Seminar 4

Optical Stretcher

Author: Bernard Pavlovič
Advisor: doc. dr. Primož Ziherl

Ljubljana, February 2012
Abstract

This seminar introduces an optical stretching device, which can be compared to an optical trap which stretches the object of observation. The following chapters describe the structure and the operating principles. The main application of this device serves the exploration of mechanical properties of cells and the detection of malignant tissue. It is an basic foundation and has great potential in further biomedical research.
1. Introduction

Laser traps are already known as an invaluable tool in biological cell research. The principle is simple. The momentum of the beam is transferred to the object, which because of Newton’s second law, exerts a force on the object. Such traps are used to trap cells, to study properties of single DNA strands or to measure forces exerted by molecular motors such as myosin and kinesin. The most known and used traps are optical tweezers. Optical tweezers operate on a single-beam laser basis [1]. The optical stretcher, on the other hand, works with a double-beam laser. This device can stretch dielectric materials, including biological cells, and measure their viscoelastic properties fast and without influence on the sample. The force caused by the beam which acts between the cell even has the necessary sensitivity to distinguish between different cytoskeleton phenotypes, which are basic components of cytoplasm. This seminar introduces the basic structure, the operating principles and the applications of the optical stretcher device [1].

2. Operating principles

The optical stretcher works with a double beam trap, where two slightly divergent laser beams of a Gaussian profile trap an object in the middle. The stabilization of the object is fulfilled, as the total force on the object is zero. This condition is sufficient if the refractive index of the object is larger than the index of the medium which surrounds the object. The beam size must certainly be larger than the size of the object. The momentum transfer in cells happens at the surface, where the net force acting on the whole object is zero (Fig. 1). It is the result of the symmetrical double-beam trap geometry [1]. If the object is sufficiently elastic, like it is in the case of organic cells, the two laser beams stretch the object along the beam axis. The force, which is responsible for the stretching is due to Newton’s second law. Take for example a ray of light passing through a cube made of optical denser material (Fig. 2). To explain the stretching force, it must be said that the diameter $r$ of the cells, which is about 10 micrometers, is much larger than the wavelength $\lambda$ of the beam, which lies in the infrared spectrum. Considering that $2\pi r/\lambda \gg 1$, ray optics can be used [1]. For an incident ray travelling through a medium with refractive index $n_1$, carries a momentum of

$$p_i = \frac{n_1 E}{c},$$

where $E$ is the energy of the ray and $c$ is the speed of light in vacuum. If the ray hits an object with a different refractive index $n_2$, some of the energy $E$ is reflected [1].
Figure 1  The difference in momentum $\Delta p$ leads to a force $F$. The net force is because of the symmetry zero [2].

Figure 2  A) A scheme of momentum conservation of one light beam for a cubical object. B) Typical values of forces on either side of object. C) Resulting force [1].

From Fresnel the reflection coefficient $R$ can be determined [3].

$$R = \frac{n_1 \cos \theta_i - n_2 \cos \theta_t}{n_1 \cos \theta_i + n_2 \cos \theta_t}, \quad (2)$$

where $\theta_i$ and $\theta_t$ are incident and transmission angles, which are generalized to be zero, and the condition $n_2 > n_1$ is fulfilled. This means that the observed object is optically denser than the medium which surrounds the object. In this case the momentum size of the reflected and the transmitted ray $p_r$ and $p_t$ can be calculated [1].
The total momentum of the ray which is passing through the object has to be conserved. Because of the difference in the refractive indexes, there has to be a momentum difference $\Delta p$ between the incident ray and the reflected and transmitted rays [Eq. (4)]. This momentum difference causes, due to Newton’s second law, a force of magnitude $F$, acting on the surface of the observing object [Eq. (5)]:

$$\Delta p = p_i + p_r - p_t;$$

$$F = \frac{\Delta p}{\Delta t} = \frac{n_1 \Delta E}{c \Delta t} = \frac{n_1 Q P}{c} ;$$

