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Biophysik-Praktikum

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.

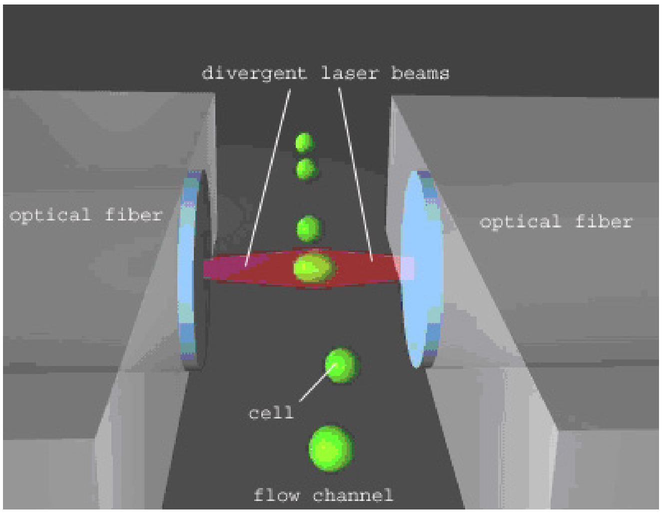

Figure 1: *Scheme of the flow chamber of the Microfluidic Optical Stretcher. Cells are delivered through the flow channel and trapped and deformed in the center of the two counter propagating divergent laser beams (source: Guck et al., 2005).*

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, ⌠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)

$$\sigma(\alpha) = \sigma_0 \cos^n \alpha \,, \qquad (2)$$

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

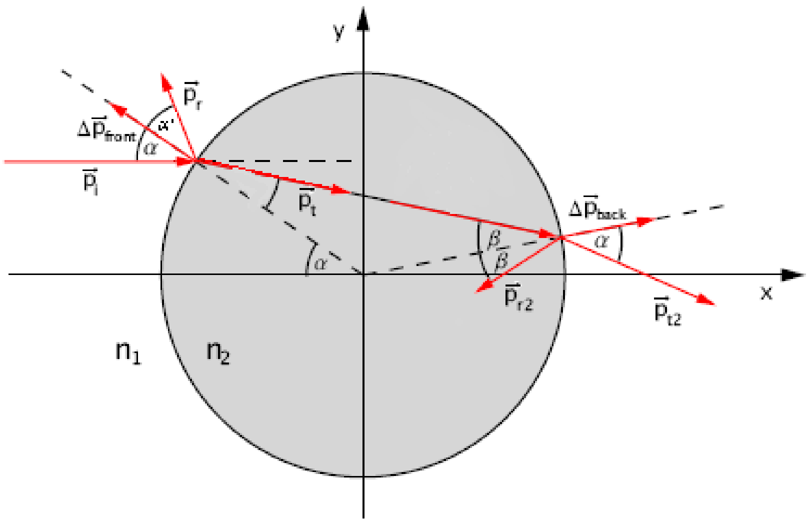

Figure 2: *A light ray being refracted by a spherical object. The incident light ray carries the momentum* $\vec{p}_i$*, the refracted* $\vec{p}_t$ *and the reflected* $\vec{p}_r$*.* $\Delta\vec{p}_{front}$ *and* $\Delta\vec{p}_{back}$ *are transferred to the surface due to conservation of momentum.*

