NTNU

Department of physics

Examination paper for TFY4265 Biophysical micromethods

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Date Signature

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Each subquestions carries equal weight. None of the questions require lengthy answers so answer as concisely and precisely as possible.

Problem 1: Optical microscopy

(a) After excitation of N_0 molecules, the number of molecules $N(t)$ that are still excited as a function of time will follow an exponential decay, $N(t) = N_0 \exp(-t/\tau)$, where τ is the lifetime (the average time a molecule resides in the excited state). The various deexcitation processes can be described by rate constants k_i where $k_i dt$ is the probability of deexcitation through process i in a small time interval dt. We can separate the deexcitation processes into radiative (k_r) and non-radiative (k_n). Write down a justified expression for the lifetime and the quantum yield Q of a molecule in terms of k_r and k_n.

You will probably see that the lifetime depends on the rate of non-radiative decay. What practical applications can this dependence have?

(b) Between the collector and the condensor lens in a microscope set up for Kohler illumination there are two adjustable apertures/diaphragma. What are they called? What is their function? What other planes in the optical system are conjugate to these two apertures (make a sketch to illustrate this)?

(c) Compare the contrast mechanism in phase contrast microscopy and differential interference contrast (DIC) microscopy. How are they similar? How are they different?

(d) Both stimulated emission depletion (STED) and ground state depletion (GSD) microscopy are two methods for achieving superresolution imaging. How are they similar in achieving this? How are they different?

(e) Fluorescence correlation spectroscopy is a method which can be used to measure the diffusivity of fluorescent molecules in a solution. Explain the main steps in the analysis of such data (you can, but are not required to, use mathematical expressions).

(f) What are the advantages of multiphoton microscopy?

Problem 2: Force based techniques

(a) In atomic force microscopy, a piezoelectric scanner is used to scan the sample under the cantilever. What is a piezoelectric material? What do we mean by hysteresis in the piezoelectric material? How do we correct for the hysteresis in a piezoelectric scanner?

(b) When approaching a sample with the AFM tip there will first be an attractive force due to van der Waal forces and then repulsion due to Pauli exclusion. Draw a curve showing the interaction force as a function of distance and indicate in which regions of the curve the cantilever is operated in the three main imaging modes (contact, oscillating, tapping)

(c) The calibration of optical tweezers is based on the theory of Brownian motion. The starting point is the equation of motion for the trapped particle in the solution (the Langevin equation),

$$mx''(t) + \gamma x'(t) + kx(t) = \eta(t)$$

Explain the physical origin of the terms γ, k, and η.

(d) Dynamic force spectra can be analysed using the idea of the *most likely unbinding force* f^* to say something about the potential energy of the molecular bonds. The result of the theory is that

$$f^* = f_\beta \ln(r_f) - f_\beta \ln(f_\beta k_0)$$

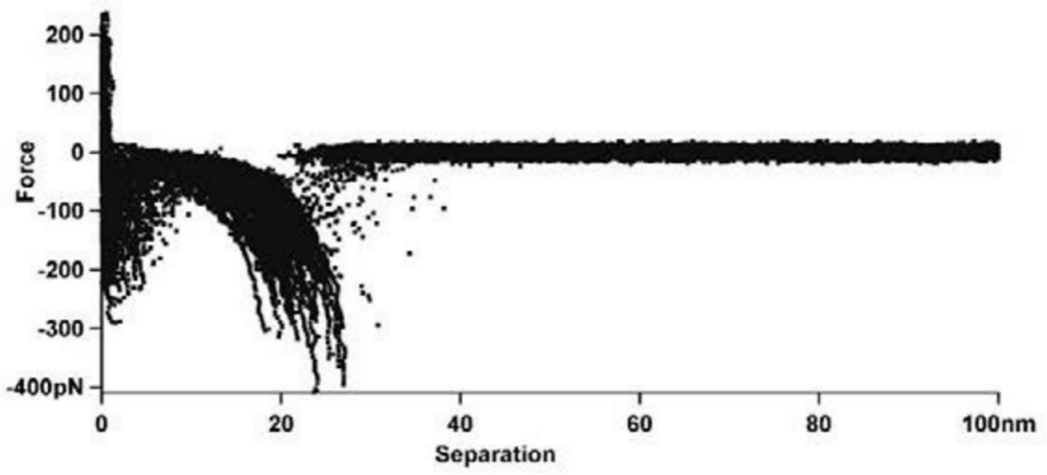

Figure 1: Approach-retract curves

where f_β and k_0 are fitted parameters. Figure 1 is a large data set of multiple approach-retract curves showing unbinding events. Explain how f^* and r_f (loading rate) are determined from this data and how the parameters f_β and k_0 are typically fitted to the data using the above equation

Problem 3: Electron microsocpy

(a) When the electron beam hits the sample in an electron microscope, both electrons and photons are emitted. Explain what we mean by secondary electrons, backscattered electrons, auger electrons, bremsstrahlung, and characteristic x-rays. Also sketch the relative sizes of the regions in the sample from where we can acquire these signals.

(b) In preparation of samples for scanning electron microscopy there are many essential steps. Three of these are fixation, critical point drying and coating. Why are these steps performed and how are they done?