MIDTERM EXAM

ANSWERS

EXPERIMENTAL BIOPHYSICS

TUESDAY, MARCH 8, 2011

1400-1800

EDEN

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THE QUESTIONS ARE ORGANIZED ALONG THE CONTENTS OF THE LECTURES. IF YOU CANNOT ANSWER ONE QUESTION, DO NOT DWELL TOO LONG. TRY THE NEXT QUESTION INSTEAD!

FEEL FREE TO DRAW FIGURES IN YOUR ANSWERS. OFTEN IT IS EASIER AND MORE EFFICIENT TO CONVEY A MESSAGE USING A FIGURE THAN IN TEXT.

TYPICALLY, FOR A PASSING GRADE [G], YOU SHOULD HAVE AT LEAST 50% CORRECT, AND A PASSING WITH DISTINCTION GRADE [VG] REQUIRES AT LEAST 75%. THE FINAL GRADING SYSTEM USED WILL DEPEND ON IN WHAT CONTEXT YOU FOLLOW THE COURSE.

MAKE SURE TO WRITE YOUR NAME ON EACH SHEET OF PAPER. USE A NEW SHEET OF PAPER FOR EACH NEW FIELD, *I.E.* ONE NEW SHEET OF PAPER FOR *BIOLOGY AND SCALES*, ONE NEW SHEET FOR *OPTICS ON THE SMALL SCALE,* ETC ETC.

NOTE THAT THE NUMBER OF POINTS ASSIGNED TO EACH QUESTION GIVES YOU AN INDICATION ON HOW MUCH TO WRITE.

HAND IN YOUR ANSWERS TOGETHER WITH THE QUESTIONS.

A FEW USEFUL NUMBERS:

$1\ k_BT \approx 4$ pN nm = 10^{-21} J AT ROOM TEMPERATURE

$N_A = 6\ 10^{23}$ MOLES^{-1}

$\eta_{water} = 1\ 10^{-3}$ kgm^{-1}s^{-1} AT ROOM TEMPERATURE

$\gamma_{LG} = 0.0728$ J m^{-1} TYPICAL SURFACE ENERGY OF THE WATER-AIR INTERFACE

$\theta_C = 20°$ TYPICAL CONTACT ANGLE OF A WATER DROPLET ON GLASS

GOOD LUCK!

JONAS T

1. OK - Biology and Scales [16p]

Use a new sheet of paper for these questions.

[a] What are the typical size scales of biomolecules (proteins, DNA), viruses, bacteria and larger cells? [6p]

proteins – 10nm

DNA, RNA – 0.34nm/bp, 1000bp per gene, several cm per human chromosome

Bacteria – 1µm

Human cells – 10-100µm

[b] Atomic force microscopy (AFM) is a powerful tool. Explain its main principle [1p] and explain three examples of uses in biology [6p]. [total 7p]

Cantilever equipped with a sharp needle. Angle of cantilever monitored by a laser.

Imaging of DNA on surfaces

Force measurement of proteins in lipid bilayer membranes

Manipulation (DNA writingt, nanodip pen…)

[c] In science critical thinking is crucial. Discuss dangers and pitfalls! [3p]

Cognitive dissonance

Social/peer pressure

Overly reliance on authorities

2. Basic Fluidics [18p]

Use a new sheet of paper for these questions.

[a] In addition to the techniques described in question 2b, describe in a few words each four additional techniques to transport sample and/or fluids! Assuming a cylindrical channel, for each one of the techniques, how does the resulting flow velocity scale with the radius of the channel? [6p]

Techniques [4p]; scalings [2p].

Pressure driven flow. Surface to volume ratio is high leading to a high fluidic resistance. Velocity ~ r^2.

Electrophoresis – charged particles will move in an applied electric field. Velocity ~ r^0.

