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LA DÉFORMATION DE GLOBULES ROUGES DANS LES PINCES OPTIQUES

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Résumé

On expose ici deux méthodes de déformation de globules rouges. La première méthode constituée de deux faisceaux laser contre propageant non focalisés est appelée « optical stretcher ». On y décrit les forces et stress appliqués à la cellule pour ensuite déterminer la déformation. La comparaison des résultats théoriques avec les expériences réalisées a permis de déterminer l’élasticité membranaire à $(20\pm2)\mu\text{Nm}^{-1}$. Une méthode originale n’utilisant aucun système d’imagerie et permettant de mesurer la déformation de la cellule déformée en utilisant le couplage résiduel dans les fibres optique est mathématiquement expliquée et les résultats théoriques utilisés pour étudier la faisabilité d’une telle expérience.

La seconde méthode appelée « oscillating tweezer » est constituée d’une trappe optique à forte ouverture numérique où un modulateur acousto-optique permet de changer discrètement la position du foyer de la trappe avec une fréquence ajustable. À basse fréquence, la cellule se dandine de gauche à droite, mais à plus haute fréquence, la cellule se déforme. On expliquera donc l’origine de la déformation causée par la distribution de la force. Une théorie approximative est présentée pour calculer la déformation. Encore une fois, une comparaison avec les expériences réalisées a été effectuée. L’élasticité membranaire pour des globules rouges humains a pu être mesurée entre $20\mu\text{Nm}^{-1}$ et $29\mu\text{Nm}^{-1}$ et autour de $11\mu\text{Nm}^{-1}$ pour des globules rouges de souris.
Abstract

Two methods for deforming red blood cell (RBC) are studied. The first method is made of a non focalised dual beam counter-propagating optical stretcher. The stress and forces applied to the cell are described and analysed to compute the resulting deformation. The comparison with experimental result is made and allows to determinate the elasticity of human RBC at \((20\pm2)\mu\text{Nm}^{-1}\). An original method using no imaging system and allowing to measure the cells deformation by using the fiber-to-fiber coupling is mathematically explained and used to study the feasibility of such an experiment.

The other method called oscillating tweezers is built from a high numerical aperture optical tweezers where an acousto-optic modulator allows to discretely changing the focal position at a given frequency. At low frequency, the cell moves from left to right, however at higher frequency, the RBC deform. The origin of the deformation is explained by calculating an approximate stress distribution. Here again, we compare the results with the experimental work. The value of elasticity found for human RBC is between 20\(\mu\text{Nm}^{-1}\) and 29\(\mu\text{Nm}^{-1}\) and around 11\(\mu\text{Nm}^{-1}\) for mice RBC.
Avant-Propos

La réalisation de ce travail n’aurait pu être possible sans le support et l’aide de personnes que je tiens à remercier.

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Je voudrais aussi mentionner au lecteur que ce travail a été publié en partie dans différents journaux. Il est donc possible, pour l’instant, de consulter les Ref. [1,2]. Le travail a aussi fait objet de conférences lors de la tenue de « Photonics North » dans la ville de Québec en juillet 2006 et aussi lors de la tenue de « Optics Within Life Science 9 » (OWLS9) dans la ville de Taipei, Taiwan en novembre 2006. À noter que 4 autres publications sont en cours de rédaction ou sont déjà soumises sur les sujets de déformation des cellules avec l’optical stretcher, avec l’oscillating tweeter et aussi sur le couplage optique comme méthode de mesure.
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### Symbol list

The symbol listed here always represents the same parameter if it is not explicitly said otherwise in the text.