$P$ is the incident ray power, whereas $Q$ describes the amount of momentum transferred ($Q = 2$ for reflection, $Q = 1$ for absorption). To simplify the calculation, the force size $F$ is described in terms of $Q$. Taking in account Eq. (1), Eq. (3) and Eq. (4), Eq. (5) becomes

$$Q_{\text{front}} = 1 + R - n(R - 1),$$

where $n = n_1 / n_2$. This force points backwards away from the object (Fig. 2B). The transmitted ray travels to the other side of the object, where it comes to a second reflection and transmission. Like at the first boundary, a force acts on the object which can be described as

$$Q_{\text{back}} = [n + Rn - (1 - R)](1 - R);$$

This force acts in the beam direction, away from the object (Fig. 2B). The total force, which acts on the object is an order of magnitude smaller than the front or the back force. This force pushes the object in the beam direction, while the back and front force stretch the object. Taking in account the angle $\alpha$ under which the beam hits the object, $Q$ becomes a function of $\alpha$, $Q(\alpha)$. According to Snell’s law, it is possible to calculate the stress $\sigma$ along the objects surface

$$\sigma_{\text{front/back}} (\alpha) = \frac{n_1 Q_{\text{front/back}}(\alpha) I(\alpha)}{c} ,$$

where $I(\alpha)$ is the light intensity of the beam and $Q_{\text{front/back}}$ represents the back or the front force acting on the objects surface. $Q_{\text{front/back}}$ is composed of the perpendicular and the parallel force component, also calculated from [Eq. (5)], with respect to Snell’s law [1].

$$Q_{\text{front/back}} = \sqrt{[Q_{\text{parallel}}(\alpha)]^2 + [Q_{\text{perpendicular}}(\alpha)]^2}$$

(9)
To obtain the total force acting on the object it is necessary to integrate over the whole object surface $\sigma$. Of course we have to take both beams into account, to calculate the total force. This force, which is in the order of few piconewtons, pulls the object into the beam direction. It also stretches the object. Because of the cylindrical symmetry of the stress profile, a gradient force is exerted on the object, if it is pushed away from the centre of the symmetry, in this case, the beam axis. This gradient force pulls the object back into the beam [1].

In case of two beams, the total force is zero, but the object is stretched. This stretching force depends on the relative index of refraction $n$ and on the ratio of the object radius $w$ and the beam radius $\rho$. The smaller the beam radius, the more intense the light, which propagates through the object and the greater the stress on the surface (Fig. 3). If the ratio between the beam radius $w$ and the object radius $\rho$ is smaller than 1, the cell trapping is unstable. The best condition for optimal trapping is, if the ratio is slightly larger than 1. In this case the calculated stress profile approximation fits almost exactly with the true profile [1].

![Stress profile of red blood cells](image)

**Figure 3** Stress profiles of red blood cells with $\rho = 3.30 \mu m$ and $n_2 = 1.378$. The power of the beam is for each profile $P = 100 \text{ mW}$. The concentric rings show the stress in N/m². $\sigma_0$ indicates the maximum stress in each profile. The first profile is unstable because $w/\rho < 1$. The dashed line in the second profile shows an approximation of the true stress profile, which is calculated using the linear theory of thin elastic membrane [1].

### 3. Experimental apparatus

The optical stretcher consists of two divergent laser beams, which are aligned one against another with an optical fiber. The initial beam, produced by an ytterbium doped laser (Fig. 4), is split into two identical opposing light beams with Gaussian profiles. The beam wavelength, which lies in the infrared spectrum, can be tuned for various measurements. To prevent
damage to the observed objects, a typical wavelength of 1.064 nm is used. To achieve optimal light intensity, the laser diameter must be little larger than the diameter of the cell. The stretching power reaches from 5 mW up to maximal 2 W on a surface of approximately 100 µm², depending on which cell is being observed [4]. Cells of the same type differ in measuring data. Therefore, it is necessary to measure more cells of the same type to get an accurate distribution of cell properties. To ensure a fast and steady measuring cell stream, two delivery systems were developed. The first uses a silicon-like structure (Fig. 5), which aligns the optical fiber to a flow channel of size 80 x 100 µm. The dimension of the channel can be easily changed by molding the polydimethylsiloxane structure over a variety of photoresist structures. The second system is based on a microcapillary tube, where the fibers are externally aligned [4].

