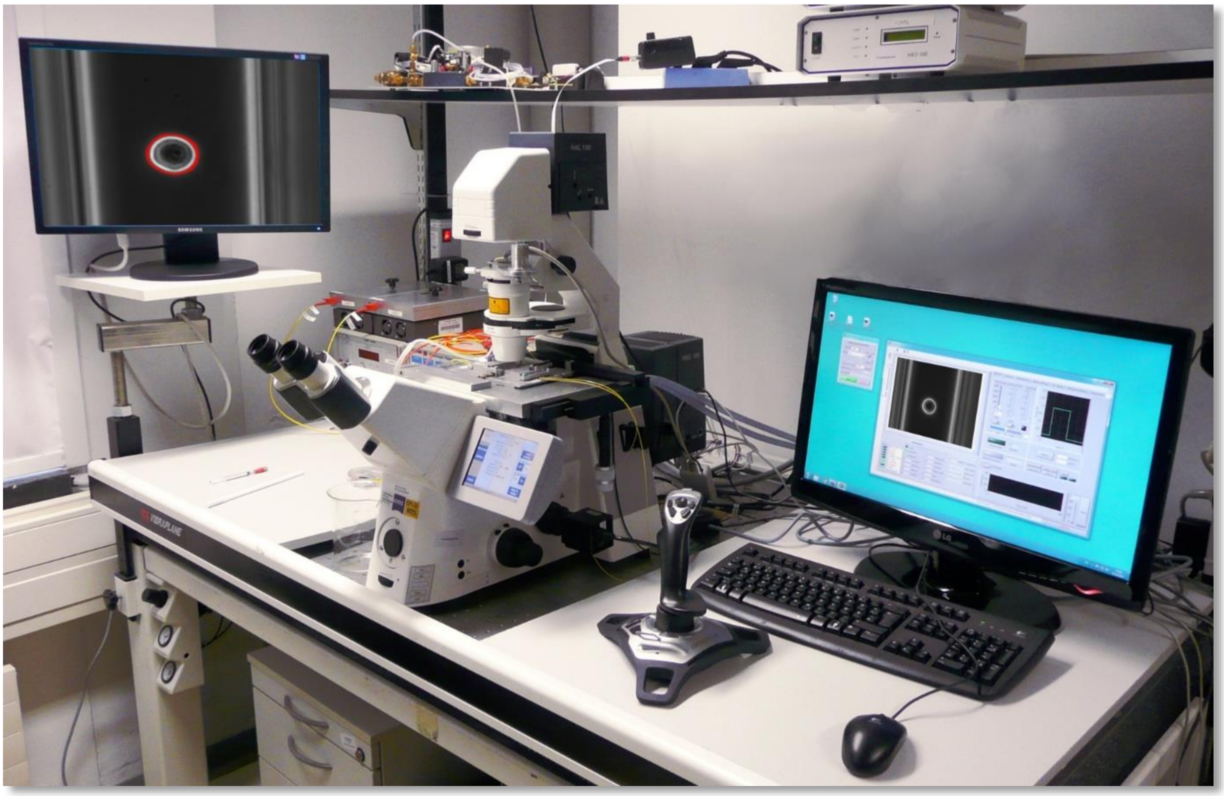
Universität Leipzig

Biophysik-Praktikum

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



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Wintersemester 2013/2014

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 fulfil in the body, they perform various complex activities. Many investigations have been driven by the desire to understand, predict, and influence cellular behaviour. 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 sub-networks 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