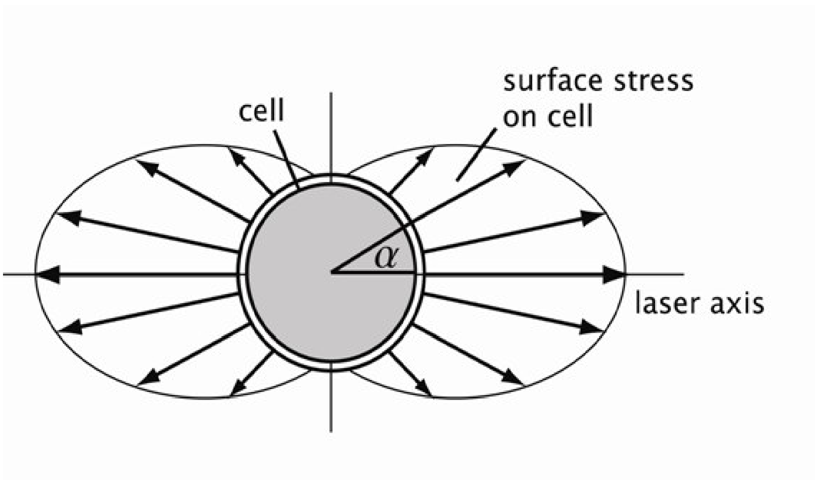

Figure 3*: Profile of the optical surface stress along the angle α of a cell in a two beam laser trap (source: (Wottawah 2006)).*

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.

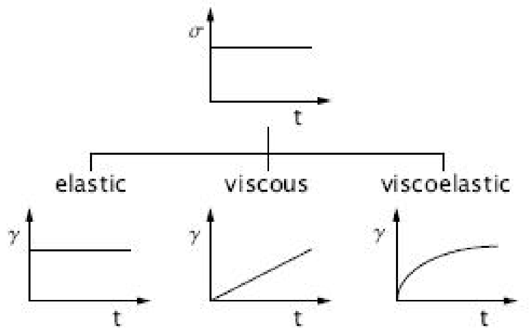

Figure 4: *Different responses to a constant stress σ. Elastic materials show an immediate response while viscous materials expand proportional to time. Viscoelasticity, as a combination of elastic and viscous behavior, response retarded to the applied stress.*

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.

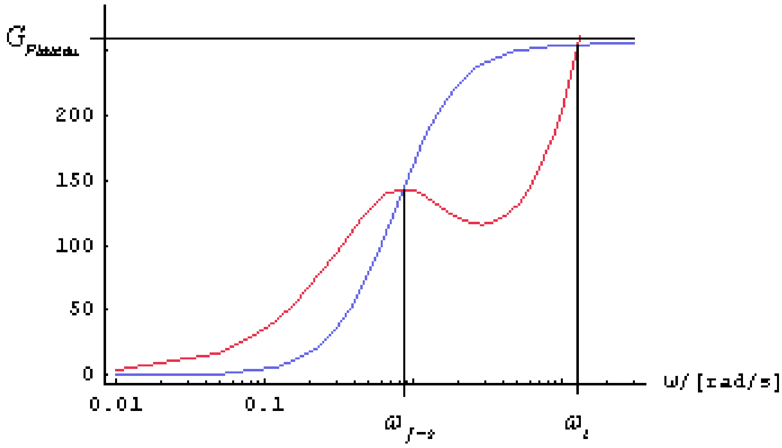

Figure 5: *The complex shear modulus. The cross-overs of the storage modulus (blue) and the loss modulus (red) are characteristic material constants as well as the plateau value of the storage modulus called rubber plateau.*

3. Tasks and experimental procedures

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

Experimental procedure and evaluation

0.) Calculations to be done in preparation for the experiment. Results will be discussed during the Antestat.

!!! This will make 25% of your final mark together with the Antestat !!!

- 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 ω_{f-s} and the inner-dynamical transition frequency ω_t.

1.) 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.vi
- Set up the program settings (assistance will be given)
- Stretch at least 50 cells with 1.0W 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
- Apply EdgeDetection 0.54 RC
- Evaluate data using Cellinspector
- Save data to CD/DVD!

2.) Calculating the material constants $G_{Plateau}$, ω_{f-s}, ω_t
- Plot relative elongation of measured cells along the laser axis (strain) over time. Create one diagram showing the plot for each cell.
- Plot average strain of all measured cells showing the standard error.

- Plot histogram of strain after 1s stretch
- Fit constitutive equation to strain (assume F_G=1.47 for geometric factor, μ=0.45 for Poisson ratio and σ_0=4.96Pa for peak stress).
- Calculate $G_{Plateau,}$ ω_f and ω_t from the fitting parameters.

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