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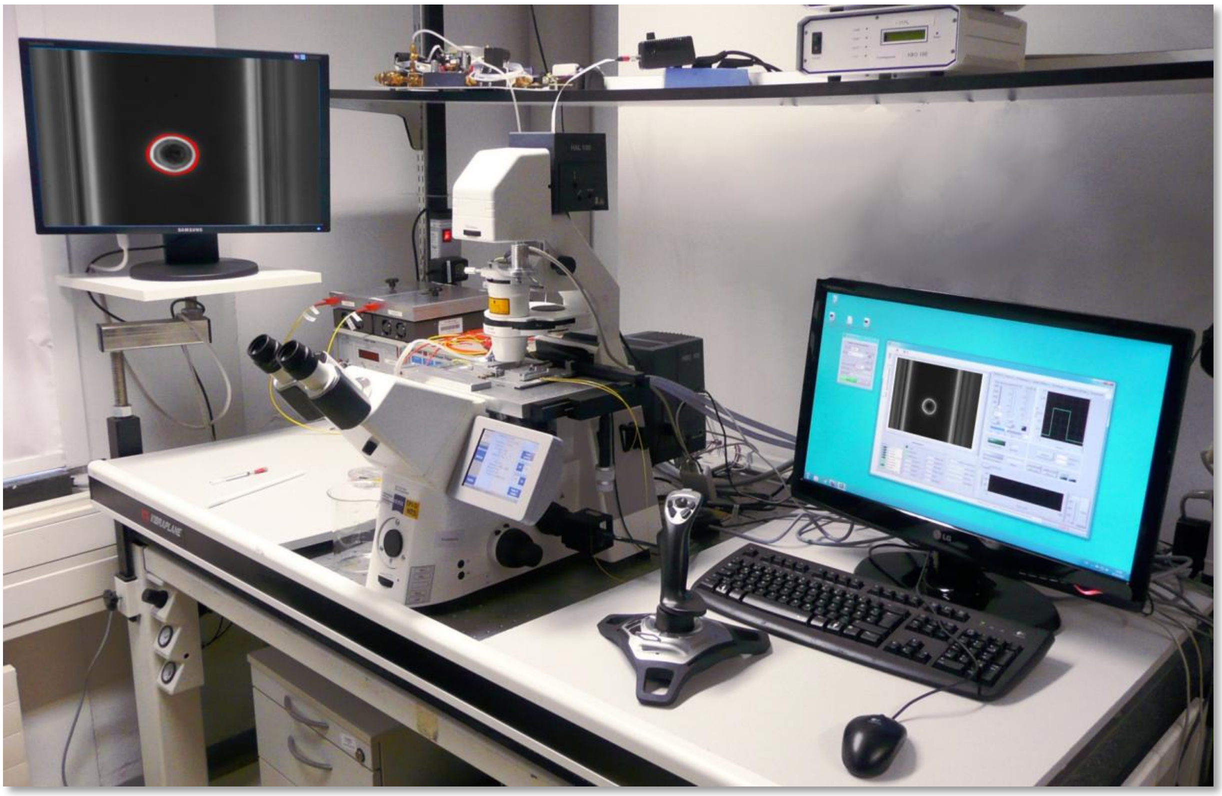
Forces on Cells in a Two-Beam Laser Trap (OS)



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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.