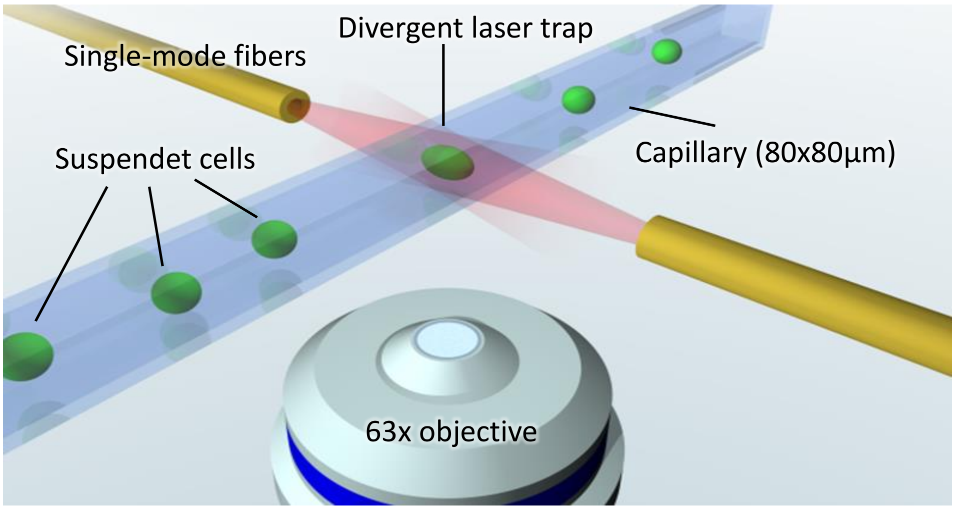

Figure 1: Scheme of the flow chamber of the AµOS. Cells are delivered through the flow channel and trapped and deformed in the center of the two counter propagating divergent laser beams.

The Automated Microfluidic Optical Stretcher (AµOS, 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.

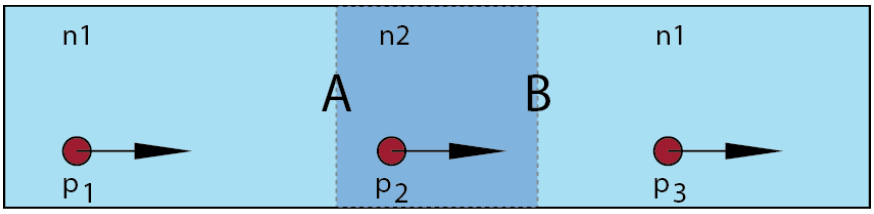

Figure 2: A photon traveling in a medium of refractive index n1, carrying a momentum of p_1. At the interface A, the photon enters another medium of refractive index n2. At interface B, the photon exits medium 2 and enters to medium 1 again.

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:

$$\vec{p}_m = \frac{E\, n_m}{c_0}\vec{e}_r \tag{1}$$

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.
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.

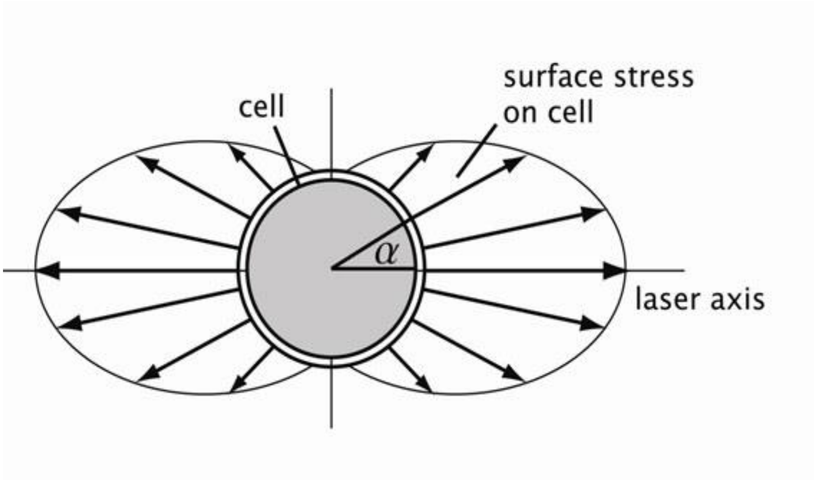

Figure 3: Profile of the optical surface stress along the angle α of a cell in a two beam laser trap (source: (Wottawah 2006)).

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 behaviour, such as cells. The different responses are illustrated in figure 4.

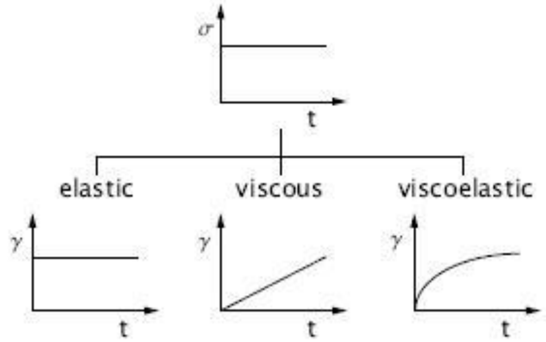

Figure 4: Different responses to a constant stress σ. Elastic materials show an immediate response while viscous materials expand proportional to time. Viscoelasticity, as a combination of elastic and viscous behaviour, response retarded to the applied stress.