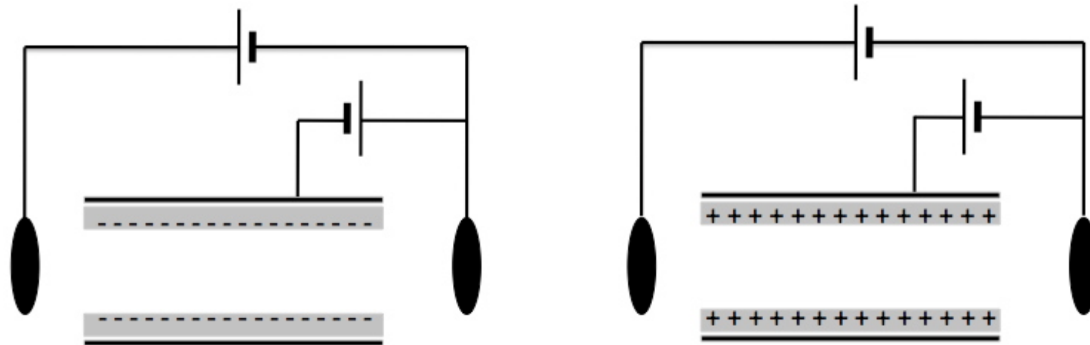
Electroendosmosis – due to surface charge of the channel, the loosely bound ions in the diffuse double layer will move, thereby dragging the fluid with them. Velocity ~ r^0.

Capillary force – surface energy drives the water into a hydrophilic channel. Velocity ~ r^1.

Dielectrophoresis – polarizable particles (charged microbeads, viruses, DNA etc) can be moved in an electric field gradient. Velocity ~ r^{-1}.

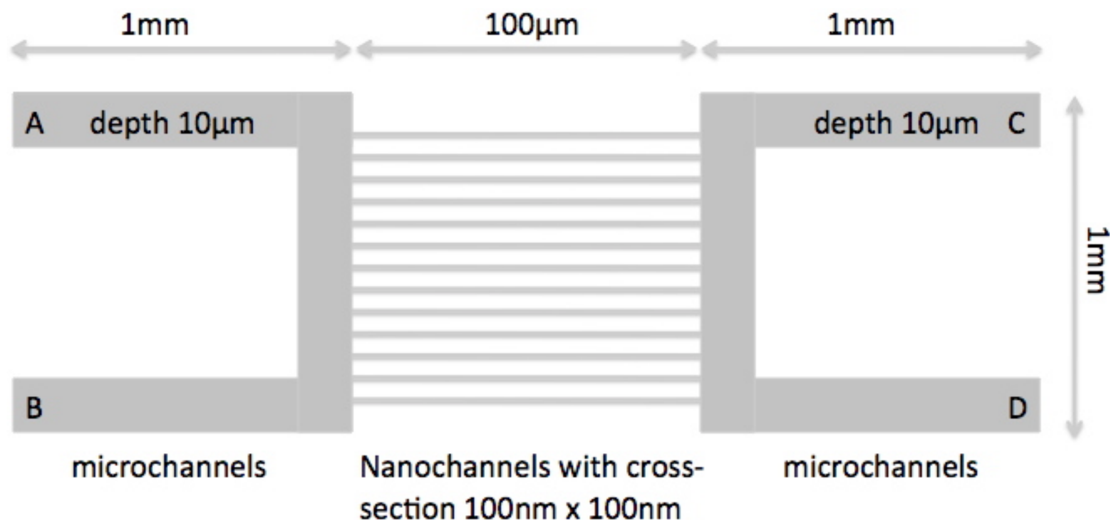
[b] With gate electrodes on the exterior of a nanochannel it is known that the charge density of the inside of the channel under certain circumstances can be tuned from positive to neutral to negative. If a voltage is applied across the channel, how can the

gate voltage be used to control the transport in the channel? What is the underlying mechanism of the transport of the fluid? [3p]



The charge of the inner walls of the nanochannel influences the electroosmotic flow. With a positively charged inner wall, the negative counter ions will carry with the fluid and with a negatively charged inner wall the positive counter ions will carry the fluid in the opposite direction.