- **$E$**: Young Modulus of elasticity in Nm$^{-1}$
- **$h$**: Thickness of the cells membrane
- **$RBC$**: Red Blood Cell or erythrocytes
- **$NA$**: Numerical aperture
- **$\lambda$**: Wavelength of the laser used
- **$\rho$**: Largest radius of a cell
- **$P$**: Momentum
- **$c$**: Speed of light
- **$n_1$**: Index of refraction outside the cell
- **$n_2$**: Index of refraction of the cell
- **$\vec{a}_i$**: Unit vector representing incidence direction of a ray
- **$\vec{a}_r$**: Unit vector representing reflected direction of a ray
- **$\vec{a}_t$**: Unit vector representing transmitted direction of a ray
- **$\vec{a}_n$**: Unit vector representing the normal to a surface
- **$A$**: Area covered by a beam
- **$w_0$**: Full width at half maximum (FWHM) of the laser beam
- **$P$**: Laser power
- **$\bar{Q}$**: Dimensionless momentum transfer vector
- **$\phi$**: Polar angle
- **$\theta$**: Azimuthal angle
- **$\epsilon$**: Incidence angle
- **$\beta$**: Refraction angle
- **$R$**: Reflectance
- **$T$**: Transmittance
- **$D$**: Distance from a fiber end to the cell center
- **$d$**: Fiber-to-fiber end-face distance
- **$\sigma_r$**: Stress in the radius direction
- **$\sigma_\phi$**: Stress in the $\phi$ direction
- **$\sigma_\theta$**: Stress in the $\theta$ direction
- **$N_{\phi}$**: Membrane stress in the $\phi$ direction
- **$N_\theta$**: Membrane stress in the $\theta$ direction
- **$w$**: Elongation in the radius direction
- **$v$**: Elongation in the azimuthal direction
- **$u$**: Elongation in the meridional direction
- **$\nu$**: Poisson coefficient
- **$\gamma_{\phi\theta}$**: Shear strain
- **$G$**: Shear modulus
- **$S$**: Shear elongation
ε_φ:  Deformation per unit length in the $\phi$ direction
ε_θ:  Deformation per unit length in the $\theta$ direction
α:   Angle from the optical axis
a:   Semi-minor axis of an ellipse
b:   Semi-major axis of an ellipse
χ:   Semi-minor axis of the biconcave cell
ψ:   Polar angle of an ellipse
m:   Angle between the horizontal and the tangent to a surface
n:   Angle between the horizontal and the normal to a surface
d_1:  Distance from a fiber end to the trap center
T:   Coupling efficiency coefficient
ψ_s:  Source field distribution
ψ'_s: Paraxial source field distribution
ψ_R:  Reception field distribution
S:   Surface occupied by a field
L:   Optical transfer function
t:   Total length of the deformed cell
f:   Focal length
W:   Aberration function
$\bar{X}$:  Vector position on a plane
W_{40}: Spherical aberration
W_{20}: Defocus aberration
r:   Entrance pupil diameter
d_o:  Distance between a principal plane and the optical fiber end
O:   Object point
O':  Image point
S_o:  Object distance
S_i:  Image distance
R_1:  Frist principal radius of curvature
R_2:  Second principal radius of curvature
Introduction

The ability of cells to sense an external tension and to react to a physical stress is intimately related to their functions and properties. Studying their deformation can then become a convenient method to test a given population. In fact, similar cells should react equally under external tension; i.e. they should display the same elasticity. Then, if a population is found to react differently the first though is that they must be different in a certain way. That difference can sometime exist because of some diseases. The red blood cells (RBCs) of diabetic patients have abnormal membrane properties [3] which could lead to different value of average cell elasticity. Measuring the elasticity of RBCs could then provide a new technique to identify certain type of diabetes. Other non-blood related diseases like cancer can impact, early in their development, on the elasticity of RBC [4,5]. Since, early detection of cancer is the key to fast recovery, having a tool to monitor such primal formation anywhere in the entire body could save countless lives. Sorting RBCs by their membrane viscoelastic properties is potentially a way to monitor our entire body. In fact, as erythrocytes circulate throughout the entire body, they are more susceptible to be affected by any abnormal change, even highly localised, happening inside the body. Measuring the average RBC elasticity could then become part of routine check-up performed just like temperature or blood pressure testing. In this paper, we present two methods used to deform cells. Erythrocytes are modeled to calculate and then to compare the deformation with the experimental data.