Figure 4 The optical stretcher, an ytterbium doped laser fibre produces an infrared beam, which is divided into two identical light beams. This beams are aligned against one the other. In the flow chamber, measuring data are being collected by a phase-contrast microscope [4].
To collect the data, video phase-contrast microscopy can be used. In this microscopy, the illuminating light is taken out of phase by passing through an object with different refraction index and thickness. This light shows a phase difference to the incident illumination light which can be detected and transformed into visible image. The properties of the observed cell is then measured by taking into account the aspect ratio of a cell over time [5].

4. Applications

To understand the mechanical properties of cells, we now briefly describe their interior structure. The cytoskeleton, which is contained in cytoplasm, is responsible for many cellular functions such as motility, organelle transport, mechanotransduction, and mitosis. It consists of three types of polymers: flexible to semiflexible intermediate filaments, semiflexible actin filaments, and rigid microtubules [6]. This polymer structures are the main origin for the mechanical properties of the cell. The optical stretcher is a perfect device to study these characteristics. For a accurate observation, a time dependent step stress $\sigma(t)$ can be applied to different cell lines. The deformation can be given by $\gamma(t) = \Delta r(t)/r$ [6]. Figure 6 shows the results of such an observation. In this case, a fibroblast NIH/3T3 cell was observed. As can be seen at Fig. 5d, the stretching stops after it reaches a plateau. This is due to an elastic component in the cell, which prevents the further fluid like expansion. This is consistent with
the fact, that after 10 seconds of stretching, the cell relaxes down to 60% of the total deformation [6].

As described, the optical stretcher reveals some important properties of cells and helps us to understand the interior structure. The processes that happen in the cytoskeleton of the cell are for each cell type specific and fine tuned. If a cell is sick, the fine tuned structure of the cytoskeleton will change. Consequently, this leads to an alteration of the viscoelastic properties of the cell. Such alterations cause capillary clogs in circulatory problems, various blood diseases, genetic disorders of intermediate filaments and their cytoskeleton networks, which leads to problems with skin, hair, liver, colon, and motor neuron diseases [7]. A good example of alteration in the cytoskeleton structure is cancer, where the cell progresses from a mature and vital to a motile and immortal state by changing the rigid and ordered cytoskeleton structure to a more irregular and compliant. More specifically speaking, a reduction of polymers and proteins, which are the basic building blocks of the cytoskeleton, take place. Consequently, the fine network structure change, which causes a different mechanical behaviour of the whole cell. Scientists have been aware of this possibilities. A lot of experiments have been made with micropipette aspiration and recently with atomic force microscopy, optical traps and magnetic bead rheology [7]. However, all these observation methods have their limits and disadvantages. For example: each mechanical intervention of the probe is leading to active cell response, adhesion and therefore to false measuring data; a low cell throughput leads to a lack of statistical data; The optical stretcher in combination with a microfluidic delivery system is characterized by fast readout, which allows a greater amount of measuring results, and a stretching way without mechanical contact to the probe which ensures unbiased observation [7].

