NORWEGIAN UNIVERSITY OF SCIENCE AND TECHNOLOGY
DEPARTMENT OF PHYSICS

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EXAM IN COURSE TFY4265
BIOPHYSICAL MICROMETHODS
5. December 2007
Hours: 0900 – 1300

Permitted aids: Simple calculator HP30S
None written books or papers

Exercise 1: Light microscopy

a) Bright field microscopy
- Describe the light path in a bright field microscope, and
- describe the functions of the main components.

b) In order to obtain optimal results in light microscopy the microscope should be adjusted according to Köhler's principle.
- Explain this principle and
- how the components should be adjusted.

c) Phase contrast microscope
- Describe the principle for phase contrast and
- describe the optical components that have to be inserted in the optical path.

Exercise 2: Fluorescence and multiphoton microscopy

a) In fluorescence microscopy an epi-configuration is normally used.
- Explain this configuration, and
- describe the difference from bright field microscopy.
- Describe the use of filters in fluorescence microscopy.

b) - Describe the principle for multiphoton microscopy, and
- the advantages compared to fluorescence microscopy.

c) Multiphoton microscopy can be used to study fluorescence and to study the second harmonic generated signal.
- Explain what the second harmonic generated signal is, and
- what criterion molecules have to fulfil to generate this signal?

Exercise 3: Light –tissue interactions, lasers, detectors.

a) When light is penetrating tissue it is attenuated due to two processes.
- Indicate these processes.
- Indicate the equation for the light intensity as a function of distance into the tissue.

b) A molecule is excited to state S_2, and fluorescence is emitted as part of the deexcitation process.
   - Indicate the various deexcitation processes that takes place when fluorescence is included, and
   - explain the processes.

c) - Explain stimulated emission, and
   - the principle for lasers

d) - Explain how the detector photomultiplier tube is operating.
   - Indicate two types of instrumentation where this detector is used.
   - Whys is a photomultiplier tube used in these cases and not a camera?

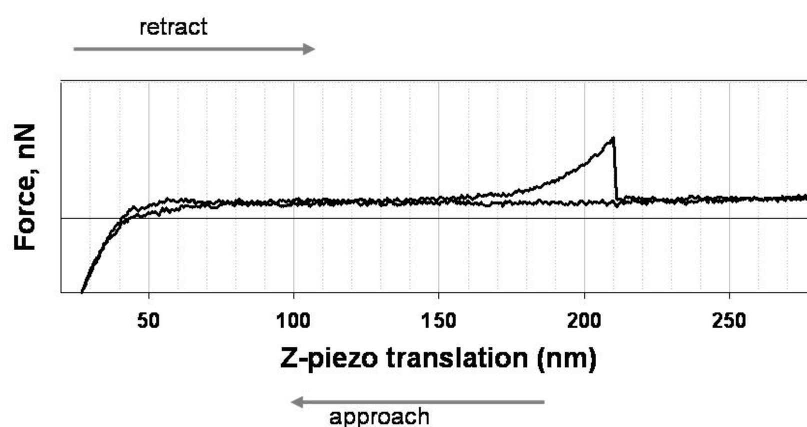
Exercise 4: Atomic force microscopy

a) AFM: principle and design
- Describe the basic principle of AFM and
- what kind of information one can obtain from this technique.
- What are the main components of the AFM and what are their functions?

b) Operational modes for AFM imaging
- Draw a schematic of the typical force-distance profile for van der Waals forces.
- Identify the two different regimes (repulsive and attractive).
- There are three main operational modes for imaging with AFM. Use the force-distance profile you have drawn and indicate in what part of the force-distance regime each imaging mode is operating in.
- Describe shortly the three modes and how they differ.
- What imaging mode is preferable for biological samples and why?

c) Force measurements with AFM
The AFM can be used to measure different types of forces.
- Outline how the elastic properties of a sample surface can be mapped using the FIEL method (Force Integrated to Equal Force Limit).

In dynamic force spectroscopy, the tip is moved vertically with respect to the sample and a force-distance curve is recorded. From a typical force curve as shown below, two types of information can easily be extracted.
- What information is this, and
- in what part of the curve is this information recorded?



Exercise 5: Electron Microscopy

a) EM: principle and design
- What is the general principle behind image formation in electron microscopy?
- What determines the theoretical resolution and
- how is this compared to conventional light microscopy?

Lenses constitute an important component in EM.
- What is the main operational principle of lenses in EM, and
- what are the differences compared to lenses in conventional light microscopy?
- How does one change the focal length of a lens in EM?

b) TEM and SEM
- What is the difference in image formation in TEM and SEM?
- In what ways does a dedicated SEM instrument differ from a dedicated TEM?
- What determines the resolution in TEM and SEM?

c) Sample damage and sample preparation for EM

EM can be disruptive to a biological sample.
- In what ways can the sample be damaged?
- Why is staining often necessary when preparing biological samples for EM, and
- in general terms, what kind of “stain” is often employed?
- What is the principle behind shadowing?
- What are the three general steps when preparing a sample for SEM?
- What is the role of the antistatic film and why is it important?