# Biophysics of Cells and Single Molecules: Assignment 4

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## Problem 1

**Calibration of optical tweezers by drag force (active).**

- **Explain the principle behind drag force calibration.**

  This is a method to obtain the value of $k$ describing the gradient force $F_g = -kx$ that the tweezers produce.

  For it to work, the bead we are trying to grab is immersed in some fluid and there is a flow at speed $v$ in said fluid. Therefore, the bead will feel a drag force $F_d = \gamma v$ pushing it alongside the flow. However, the bead is also feeling a gradient force $F_g = kx$ (with $x$ measured with respect to the focus point of the tweezers). Therefore, the bead will get to equilibrium when the forces cancel each other:

$$\begin{aligned} & F_d = F_g \\ \Rightarrow\ & \gamma v = kx \\ \Rightarrow\ & k = \frac{\gamma v}{x}. \end{aligned}$$

  Therefore, if we know the velocity $v$ and the point $x$ in which the bead reaches equilibrium, we can find $k$.

- **Consider a particle in water. Find the displacement of the particle from the trap center when $R_p = 5\mu m$, $\kappa = 0.02pN/nm$ and $v_f = 100\mu m/s$. What is the displacement of the particle in air under the same flow velocity and same trapping strength?**

  We use the last result along with the fact that for a spherical particle, the drag coefficient $\gamma$ is $6\pi\eta R$, with $\eta$ the viscosity of the fluid. Therefore:

$$k = \frac{\gamma v}{x} = \frac{6\pi\eta R v}{x}.$$

Isolating $x$ we get:

$$x = \frac{6\pi\eta Rv}{k}$$

For water, the viscocity is $\eta = 0.01 Ns/m^2$, so that:

$$\begin{aligned} x_{water} &= \frac{6\pi(0.01Ns/m^2)(5 \times 10^{-6}m)(100 \times 10^{-6}m/s)}{0.02 \times 10^{-12}N/10^{-9}m} \\ &= 4.7\mu m, \end{aligned}$$

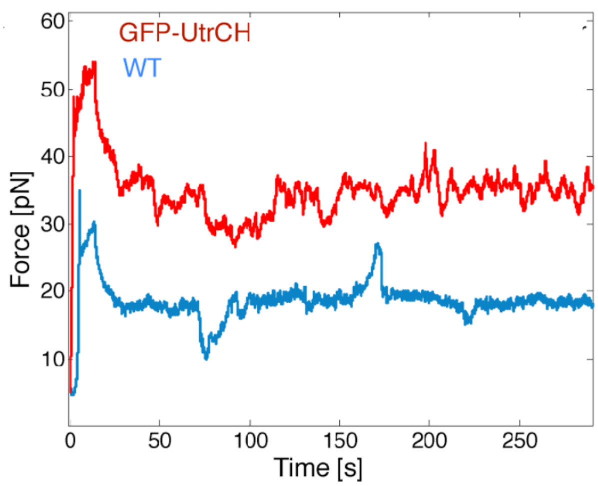
and for air the viscocity is $1.8 \times 10^{-5} Ns/m^2$, so we get:

$$\begin{aligned} x_{air} &= \frac{6\pi(1.8 \times 10^{-5}Ns/m^2)(5 \times 10^{-6}m)(100 \times 10^{-6}m/s)}{0.02 \times 10^{-12}N/10^{-9}m} \\ &= 8.5nm. \end{aligned}$$

## Problem 2

**The graphs below shows the force curve from a cell pulling on a bead. The cell has a finger-like structure (filopodium) growing out of the cell surface which grabs the particle in the trap and starts to pull the particle out of the trap**

a) **From the red force curve, estimate the maximum and minimum force. What is the maximum excursion of the particle from the center of the optical trap.**




The maximum force seems to be of around $54pN$ and the minimum force is around $27pN$.

The maximum excursion will happen when the force pulling the bead is maximum, which is $54pN$. The force of the cell pulling the bead must balance out with the force of the trap, which has the form $F = kx$, therefore, we conclude that:

$$kx_{max} = 54pN$$

$$\Rightarrow x_{max} = \frac{54pN}{k} = \frac{54pN}{0.02\frac{pN}{nm}} = 2700nm = 2.7\mu m.$$

Meanwhile, for the minimum excursion, we get:

$$x_{min} = \frac{27pN}{k} = \frac{27pN}{0.02\frac{pN}{nm}} = 1350nm = 1.35\mu m.$$

b) **Use these values to estimate the maximum and minimum energy of pulling, given that the trap constant is** $0.02pN/nm$.