[c] Micro and nanofluidic devices are often filled using capillary forces. To avoid the formation of bubbles in the device it is important that no air is trapped. For example, in the below device (made in glass), it is necessary to first fill one of the large channels (let's choose channel A to B) and the nanochannels before introducing any liquid into the other microchannel (channel C to D). Estimate (to within an error of a factor of two) how long time it will take to fill the microchannels to the left (channel A to B) and all the nanochannels in the below device. Water is introduced in reservoir A at time t=0. [6p]



Derive the time it takes the fluid to move through the device, x vs t. [3p]

Since the microchannels are so big, neglect their influence on the drag force that the liquid in tehnanochannels experience. [1p]

Calculate the following: [2p]

1. Time to fill the micro-channel

2. Time to fill the micro-channel until the last nanochannel

3. Time to fill one nanochannel

The minimum of (1) the time to fill the micro-channel and (2) the time to fill the micro-channel until the last nanochannel + the time to fill a nanochannel.

[d] The herring-bone mixer is a chaotic mixer based on alternating vorteces. Explain how it works. How does the mixing length change along the device? [3p]

A channel is created with walls that have a pattern that forces the liquid to rotate.

Draw figure..

After each cell the mixing length is cut in half.

3. Fluidics Applications [22p]

Use a new sheet of papers for these questions. Draw figures!

[a] Describe the basic idea of the bumper array (also known as a device based on deterministic lateral displacement). How does it work? [3p] What forces influence the separation of for example bioparticles during normal operation at high speeds (but not so high that the Reynolds number ≥ 1)? [2p] Give one example of an external force that can be added. [1p]

[in total 6p]

Tilted array of posts. Large particles move with the device. Small particles move with the flow. Critical size. Sharp transition. [3p]

Steric forces. Deformation of the particles. [2p]

DEP [1p]

[b] In a fractionation device that sorts in space, such as the bumper array, the Péclet number can be used to characterize its level of performance. How is the Péclet number defined in this case? [3p]

Péclet number is the ratio of the convective transport and the diffusive transport.

$Pe=uL/D$

[c] Normally microfluidics works at low Reynolds number conditions where viscous forces dominate over inertial forces. However, inertial effects may sometimes be used to sort particles. What are the basic mechanisms involved? [3p] What is the general layout of the device? [1p] What are the advantages and drawbacks as compared to deterministic lateral displacement devices? [2p]

[in total 6p]

$1<Re<2000$; laminar flow inertial flow; wall effect; shear effect; Dean flow; asymmetric flow

High speed and throughput; low resolution; only one fraction is focused.

[d] Two-phase flow is becoming increasingly important in microfluidics. Typically droplets of water are made in oil. Describe the basic mechanism behind the formation of the drops. [2p] Give examples of three key benefits of droplet-based fluidics. [3p]

[in total 5p]

Shear versus interfacial forces. T-junction or three input channels.

Single-cell biology - One cell per drop.

Single-molecule biology - PCR in a drop.

Isolated "test-tube" of chemicals. - Combinatorial chemistry; try out a wide range of chemistries.

[e] Cell lysis and sequential treatment of cells in different chemical environments is a key component in a *Lab on a Chip*. How can this be implemented using deterministic lateral displacement? [2p]

Keith Morton work!

4. Optics on the small scale [20p]

Use a new sheet of paper for these questions.

[a] What is fluorescence resonance energy transfer (FRET)? What is it good for? What additional information does single-molecule FRET give as compared to bulk FRET? [5p]

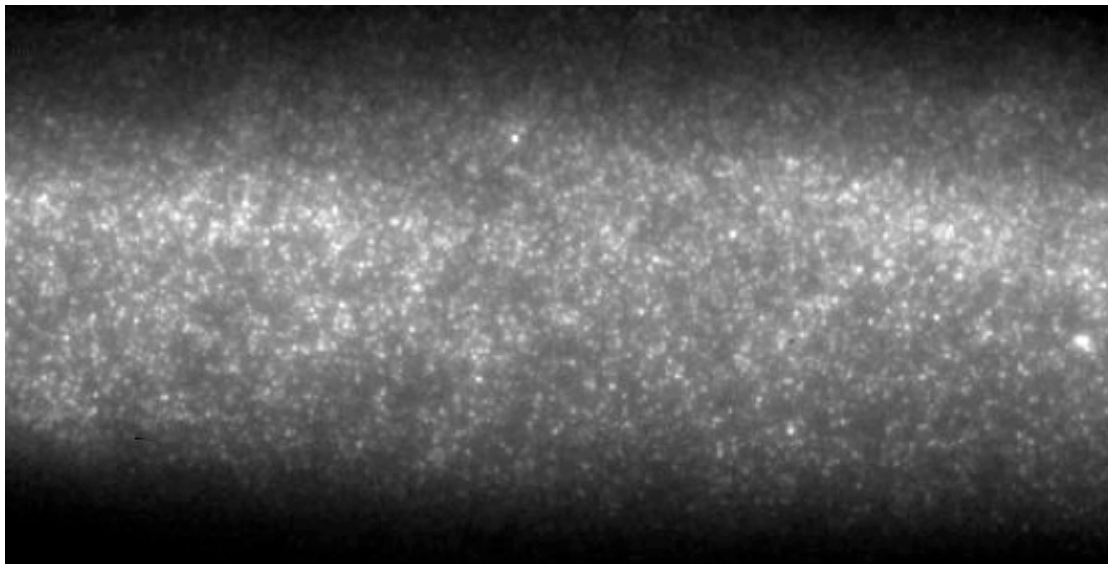
FRET – blue excitation green emission -> green excitation red emission. [2p]

