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Forces on Cells in a Two-Beam Laser Trap (OS)

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1. Introduction

Biological cells are the functional building blocks of life. In large structures they form tissue and organs which, taken together, make up the human body. Due to the numerous individual tasks cells have to fulfill in the body, they perform various complex activites. Many investigations have been driven by the desire to understand, predict, and influence cellular behavior. In order to comprehend the mechanisms of biological functioning of the cellular unit it is necessary to investigate the interaction of the underlying subsystems. One of the major underlying functional systems of the cell is the cytoskeleton. It is a polymer network consisting of individual subnetworks and essential for cellular functions such as cell motility, organelle transport, mechanotransduction, and cell division. Cytoskeletal characteristics are reflected in its mechanical properties, which can be probed by rheology.
The Optical Stretcher can be used to do whole cell elasticity measurements. In contrast to other techniques the measurements can be done without touching or modifying the cell (Guck 1997). Two counter propagating divergent laser beams create an optical trap in which particles can be trapped and stretched.

2. Theoretical Background

The Microfluidic Optical Stretcher (MOS, see figure 1) is a further developement of the former setup advanced by a microfluidic delivery system which was invented by Bryan Lincoln (Lincoln et al 2005). It enables measurements of about 100 cells per hour and therefore empowers statistically significant results.

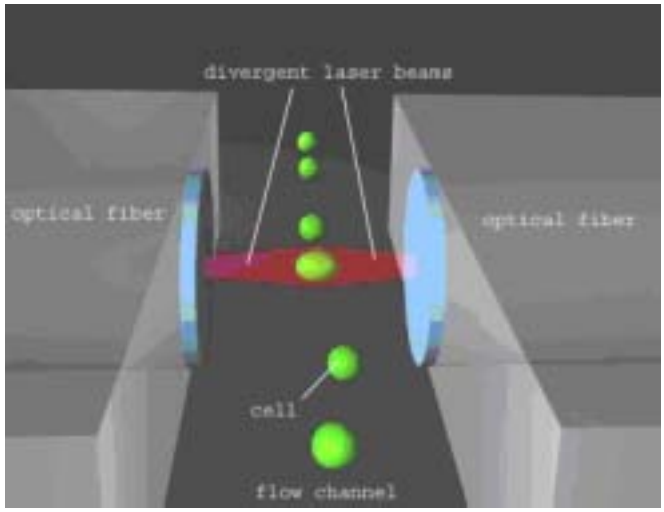

Figure 1: *Scheme of the flow chamber of the Microfluidic Optical Stretcher. Cells are delivered through the flow channel and trapped and deformed in the center of the two counter propagating divergent laser beams (source: Guck et al., 2005).*

When light passes from an optically thinner medium (e.g. water) to an optically denser sphere (e.g. cell), a force is induced to the surface of the sphere due to momentum transfer. These transferred momenta are always normal to the surface (Wottawah 2006). They create a surface stress (force per unit area) which can be expressed by

$$\sigma(\alpha) = \frac{F(\alpha)}{A} = \frac{\Delta p(\alpha)}{\Delta t A} = \frac{\Delta p(\alpha)}{E}\frac{E}{\Delta t A} = \frac{\Delta p(\alpha)}{E} I \,, \qquad (1)$$

with I being the intensity of the incident ray light, ^{1}p the transferred momentum and E the energy of the incident light ray. For a two beam laser trap the entire stress profile can be approximated by the analytic expression (see also figure 3)

$$\sigma(\alpha) = \sigma_0 \cos^n \alpha \,, \tag{2}$$

where n is an even number (Schinkinger and others 2004).