Since the trap acts with a force that has the same form as that of a spring, the energy expression will also have the form that it has for a spring, which is $\frac{1}{2}kx^2$. Therefore:

$$U_{max} = \frac{1}{2}kx_{max}^2 = \frac{1}{2}(0.02pN/nm)(2700nm)^2 = 72900pNnm,$$

and for the minimum:

$$U_{min} = \frac{1}{2}kx_{min}^2 = \frac{1}{2}(0.02pN/nm)(1350nm)^2 = 18225pNnm.$$

c) **Consider the particle trapped at room temperature with a trapping constant of $0.02pN/nm$. What is the relative probability of finding the particle at the center of the trap relative to finding the particle at the position found in (a), in absence of the external force provided by the cell?**

Considering only the energy of the optical trap $\frac{1}{2}kx^2$, the energy at the center of the trap is $U_{center} = 0$. On the other hand, the energy at the maximum excursion point is, as we found in (b), $U_{max} = 72000pNnm$. We know that the probability follows the Boltzmann distribution, so that $P(x) \propto e^{-\beta U(x)}$ (there is a normalization constant, but it cancels out when considering the ratio between the two probabilities). Therefore, the relation between the probabilities is:

$$\begin{aligned}\frac{P(x_{max})}{P(x_{center})} &= \frac{e^{-\beta U_{max}}}{e^{-\beta U_0}} \\ &= \frac{e^{-(1/4.1pNnm)(72600pNnm)}}{e^0} = e^{-17700},\end{aligned}$$

which is an incredibly small number. That means that without the force, the probability of the excursion being so high is ridiculously small.

## Problem 3

**The optical force originates from the gradient in the light: $F = \alpha \nabla I$. However, this expression does not tell us much about the nature of the potential (whether it is spring-like or not). Most optical traps are based on a focal light distribution which is a normal distribution: $I(x) = I_0 \exp(-x^2/2\sigma^2)$ where $\sigma$ is the standard deviation. Use Taylor expansion (to 1st order) of the intensity distribution to show that gradient force can be approximated to be spring-like near the center of the trap.**

The Taylor expansion to second order (I take it to second order so that after differentiation, $F$ is to first order and because the first order term for $I(x)$ vanishes) of $I(x)$ is:

$$I(x) \simeq I(0) + \left.\frac{dI}{dx}\right|_0 x + \frac{1}{2!}\left.\frac{d^2 I}{dx^2}\right|_0 x^2.$$

And we have that:

- $I(0) = e^0 = 1$
- $\frac{dI}{dx} = \frac{d}{dx}\exp(-x^2/2\sigma^2) = -\frac{x}{\sigma^2}e^{-x^2/2\sigma^2}$, which evaluated at 0 is clearly 0.
- $\frac{d^2 I}{dx^2} = \frac{d}{dx}\left(-\frac{x}{\sigma^2}e^{-x^2/2\sigma^2}\right) = -\frac{1}{\sigma^2}e^{-x^2/2\sigma^2} + \frac{x^2}{\sigma^4}e^{-x^2/2\sigma^2}$, which evaluated at 0 gives a result of $-\frac{1}{\sigma^2}$.

Therefore, the Taylor expansion becomes:

$$I(x) \simeq 1 + \frac{1}{2}\left(-\frac{1}{\sigma^2}\right)x^2 = 1 - \frac{x^2}{2\sigma^2}$$

Then we differentiate to obtain the force:

$$F = \alpha\frac{d}{dx}I = -\alpha\frac{x}{\sigma^2}$$

which resembles a spring like force $F = -kx$ with $k = \frac{\alpha}{\sigma^2}$.

## Problem 4

**Compute the mean square displacement, $\langle x \rangle$, of a bead in a one dimensional optical trap and show that we can determine the trap stiffness as $\kappa = \dfrac{k_B T}{\langle x^2 \rangle}$.**

We know that modeling the trap as a spring, the energy is given by $E = \dfrac{1}{2} k x^2$. Then, Boltzmann distribution for the probability of having the particle a distance $x$ is:

$$P(x) = P_0 e^{-E/k_B T} = P_0 e^{-\frac{kx^2}{2k_B T}},$$

with $P_0$ a normalization constant such that the integral of $P(x)$ over $(-\infty, \infty)$ is 1.

Then, the expected value $\langle x \rangle$ is:

$$\langle x \rangle = \int_{-\infty}^{\infty} x P(x) dx = \int_{-\infty}^{\infty} x P_0 e^{-\frac{kx^2}{2k_B T}} dx$$

This integral is clearly equal to zero, since the function inside it is an odd function (changing $x$ by $-x$ changes the sign of the function) and therefore integrating over the whole real line $(-\infty, \infty)$ gives a result of 0.

$$\boxed{\langle x \rangle = 0.}$$

Now we calculate the second moment of the distribution:

$$\langle x^2 \rangle = \int_{-\infty}^{\infty} x^2 P(x) dx = P_0 \int_{-\infty}^{\infty} x^2 e^{-\frac{kx^2}{2k_B T}} dx$$

We will use integration by parts $\int u dv = uv - \int v du$, with $u = x$, $du = dx$ and $dv = x e^{-\frac{kx^2}{2k_B T}}$.

