *phase plate*. Explain why these two components have to be placed in conjugate planes.

(d) Explain how (pulsed) STED uses two laser beams to achieve resolution which is better than the diffraction limit.

(e) Explain briefly how *fluorescence correlation spectroscopy* can be used to determine the diffusivity of fluorescent molecules in a solution.

(f) Assume you have designed a nanoparticle which carries a cancer drug. The surface of the drug is covered with a molecule (ligand) that you think binds specifically to a surface receptor on cancer cells. Now you ask yourself two questions: 1) Is the nanoparticle actually binding to the receptor I believe? 2) Is the cell taking up (internalizing) the nanoparticle or is it just sticking to the cell surface. Describe experiments where you would be able to answer these questions. Assume you can tag both the nanoparticle and the receptor with fluorophores without modifying their function.

Problem 2: Force based techniques

(a) What is the difference between *constant force* and *constant height* imaging modes in AFM?. Discuss the advantages and disadvantages of both modes.

(b) Why is the loading rate important in dynamic force spectroscopy?

(c) Explain using geometrical optics why a particle trapped with optical tweezers needs to have a higher refractive index than the surroundings.

Problem 3: Electron microscopy

(a) Explain what the difference is between *backscattered electrons* and *secondary electrons*. Explain why we use different detectors for these two interactions and how these detectors work.

(b) Outline the steps that are necessary for preparing a sample for *scanning electron microscopy*, including the rational behind each step.

(c) In environmental scanning electron microscopy (ESEM) it is possible to image samples under much higher pressure than in conventional scanning electron microscopy. Explain how this is possible.