Very precise distance measurement on the 1-10nm scale. [1p]

Heterogeneity; … [2p]

[b] The picture below is one frame from a movie of GFP molecules embedded in agarose gel. How do you tell the difference between aggregates of fluorescent molecules and single fluorescent molecules from these types of data? Note that in the real case you would have access to the entire movie.

In other words, how do you make sure that these spots indeed represent the fluorescent signal from individual GFP molecules? [3p]



Blinking, ON/OFF behavior – the intensity is either on or off, never in between

Reasonable photon count

Antibunching – the photons come one by one, not two at the same time.

[c] How can single-molecule imaging be used to distinguish between strongly and loosely bound molecules in, for example, a bacterium and to measure the effective diffusion coefficient of the molecules? [3p]

Localization enhancement – a diffusive molecule will be too blurred to be detectable for long exposure times, whereas a molecule bound to one position will be clearly

visible especially for long exposure times. The blurred spot size x is related to the exposure time t as $\langle x^2 \rangle = 4D_{eff}t$.

[d] What is total internal reflection fluorescence (TIRF) microscopy? Why is it useful for single-molecule studies? [4p]

Snell's law… Angle of incidence is greater than the critical angle as defined by n_prism sin theta = n_water [2p]

Small excitation volume gives low background fluorescence. [2p]

[f] The resolution limit of standard microscopy is set by the Abbé limit to $r \sim \frac{0.66\lambda}{NA}$

How can this limit be circumvented using stimulated emission?

Describe the setup and key features.

What resolution is it possible to achieve typically in biological samples using this technique? [5p]

Principle: Excitation in a standard Gaussian mode. Stimulated emission in a ring-like mode. The stimulated emission is at a wavelength that is filtered out and does not reach the photodetector. In effect this means that the central spot is decreased in size. To go beyond the diffractin limit it is important that the stimulated emission is depleted, i.e. that the STED beam has an intensity greater than saturation.

Performance: High-resolution, sub-diffraction limit imaging in the far-field. [4p]

Basicly the standard diffraction limit is improved by the STED factor.

$$r \sim \frac{0.66\lambda}{NA} \frac{1}{\sqrt{1 + \frac{I_{STED}}{I_{SAT}}}} \text{ [1p]}$$

In practice ~50nm resolution, but requires skill and expensive equipment.[1p]

5. Molecular Motors [21p]

Use a new sheet of paper for these questions.

[a] Name and explain three key differences between a Brownian or molecular motor on the one hand, and a combustion engine on the other hand. [3p]

1. *Combustion engines use chemical energy to produce heat, and the heat is then converted into work. Molecular motors skip the heat step, but work isothermally.*
2. *The kinetic energy in mobile parts of a combustion energy (for example the piston) vastly exceeds the thermal kinetic energy.*
3. *Brownian motors use Brownian (thermal) motion to move forward. The input power is used not to generate motion, but to rectify random thermal motion.*

[b] How does FIONA work, and how can it be used to study details of the stepping mechanism of motors such as myosin and kinesin? [4p]

1. *Principle of FIONA (accuracy ≠ resolution); localization of centroid*

$$r \sim \frac{0.66\lambda}{NA} \frac{1}{\sqrt{N}} \text{ [2p]}$$

2. *By attaching a fluorophore either to a motor head, or to the center of the motor, and comparing the observed step sizes, one can distinguish between hand-over-hand and inchworm stepping processes. [2p]*

[c] Describe and discuss the two basic models for how stepping is achieved in two-headed motors such as kinesin or myosin V. [3p]

In one model, the motor has a power stroke: it performs a directed stepping motion, powered by some elastic energy stored in the molecule. In the other model, stepping is entirely due to rectified diffusion.