First we need to find $v = \int x e^{-\frac{kx^2}{2k_B T}} dx$, for which we can use the substitution $z = -\frac{kx^2}{2k_B T}$ and therefore $dz = -\frac{kx}{k_B T} dx$, so that $x dx = -\frac{k_B T}{k} dz$. Therefore, the integral turns into $v = \int -\frac{k_B T}{k} e^z dz$, which is equal to $v = -\frac{k_B T}{k} e^z = -\frac{k_B T}{k} e^{-\frac{kx^2}{2k_B T}}$.

Therefore, the integration by parts tells us that:

$$\begin{aligned} P_0 \int_{-\infty}^{\infty} x^2 e^{-\frac{kx^2}{2k_B T}} dx &= P_0 uv \Big|_{-\infty}^{\infty} - P_0 \int_{-\infty}^{\infty} v du \\ &= -P_0 \frac{k_B T}{k} x e^{-\frac{kx^2}{2k_B T}} \Big|_{-\infty}^{\infty} - P_0 \int_{-\infty}^{\infty} -\frac{k_B T}{k} e^{-\frac{kx^2}{2k_B T}} dx \end{aligned}$$

Since $e^{-\frac{kx^2}{2k_BT}}$ becomes 0 at $x = \pm\infty$, the first term is equal to 0, so we get:

$$\begin{aligned} P_0 \int_{-\infty}^{\infty} x^2 e^{-\frac{kx^2}{2k_BT}} dx &= P_0 \frac{k_BT}{k} \int_{-\infty}^{\infty} e^{-\frac{kx^2}{2k_BT}} dx \\ &= \frac{k_BT}{k} \int_{-\infty}^{\infty} P(x) dx \end{aligned}$$

However, the total integral of $P(x)$ is normalized to 1, so we conclude that:

$$\langle x^2 \rangle = \frac{k_BT}{k},$$

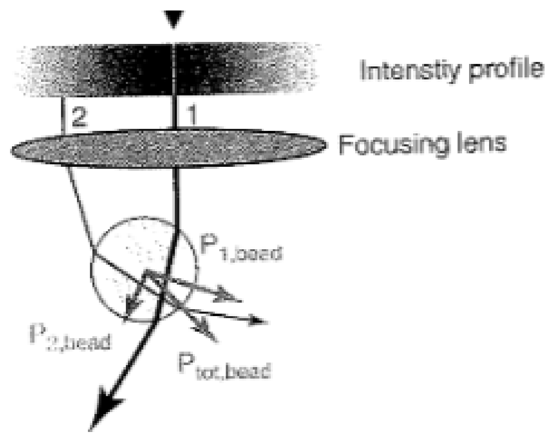
and therefore:

$$k = \frac{k_BT}{\langle x^2 \rangle}$$

## Problem 5

**Is it possible to optically trap an air bubble in water using a focused laser beam? Argue which forces are at play.**

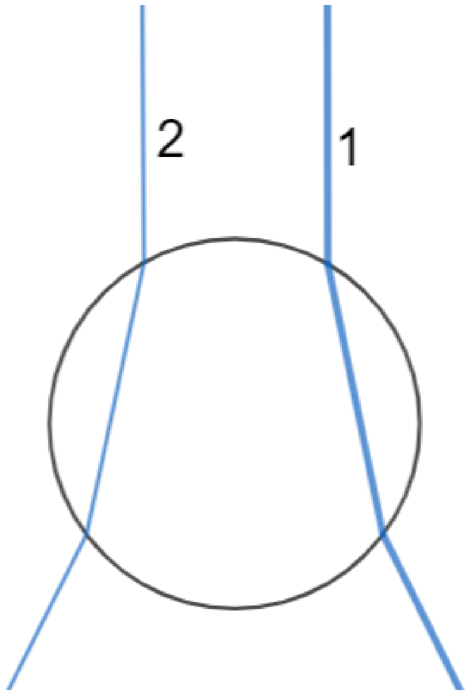
As we saw in class, in the Mie regime, optical trapping is explained by the fact that when a ray passes through the bead, it deflects and gives some momentum to the bead. The classical setup we saw in class looks like this:




The bead is slightly to the left of the center and we obtained the result that the laser would pull the bead back to the center. The ray 1 refracts in such a way that it ends up pointing slightly to the left, therefore giving the bead a force to the right. On the other hand, the ray 2 deflects to the right and therefore gives the bead a force to the left. However, since ray 1 is stronger (since it comes from a stronger part of the intensity profile), there is a net force to the right that takes the bead back to equilibrium.

However, the ray-tracing for this image is based on supposing that the bead has a higher index of refraction than the surrounding water.

Nevertheless, for air, the index of refraction is smaller than that of water, so the bending happens the to the opposite direction. Instead of the rays bending inwards when they reach the bead, they bend outwards. In this case, the rays will look something like this:




Therefore, now ray 1 is changing direction to the right, giving the bead a force towards the left. Meanwhile, ray 2 deflects towards the left, giving the bead a force towards the right. However, since the first ray is stronger (it comes from the center of the intensity profile), it dominates and the bead will have a net force towards the left direction. Therefore, we conclude that if it starts a little bit to the left, it feels a force that also points to the left, pushing it farther away from the center and will not be trapped.