



NTNU – Trondheim
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Department of physics

Examination paper for TFY4265 Biophysical micromethods

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Examination date: 4.12.2014

Examination time (from-to): 0900-1300

Permitted examination support material: D

Other information:

Language: English

Number of pages (front page excluded): 1

Number of pages enclosed: 0

Checked by:

Date

Signature

Exam TFY4265.

Fall 2014

Each subproblem has equal weight towards the grade. Make an effort to be as precise and concise as possible in your answers. There are no extra points for irrelevant information. Label questions clearly

Problem 1 **Basic microscopy and light-matter interactions**

- (a) Explain the Franck Condon principle and how this is related to the mirror images of fluorescence absorption and emission.
- (b) Make a drawing which illustrates the location of the two sets of conjugate planes when a microscope is adjusted for Kohler illumination. Label all components.
- (c) What are the steps which are necessary to adjust a microscope for Kohler illumination?

Problem 2 **Confocal microscopy**

- (a) What is special about confocal microscopy? What is the advantage of confocal microscopy?
- (b) In confocal laser scanning microscopy we adjust gain and offset in the software. What do these settings actually adjust and why do we do it?
- (c) What is fluorescence resonance energy transfer and what can it be used for?
- (d) Explain the origin of the shape of a typical autocorrelation curve in fluorescence correlation spectroscopy.
- (e) Explain how STED microscopy works.

Problem 3 **Force microscopy**

- (a) Atomic force microscopy (AFM) can be operated in at least three different modes: contact, oscillating and tapping mode. Describe the differences between these modes
- (b) What is the difference between constant height and constant force measurements in AFM?
- (c) Describe how the piezoelectric scanner and the spring constant of the cantilever are typically calibrated.
- (d) Using the geometrical optics (rays) approximation explain how we achieve lateral and axial trapping in an Optical Tweezers setup.

Problem 4 **Electron microscopy**

- (a) In electron microscopy we can measure secondary and backscattered electrons. Explain the physical origin of these signals and which are used in SEM and TEM.
- (b) Explain briefly the steps which are necessary in preparing a sample for SEM