The first part of this thesis is an introduction to the basic concept that will be used repeatedly throughout the paper. The basic shape and properties of the erythrocytes are shown. Also, we explain the concept of optical trapping and how forces are generated by light beams.

The first tool to be studied is the dual-beam fiber-optic stretcher consisting of two non-focused counter-propagating laser beams from two well aligned single-mode optical fibers. Yeast cells [6], red blood cells (RBCs) [7] and Chinese hamster ovary cells (CHOs) [8] have been studied with this tool. In this optical stretcher, the cell is confined on the common optical axis of the two beams by the transverse gradient forces, and is stabilized at a point on the optical axis where the two laser beam scattering forces balance each other.
As the index of refraction inside the cell is usually greater than that of the surrounding medium, the changes in the photon momentum due to Fresnel reflection and refraction at the interface tend to stretch the cell. The other tool is the oscillating tweezers. It is made from a single beam high numerical aperture trap. Then an acousto-modulator makes the beam oscillate between two given points. At high frequency, the cell cease to move as the beam oscillate and begin to stretch; the cell then sees two beams and here again the change in the photon momentum will be responsible for this phenomenon. In these applications, the optical stretcher applies forces that are less localized, smoother and with less disturbance to the cells, in comparison with other approaches such as optical tweezers with beads attached to the cells [9-11], atomic force microscope [12] or micropipette [13] so that the elasticity of the whole cell is measured in a more natural environment.

To study the cell’s response to external forces in an optical stretcher, the precise stress distribution on the cell surface needs to be determined. The studies on the photonics radiation pressure of a laser trap on a dielectric sphere in the ray optics regime by Ashkin and many authors [14-16] are well known. Most works model the trapped particle as a tiny rigid non-deformable sphere. Consequently, only the total force applied to the trapped object is of interest. However, in the dual-beam optical stretcher applications, we are interested in the local stress distribution on the cell’s surface and in the concomitant cell’s deformation [7]. Guck gave the first comprehensive but approximate theory on the photonics stress distribution in the dual-beam fiber-optic stretcher on a spherical deformable red blood cell [7].

In this paper, we present a more precise theory of the scattering stress distribution on the surface of a spherical cell. Our results show local peaks in the stress profile that were not previously outlined. The peaks result from the focusing power of the spherical cell acting as a thick convergent lens. An analytical proof is given in the appendix to show that the local scattering stress is perpendicular to the spherical surface, independent of the incident angle, polarization of the incident beam, as well as the reflectance and the transmittance at the cell surface. In addition, we consider the divergence of the beams from the fibers, and express the stress distribution as a function of fiber-to-cell distance, which is directly measurable in experiments, for a given fiber numerical aperture. We then show
how to calculate the deformation of the cells according to the stress calculated previously. The method, as opposed to the one presented in [7], allows simulating the deformation for any physical parameter for which the dual beam optical stretcher is used. The theory does not use a fit of the applied stress and is not limited to the case where such a fit exist. It uses a numerical method to compute the cells deformation from the stress profile. Then, we apply this model to fit the experimental data, obtained from the measurement of the deformation of a spherical human red blood cell trapped and stretched in a fiber-optical dual-beam trap, with a single fitting parameter $E_h$ where $E$ is the Young’s modulus and $h$ is the thickness of the cell membrane.

We also present an original method to experimentally quantify the deformation of cells in the optical stretcher without the need for an imaging system. Instead of using a picture of the deformed cell to measure its shape, we study the coupling between both fibers resulting from the RBC trapped in the middle. This technique could allow obtaining the elasticity of a large number of cells by rapidly measuring the light coupling efficiency as a function of the laser beam power and the fiber end-to-fiber end separation. We model the deformed cell as an ellipsoidal bull lens and compute the coupling coefficient between the two fibers as the cell is deforming. A fully complete calculation taking the aberrations into account is presented. The sensibility of the system is studied and means to improve the absolute coupling efficiency are given.