Theoretical Background

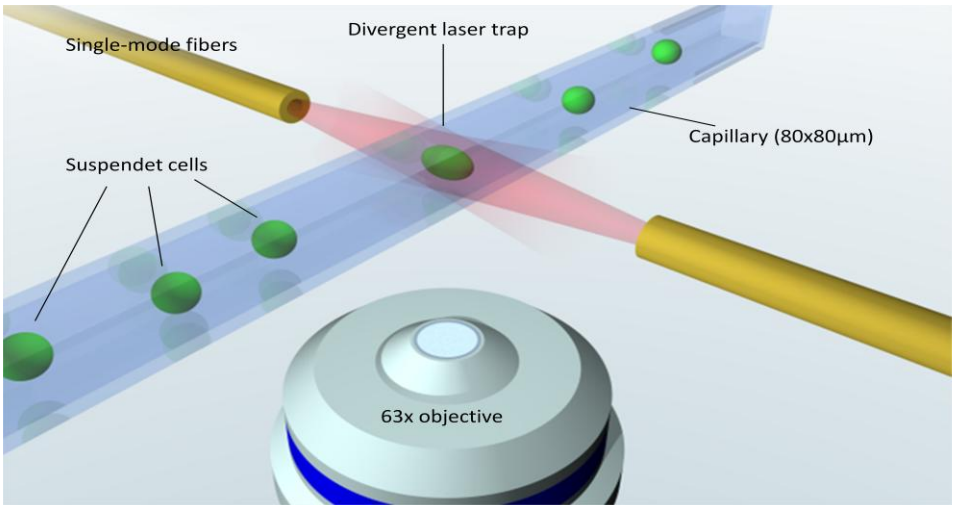

Figure 1: *Scheme of the flow chamber of the AMOS. Cells are delivered through the flow channel and trapped and deformed in the center of the two counter propagating divergent laser beams.*

The Automated Microfluidic Optical Stretcher (AMOS, see figure 1) is a further development of the former setup advanced by a microfluidic delivery system and a computer-controlled pumping system as well as sophisticated software tools. It enables fully automated measurements of about 200 cells per hour and therefore empowers statistically significant results.

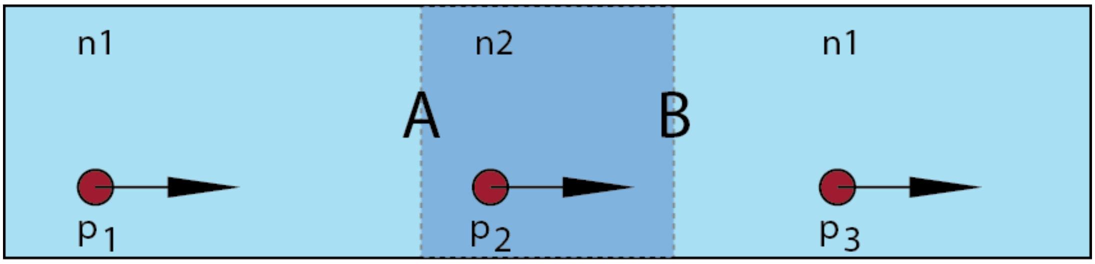

Figure 2: *A photon traveling in a medium of refractive index n1, carrying a momentum of p_1. At the interface **A**, the photon enters another medium of refractive index n2. At interface **B**, the photon exits medium 2 and enters to medium 1 again.*

The occurrence of optical forces can be explained by conservation of momentum. Einstein's explanation of the photoelectric effect is based on the ascription of momentum to photons. Contrary to Newton's second law, massless quasi- particles are therefore able to apply forces, according to classical transfer and conservation of momentum.

Following this explanation, a photon travelling in media of refractive index n_m carries a momentum $\vec{p}_m$ given by:

$$\vec{p}_m = \frac{E\, n_m}{c_0} \vec{e}_r$$

where $E = h\nu$ is the energy of a photon, $\vec{e}_r$ is the unit vector in direction of propagation and c_0 is the speed of light in vacuum. This means that the momentum of a photon depends on the refractive index of the medium it travels in. Consequently, at the interface of two media with different refractive indices, the momentum of a photon is changed while its passing the interface. The law of conservation of momentum requires the appearance of a resulting momentum $\vec{p}_{res}$, which is transferred to the interface, according to:

$$\vec{p}_{res} = \vec{p}_1 - \vec{p}_2$$

In case of a cell experiencing forces in a two beam laser trap, more complicated calculations yield a stress profile acting on the cell's surface as shown in figure 3.