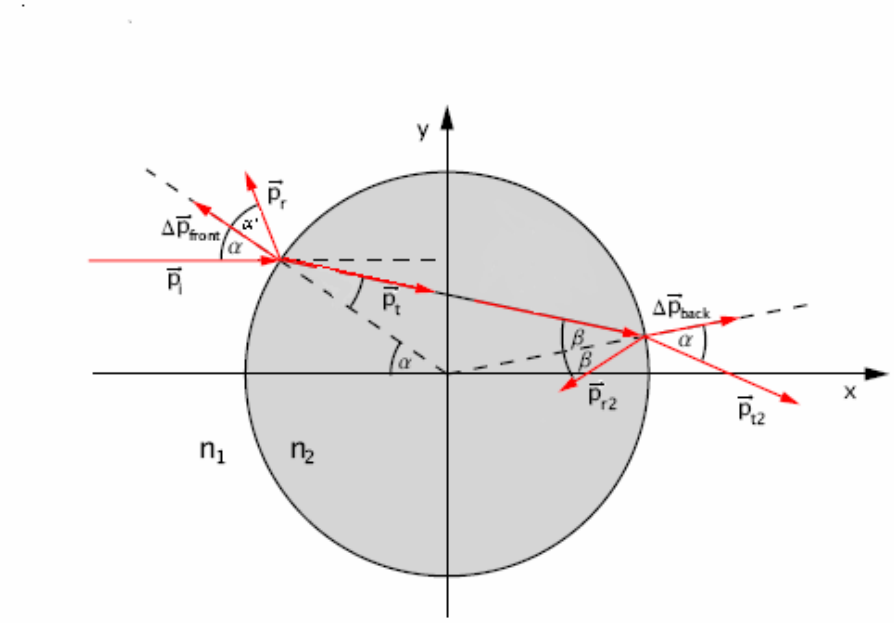

Figure 2: *A light ray being refracted by a spherical object. The incident light ray carries the momentum $\vec{p}_i$, the refracted $\vec{p}_t$ and the reflected $\vec{p}_r$. $\Delta\vec{p}_{front}$ and $\Delta\vec{p}_{back}$ are transferred to the surface due to conservation of momentum.*

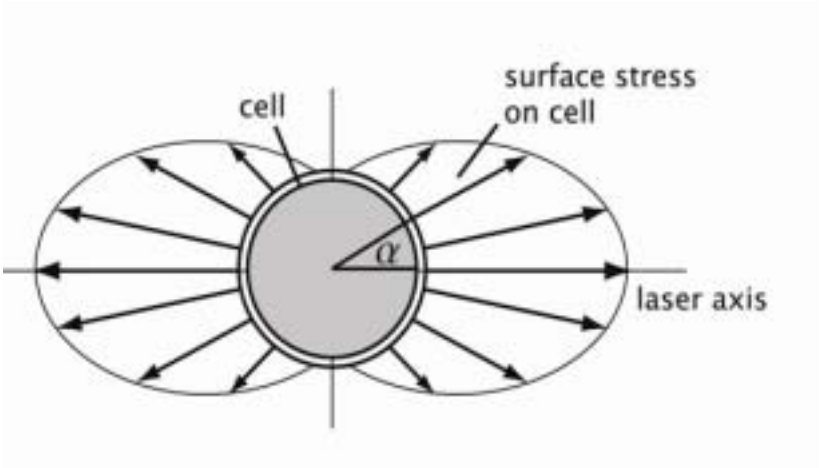

Figure 3*: Profile of the optical surface stress along the angle α of a cell in a two beam laser trap (source: (Wottawah 2006)).*

When a deforming force is applied to a material, it will respond either elastic, viscous or viscoelastic, an intermediate form of purely elastic or viscous behavior, such as cells. The different responses are illustrated in figure 4.

