NTNU

Department of physics

Examination paper for TFY4265 Biophysical micromethods

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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) What is the advantage of using *phase contrast microscopy* compared to bright field microscopy? What is the principal function and structure of a phase contrast microscope?

(b) The Rayleigh criterion is often used in microscopy to determine the resolution of the system. Explain what is meant by this criterion and why it is appropriate to determine the resolution

(c) In confocal laser scanning microscopy important settings during image acquisition are 1) laser power, 2) scan speed, 3) detector gain, and 4) pixel size. Explain how these settings result in trade offs in the resulting image quality with regards to image quality parameters such as 1) photobleaching, 2) noise, and 3) tracking fast kinetics in live cell imaging.

(d) What is the principle behind structured illumination microscopy?

(e) How does fluorescence recovery after photobleaching (FRAP) work? What can this technique be used for?

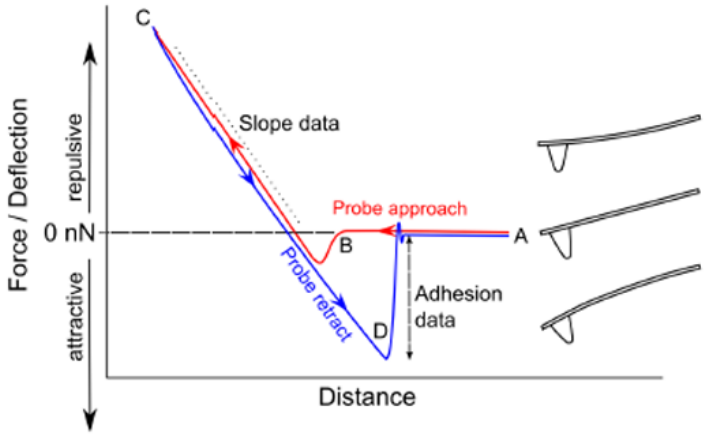

Figure 1: Problem 2a. A typical force-distance curve

Problem 2: Atomic force microscopy

(a) Figure 1 shows a typical force distance curve for an AFM tip. Explain what physical interactions give rise to the various features of this curve.

(b) Explain how the scanner in an AFM works and how it is calibrated.

(c) In dynamic force spectrosocpy we say that a rupture event is a stochastic process. What is meant by this? Why is the *loading rate* important for the analysis?

Problem 3: Electron microscopy

(a) Explain what the main necessary steps are when processing a biological sample for transmission electron microsocpy.

(b) What do we mean by *backscattered* and *secondary electrons* in electron microscopy?

(c) When preparing a sample for scanning electron microscopy, *critical point drying* is typically used. What is critical point drying, how is it performed, and why is it used?