Considering the abilities of the optical stretcher, the device can serve as an indicator of different cell types. Furthermore, it can distinguish between healthy and ill cells such as cancer cells. Cancer can be cured if the disease is discovered in an early stage. The problem with present pathology evaluations remains, that cancer can be discovered by change in the suspected tissue. At this state of cancer evolution, it is almost too late to cure it. The optical stretcher allows the observation of cells acquired after they have been spontaneously shed by the body [7].
Figure 6  (a) A relaxed and stretched NIH/3T3 cell. The image with 10µm resolution was captured by phase contrast microscopy. (b)-(d) Measurement data of time dependent stretching $t = 0.2$ s; $2.5$ s; $10$ s. (b) When stretching lasts a short time period, the state of deformation returns quickly to normal. c) By increasing the stretching time, the period lasts longer until the cell returns to normal form. d) After 10 seconds a permanent deformation occurs. The cell relaxes down to 60% of the total deformation [6].

To study the differences in mechanical properties of healthy and malignant cells, human breast epithelial cells and their cancerous counterparts were observed [7]. The result can be seen in Fig. 7. It was possible to distinguish the healthy cells from cancer cells. Furthermore,
the results show that the metastatic cells had been even more deformed than the non-metastatic cancer cells. The three cell types had been to 99.9% distinguished [7].

Figure 7 Stretching of healthy and cancer diseased breast epithelial cells. The left panels show unstretched cells trapped by 100 mW incident beam power. On the right panels are trapped cells stretched by 600 mW: A, B – healthy cells; C, D – initial cancer cells; E, F malignant cancer cells. The difference in diameter which allows to differentiate the cells is clearly seen. The scale bar is 10 µm [7].

A great problem and also very widespread is the oral squamous cell carcinomas (OSCC). This type of cancer belongs to the 10 most common cancers in the world. Patients with OSCC have a under 50% survival rate of 5 years. The only hope to increase this rate is by early diagnostics [8]. Conventional diagnostic requires a scalpel biopsy, which is difficult and awkward to perform in screening tests. Because of the smaller number of cells required for an optical stretcher analysis, a surgical intervention would not be necessary. Figure 8 shows the measuring data of several primary human keratinocyte (PHK) cells from four healthy persons in comparison with cells of five persons with OSCC, \( D(t) \) is the time dependent mean deformability of the cells with units of Pa\(^{-1}\). The differences in cell deformation between PHK and OSCC can be clearly seen. At 0.2 s, the cancer cells were 3.5 times more deformed then the healthy cells (Fig. 8A), which can be seen as a additional characteristic. The deformability between the non-metastatic tumours OSCC3 and the metastatic OSCCs is also clearly distinguishable (Fig.8B). In order to compare all healthy and all OSCC cell lines, an average was computed (Fig. 8C) [8].
Figure 8  A) The average compliance of PHK and OSCC. At 0.2s cancerous cells achieved a deformability of more than 3.5 times compared with healthy cells. B) Compliance between metastatic and non-metastatic OSCC. C) Deformation response of the average of all OSCC-s and healthy cells. At 0.5 s the deformability difference of the OSCC-s average compared with the healthy cells average is 2.5 times greater and can be clearly distinguished [8].

The optical stretcher can be used as a dual beam cell rotator. In this mode an asymmetric laser beam, controlled by a dual mode fibre, causes a rotation around the axis perpendicular to the beam axis. Some experiments with red blood cells (Fig. 9) and MCF-7 cell clusters (Fig. 10) had been made to analyse the stability of cell rotation [9].
Figure 9 The image shows a 90° rotation of red blood cells caused by an optical cell rotator. Time between a step is 200 ms. The turning process is marked by arrows [9].

Figure 10 An optical rotation of an MCF-7 cell cluster inside a microcapillary. The image size is 10 μm [9].

Conclusion

The optical stretcher is a device with great potential in different medical branches. The greatest advantage of this device still remains the fast and uncomplicated diagnostic of malignant tissue. The optical stretcher is already in use for oral cancer diagnostics. In addition to diagnostics, this device allows microbiologist a quick exploration of cells without changing the structure with mechanical interventions or destroying the cell interior structure by observation preparations. It serves as a cornerstone for innovative observation devices, as the optical cell rotator. With a complicated ray configuration, their is even a more complex deformation able, which will in the future deceive a lot of attention.
5. Literature