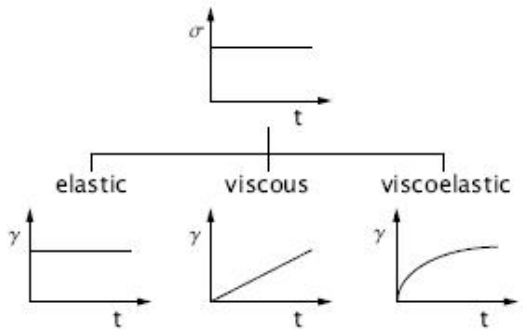

Figure 4: *Different responses to a constant stress σ. Elastic materials show an immediate response while viscous materials expand proportional to time. Viscoelasticity, as a combination of elastic and viscous behavior, response retarded to the applied stress.*

For linear elastic deformation, the tensile creep compliance D(t) can be described in terms of stress σ(t) and the relative deformation (strain) γ(t)=Δx/x:

$$\sigma(t) = E\gamma(t) = \frac{1}{D}, \tag{3}$$

where E(t) is the Young's Modulus. Linear viscous deformation is given by

$$\sigma(t) = \eta \frac{d\gamma(t)}{dt}. \tag{4}$$

There are various types of viscoelastic behaviour. The general relationship between stress and strain can be expressed by

$$\sum_{i=0}^{n} a_i \partial_t^i \gamma(t) = \sum_{j=0}^{m} b_j \partial_t^j \sigma(t). \tag{5}$$

Terminating equation (5) at the second order expansion of the strain and at first order expansion of the stress and neglecting zeroth-order term of strain leads to

$$a_1 \partial_t \gamma(t) + a_2 \partial_t^2 \gamma(t) = \sigma(t) + b_1 \partial_t \sigma(t), \tag{6}$$

which has been found sufficient to fit the typical response of a cell to a temporal stress (Wottawah and others 2005). A temporal step stress can be described by

$$\sigma(t) = F_G \sigma_0 \theta(t) \theta(t_1 - t), \tag{7}$$

where F_G is a geometric correction factor which takes into account the geometry of the cell and the shape of the stress applying field with respect to the peak stress, σ_0 (Ananthakrishnan

2003). t is the time since the step stress was initiated, t_1 the length of time of the applied stress. Suspended cells comprise a nearly isotropic actin cortex which mainly attributes to the deformation and therefore allows modeling these cells as thick shells (Ananthakrishnan and others 2005). Combining equation (6) and (7), the strain then turns out to be

$$\gamma(t) = F_G\sigma_0\left(\frac{b_1}{a_1} - \frac{a_2}{a_1^2}\right)\left(1 - \exp\left(-\frac{a_1}{a_2}t\right)\right) + \frac{F_G\sigma_0}{a_1}t \tag{8}$$

for 0<t<t_1, the interval the stress is applied, and

$$\gamma(t) = F_G\sigma_0\left(\frac{b_1}{a_1} - \frac{a_2}{a_1^2}\right)\left(1 - \exp\left(-\frac{a_1}{a_2}t_1\right)\right)\exp\left(-\frac{a_1}{a_2}(t - t_1)\right) + \frac{F_G\sigma_0}{a_1}t_1 \tag{9}$$

for t>t_1.

In order to get the constants a_1, a_2 and b_1, the function is fitted to the measured data of the time dependent strain. The time dependent shear modulus, G(t), is defined by the ratio of mean stress to mean strain. Considering the described strain behavior, G(t) is given by (Aklonis 1972; Wottawah et al 2005)

$$G(t) = \frac{1}{2(1+\mu)}\left(\frac{a_1b_1 - a_2}{b_1^2}\exp\left(-\frac{t}{b_1}\right) + \frac{a_2}{b_1}\delta(t)\right), \tag{10}$$

where μ is the Poisson ratio which is considered to be μ=0.45 for shell-like actin cortex (Mahaffy et al 2000).

G(t) can be converted via Fourier transformation into a frequency dependent complex shear modulus G*(ω)=G'(ω)+iG''(ω) with

$$G'(\omega) = \frac{1}{2(1+\mu)}\left(\frac{\omega^2(a_1b_1 - a_2)}{1+\omega^2b_1^2}\right), \tag{11}$$

$$G''(\omega) = \frac{1}{2(1+\mu)}\left(\frac{\omega a_1 + \omega^3a_2b_1}{1+\omega^2b_1^2}\right). \tag{12}$$

The storage modulus, G'(ω) yields the elastic component of the cell's response to the applied stress, the loss modulus, G''(ω), the viscous component. From these functions, one can obtain characteristic material constants (Wottawah and others 2005) such as the so-called rubber plateau, the fluid-to-solid transition frequency, and the inner-dynamical transition frequency.