A common model to describe material response assumes that materials can be described by a set of ideal springs and dash-pots in series and/or parallel leading to equations for complex stress-strain relations. An ideal spring follows Hooke's law

$$\sigma_S(t) = E\varepsilon_S(t) \tag{2}$$

and an ideal dash-pot follows Newtonian friction

$$\sigma_D(t) = \eta\dot{\varepsilon}_D(t) \tag{3}$$

with the stress σ the strain ε, the Young's modulus E (spring constant) and the viscosity η (friction coefficient). To get intensive (instead of extensive) material properties E and η, the units in these constitutive equations are Pa for stress, 1 for (relative) strain, Pa for Young's modulus and $\mathrm{Pa \cdot s}$ for the viscosity.

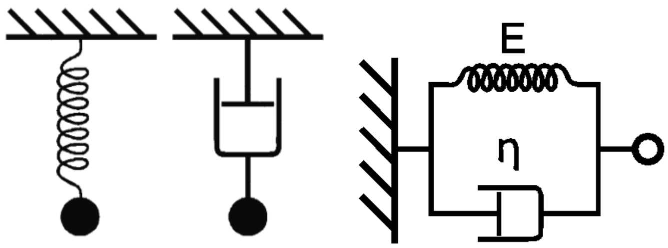

Figure 5: Sketch of a spring and a dash pot (left) and a sketch of the Kelvin-Voigt model (right). The point of action of the applied stress and/or strain is the black circle.

The most basic viscoelastic models for step stress experiments is the Kelvin-Voigt model (spring and dashpot in parallel). It is used to model the resulting strain response due to sudden stress (step stress).

Another way to model the strain response is based on a power-law behaviour, here the Bailey-Norton law, given by

$$\varepsilon(t) = A\sigma_0^n t^m$$

with time t, constant stress σ_0, material constant A and rational exponents n and m ($0 < m < 1$ in most cases).

Tasks and experimental procedures

In experimental physics, your work is not done by creating an experiment; mostly, this is merely the beginning. After you measured "some numbers", you have to interpret them and show the significance of your measured/calculated values. In this experiment, the main focus is neither preparing an experiment nor the acquisition of data (this will be mostly done by the computer or the assistants), but to analyse the data and to test for its significance. For this, a rigorous theoretical understanding and preparation is recommended.

Calculations to be done IN PREPARATION for the experiment. Results will be discussed during the Initial Test. You have to hand in your sheet!

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

- Consider a photon as shown in **figure 2**, travelling in a medium with refractive index n_1. What momentum is transferred to interface A if the photon is totally reflected?
- 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 $n_2 > n_1$.
- Given a laser power of $P = 1.0$ W. Calculate the appearing forces at interface A, given $n_1 = 1.33$ and $n_2 = 1.37$. Mind the relation $\vec{F} = \mathrm{d}\vec{p}/\mathrm{d}\vec{t}$. Reflections can be ignored.
- Draw a sketch of a Kelvin-Voigt model and write down the stress-strain relation (see eq.(2) and eq.(3)) for the point of action. (Newton's force diagrams might help)
- Solve this differential equation for the strain $\varepsilon(t)$ for a step-stress $\sigma(t) = \sigma_0 \mathrm{H}(t)$ where $\mathrm{H}(t)$ is the unit step function.
- (Bonus: we will ask some "physics vocabularies" related to the Theoretical Background chapter)

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 in time. Use an appropriate data processing program (e.g. Origin, MatLab, Mathematica, QtiPlot, …) for data evaluation.

Deformation over time (10%)

- Determine the *relative* deformation of the cell with time (graph). Use initial diameter of the cells during trap for normalisation. Calculate/Estimate the acting forces for both data sets (see initial test).

Histograms (25%)

The maximum deformation at the end of stretch (not relaxation) is of special interest.

- Plot histograms of the maximum deformation for both data sets.
- Determine mean value and standard deviation of the maximum deformation.
- Do the histograms resemble a Gaussian distribution? Use a statistical tool to verify your answer.
- (Bonus: Which probability distribution would eventually better describe your data? Show with fit of distribution curves the better matching.)

Significance (15%)

- How much do the mean values of the maximum deformation of the two sets differ from each other?
- Is this change significant? Use a statistical tool (e.g. Student's t-test ...) to verify.

Viscoelastic model (25%)

- Plot mean/median values of deformation over time for the data sets of the two laser powers.
- Is there a change in deformation behaviour (shape of deformation curve)?
- Use (3) to calculate the mean/median apparent viscosity in the last half of a second of the stretch for both data sets
- Use the Kelvin-Voigt model to determine the strain retardation time τ for the stretch process. Determine η and E of the model. Does the Model fit the data well?
- (Bonus: Use the power-law model and determine the exponent m of t for both data sets.)
- Compare both data sets. Do cells react more viscous or more elastic at higher forces?

References

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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.

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