[d] What is "gating" in a bipedal (two-headed) molecular motor? Give an example of a gating mechanism. [4p]

A mechanism that prevents the motor from detaching from its track by making sure that at least one head is always bound. This is achieved by letting the binding state of one head influence the unbinding rate of the other head.

An example is intramolecular strain. Binding of one head induces strain that is communicated to the other head, changes its conformation and thereby its binding strength.

[e] Give at least one useful definition of a "stall force" in a molecular motor. [2p]

One definition is: the force under which the rate for forward stepping equals the rate for backward stepping.

Another, of course: force when average motion ceases, or when the direction of motion changes sign.

[f] The sketch below indicates a possible representation of the binding potential of a molecular motor. Each dip is binding site. Between dips, the motor can perform free, one-dimensional diffusion. Motor stepping corresponds to motor motion from one dip to the next, over a step length *L*.

- Redraw and briefly explain what the potential would look like when small force *f* to the left is exerted on a motor.
- Roughly how large can the force *f* be, to still allow the motor to step diffusively to the right? Assume room temperature and L = 10 nm. Show all steps.

[in total 5p]

[2p]

$fL < \frac{1}{2} kT$

$f < \frac{1}{2} kT/L \approx 2\text{pN nm}/10\text{ nm} \approx 0.5\text{ pN}$ [2p]

Since in reality several ½ kT are available occasionally, the motor can step against a few pN. [1p]

6. NanoSafety [22p]

Use a new sheet of paper for these questions. Draw figures!

[a] Cite three examples of manufactured (useful) nanoparticles together with their applications. [6p]

Manufactured nanoparticles: Semi-conductor nanoparticles (Quantum dots), Polymer NP (drug delivery), nanowires (transistors, LED), carbon nanotubes (strengthening material, nanoelectronics), metal nanoparticles (Au imaging, cancer treatment, Ag, antibaterial).

[b] Some nanoparticles are produced as byproducts of combustion reactions. Can you name three examples? [3p]

- *Carbon nanoparticles (including nanotubes) when burning wood.*
- *Diesel particles from diesel engines combustion.*
- *Soot from burning candles.*

[c] There have been many concerns about the safety of carbon nanotubes due to their shape that resembles that of asbestos. Why is asbestos hazardous for the health? [4p]

The long and thin asbestos fibers can penetrate deep into the lungs where they are no clearance mechanism by coughing or mucus. Asbestos fibers are longer that a microphage, which leads to the occurence of frustated phagocytosis (the macrophages cannot phagocytize the fibers). This in turns leads to chronic inflammation and fibrosis in the lung (asbestosis). Exposure to asbestos also leads to the formation of granuloma (organization of immune cells). Finally, asbestos is a carcinogen: most of the mesothelioma (cancer of the protective sac of internal organs) are due to asbestos exposure. Most mesothelioma occur in the pleura or the peritoneum.

[d] Give five examples of characteristics of nanoparticles that are important for their effects on a biological system? [5p]

Shape, Size, charge, zeta potential, hydrophobicity (including aggregation state in the medium that you are going to exposure the bio entity to), protein corona, particle core material, surface material, surface reactivity, radioactivity, crystalline structure (when applicable).

[e] Give four examples of different parameters that one can investigate when studying the effects of nanoparticles on a biological system? [4p]

- *Cytotoxicity (apoptosis, necrosis, cell survival rate compared to control)*
- *Cellular response (endocytosis, cell proliferation)*
- *Organ distribution*
- *Metabolic pathways (cellular respiration)*
- *Clearance*

- *Immuno-suppression / immuno-stimulation*
- *Formation of reactive oxygen species*