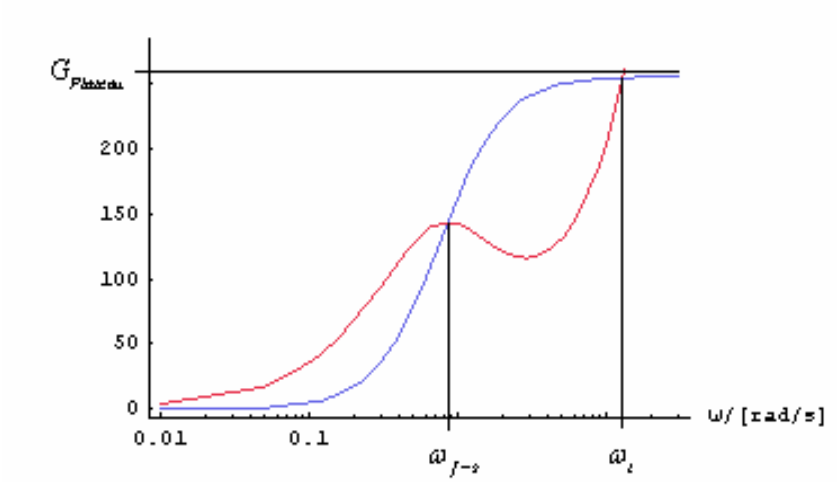

Figure 5: *The complex shear modulus. The cross-overs of the storage modulus (blue) and the loss modulus (red) are characteristic material constants as well as the plateau value of the storage modulus called rubber plateau.*

The refractive index of cells is - besides their size and the distance between the fibers - necessary to calculate the exact forces acting on the cell.
To measure the refractive index, a technique first described by Barer and Joseph is used (Barer 1955). Here, cells are suspended in bovine serum albumin (BSA) dissolved in phosphate buffered saline (PBS) in various concentrations. If the refractive index of this solution is equal to the refractive index of the cells, they match to the surrounding and are not visible. But cells with a refractive index different from that of the solution appear brighter or darker and can be counted. By systematically increasing the refractive index of the solution, the turning point from 'all cells appear brighter than the surrounding' to 'all cells appear darker' can be detected. This corresponds to the main refractive index of this cell type.
The concentration of the solution without cell obeys the relation

$$C_{BSA} = \frac{n_{BSA} - n_{PBS}}{\alpha} \tag{13}$$

where n_{BSA} is the refractive index of the BSA-solution with the concentration C_{BSA} and n_{PBS} the refractive index of pure PBS. The concentration C_{BSA} is the number of grams of BSA dissolved in 100 ml. The constant $\alpha = 0.00187$ [1/ (g/ml)] is the specific refraction increment for BSA (i.e. the increase in refractive index of the solution for every one percent increase in concentration of the BSA-solution).
For the determination of the cells' refractive index, the cells are initially suspended in PBS solution. Samples with different amounts of cell solution and BSA-solution are then mixed. The concentration of BSA in the mixture decreases with addition of cell/PBS- solution as well as the resulting refractive index n_{Total}. The number of cells that appear brighter or darker than the surrounding medium are counted and the resulting refractive index n_{Total} of each sample is subsequently measured using an Abbe-refractometer. Since the refractive index is not exactly the same for each cell a normal distribution of the refractive index is found. Integrating over this distribution results in an error function given by

$$erf(n) = \frac{2}{\sqrt{\pi}} \int_0^n e^{-t^2} dt \tag{14}$$

In Figure 6 the integrated distribution of NB4 cells is given as example. The inflexion point determines the resulting refractive index n_{Total} . It is the mean value of all refractive indices of the cells in the whole sample.