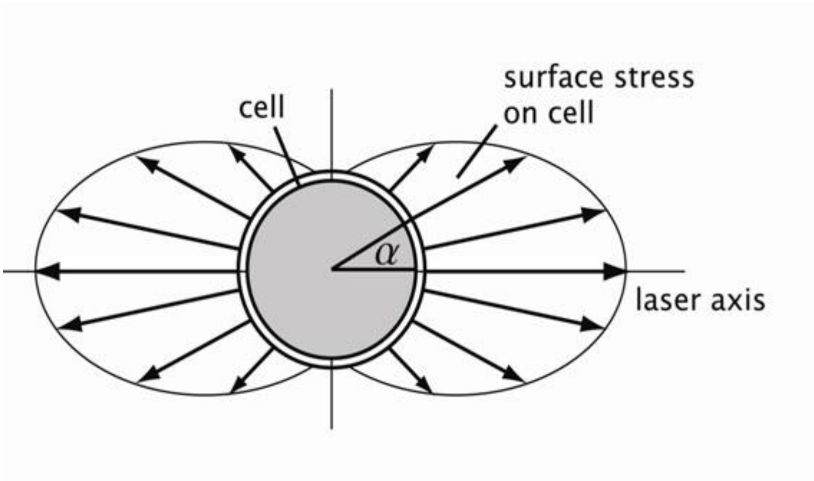

***Figure 3**: Profile of the optical surface stress along the angle α of a cell in a two beam laser trap (source: (Wottawah 2006)).*

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.

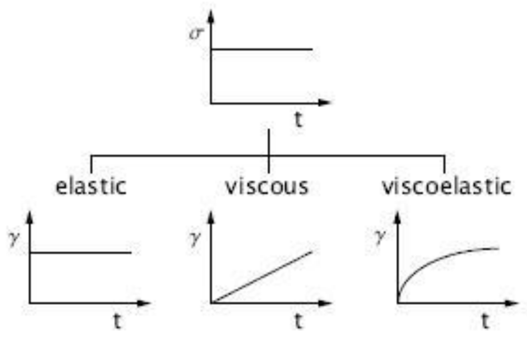

***Figure 4:** Different responses to a constant stress σ. Elastic materials show an immediate response while viscous materials expand proportional to time. Viscoelasticity, as a combination of elastic and viscous behavior, response retarded to the applied stress.*

The biomechanical behaviour of cells can be used to study cytoskeletal structures as well as diseases, known to alter cytoskeletal compositions such as cancer (Zink, Fritsch et al. 2010).

Tasks and experimental procedures

1.) **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 !!!

Consider a photon as shown in figure 2, traveling in a medium with refractive index n1.

a) What momentum is transferred to interface A if the photon is totally reflected?
b) What momentum is transferred to interface A when the photon traverse interface A. What momentum is transferred to interface B when the photon traverse interface B. Compare the direction of the induced momenta at interface A and B, under the assumption of n2 > n1.
c) Given a laser power of P = 1.0 Watt. Calculate the appearing forces at interface A, given n1 = 1.33 and n2 = 1.37. Mind the relation $\vec{F} = \frac{\mathrm{d}\vec{p}}{\mathrm{d}t}$. Reflections can be ignored.

2.) **Evaluation of two sets of data from cells stretched at different laser power.**

You will get raw data from cells stretched at 800 and 1200 mW showing the diameter of the cells along the laser axis with time. Choose an appropriate data processing program (e.g. Origin, MatLab, Mathematica) for data evaluation.

a) Determine the relative deformation of the cell with time. Use average diameter of cell during trap for normalization.
b) The maximum deformation at the end of stretch is of special interest. Plot histograms of this value for both sets of data. Determine mean value and standard deviation.
c) Do the histograms resemble a Gaussian distribution? Use statistical tools to verify your answer. *Bonus: Which probability distribution would eventually better describe your data? Show with fit of distribution curves the better matching.*
d) How much do the mean values of the two measurements differ from each other? Is this change significant? Use statistical tools (e.g. Student's t-test) and give significance level. What are the limitations of your chosen significance test?

3.) What is the impact of laser power on the acting forces?

a) Plot mean values of deformation over time for the data sets of the two measured laser powers. Does the difference mirrors your expectation of the influence of the laser power on the applied force?

b) Is there a change in deformation behavior (shape of deformation curve)? *Bonus: Do cells react more viscous or more elastic at higher forces?*

References

Guck, J. (1997). The optical stretcher, a novel, noninvasive tool to manipulate biological materials. Department of Physics. Austin, TX, University of Texas at Austin.

Wottawah, F. (2006). Optical Cell Rheology: From the Microscopic Origins of Cellular Elasticity to Oral Cancer Diagnosis. Institute of Soft Matter Physics. Leipzig, University of Leipzig**:** 118.

Zink, M., A. Fritsch, et al. (2010). "Probing the physics of tumor cells from mechanical perspectives." Cell News - Newsletter of the German Society for Biology **36**(4/2010): 17-21.