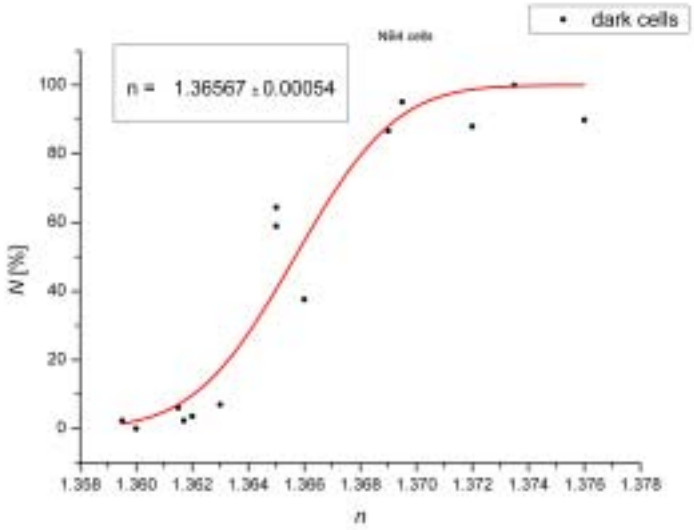

Figure 6: *Determination of the refractive index of NB4 cells by index matching (Barer 1955). This method works by counting the number of cells that are brighter or darker than the background defined by a BSA/PBS reference. The mean index of refraction of this population is given by the inflexion point of the fit.*

3. Tasks and experimental procedures

0.) Calculations to be done in preparation for the experiment
1.) Measuring the refractive index of HL60 cells.
2.) Trapping and stretching HL60 cells using the Microfluidic Optical Stretcher.
3.) Calculating the material constants $G_{Plateau}$, $\omega_{f\text{-}s}$, ω_t.

Experimental procedure and evaluation

0.) Calculations to be done in preparation for the experiment

- Determine the momenta transferred by a light ray which is refracted by a spherical object according to figure 2. Show that the transferred momenta are normal to the surface.
- Starting from equations (11) and (12) derive the plateau modulus $G_{Plateau}$, the fluid-to solid transition frequency $\omega_{f\text{-}s}$ and the inner-dynamical transition frequency ω_t.

1.) Measuring the refractive index of HL60 cells

- Prepare a BSA stem solution with a refractive index of about 1.39.
- Determine the mixing ratio of the stem solution and PBS in order to get refractive indices varying from 1.355 to 1.385 in steps of 0.001.
- Prepare appropriate solutions (total amount of 100µl each) using HL60 cells suspended in PBS.
- Put solution on a glass slide and count number of darker and brighter cells using a phase contrast microscope. (Why would a light-optical microscope not work?)
- Fit error function to data points and thus determine the refractive index of HL60 cells.

2.) Trapping and stretching HL60 cells using the MOS

- Flush microfluidic system first with Millipore and then with PBS
- Start custom made LabView based data recording program stretcher.V3.3
- Measure fiber distance.
- Inject HL60 cells into the flow chamber.
- Stretch at least 20 cells with 1.2W for 2sec. During the stretch the cell should not move or rotate. Be aware that the flow is entirely stopped during stretching.
- Flush system with Millipore when you finished your measurement
- Images are recorded as binary files during the measurement. Extract data using a custom made program stack_extractV.1.0.
- Apply edge_detection_tool_v3.02.
- Save data to CD/DVD!

3.) Calculating the material constants $G_{Plateau,}$ $\omega_{f\text{-}s,}$ ω_t

- Calculate peak stress σ_0 (assistance will be given).
- Plot relative elongation of measured cells along the laser axis (strain) over time. Do so for every measured cell in one diagram.
- Plot average strain of all measured cells showing the standard error.
- Fit constitutive equation to strain (assume F_G=4.63 for geometric factor and μ=0.45 for Poisson ratio).
- Calculate $G_{Plateau,}$ ω_f and ω_t from the fitting parameters.

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