We also present the first theory calculating the stress distribution on the surface of a biconcave RBC in the case of an oscillating tweezers. An approximate RBC shape is used to calculate a three dimensional stress distribution. This simple model is helpful to understand how forces are generated and affected by the shape. Then it serves as a basis to describe the stress distribution in the biconcave configuration. We use this stress distribution to numerically calculate the final shape of the cell as a function of oscillating distance, power, numerical aperture, etc. Finally, we apply this model to fit the experimental data, obtained from the measurement of the deformation of a spherical human red blood and mice red blood cell trapped and stretched in an oscillating tweezers trap, with a single fitting parameter $E_h$ where $E$ is the Young’s modulus and $h$ is the thickness of the cell membrane.
1. Red blood cell and optical tweezers

The red blood cells (RBCs) or erythrocytes play an essential role in our body even though they are not as sophisticated as other cells like white blood cells. In fact, around 4 to 6 million RBC per microliter navigate our body transporting roughly 270 million hemoglobin molecules that can deliver the oxygen and help remove the waste [17]. Mature RBC lack nucleus and thus have no DNA. They also lack organelles which makes their cytoplasm looks fairly homogeneous. This homogeneity allows us to treat the RBC as having a constant index of refraction. It is then modeled as a thin membrane shell filled with homogeneous and isotropic fluid [18]. The membrane shell appellation itself comes from the small ratio of the membrane thickness $h$, composed of a phospholipid bilayer sandwiched between a triagonal network of spectrin filaments on the inside and glycocalix brushes on the outside [19,20], on the cell radius $\rho$, $h/\rho \sim 0.01$.

Another important property of the erythrocytes is their flexibility. That allows them to circulate through capillaries much smaller than themselves in every part of the body. Their ability is exceptionally important as the survival of tissues and the human body itself depends on it. Lots of diseases result from dysfunctional RBCs or can affect them such as thalassemia, hemolysis, spherocytosis or malaria. The most common one is anaemia when the body lack haemoglobin, but other unrelated diseases like cancer can have an impact on the elasticity of RBC [4,5].

The erythrocytes in their natural environment at 300mOsm have a biconcave disk-like shape represented by Fig. 1.1. The top view can be represented by a disk between 6-8μm in diameter, the maximum thickness is around 2.5μm and the minimum thickness in the hollow is around 0.8μm [21]. However, when the buffer is dilute around 131mOsm the RBC can be swollen by osmosis to a spherical shape. As we will see, this property is helpful to simplify the calculation as the biconcave shape will scatter laser light in a totally different and substantially more complex way than the spherical shape.
To minimize the damage to the cell caused by light absorption [22] specific wavelength must be used in the infrared band. The least damageable wavelength varies with every type of cell as different structure, water concentration or composition can affect light absorption. However, to keep the costs low, the easily available wavelength of $\lambda=1.064 \, \mu\text{m}$ is generally chosen [7,8,10,14].

In order to compute the light scattering on the cells, many mathematical methods can be use. The most popular are FDTD, Generalized Lorentz-Mie theory, Raleigh regime and the ray optics regime. Every one of these tools has their advantages. FDTD can be applied to any problem and is useful for very complex geometry since the calculation directly uses Maxwell’s equations. However, this technique requires very powerful computer, expensive software or long coding time and does not clearly show the physics between reality and the equations. The Generalized Lorentz-Mie theory is a semi-numerical theory that applies for any problems where a spherical object scattered light. That method has the advantage of being faster to code than the FDTD technique, as it is simpler, and it also requires less computing time. The Rayleigh regime is an approximation that only applies when the size of the object is much smaller than the wavelength. This regime is not useful in our experiments since the radius of a RBC is around $\rho=3 \, \mu\text{m}$ and the wavelength used is close to $1\, \mu\text{m}$. However, the ray optics regime criterion $(2\pi \rho / \lambda >> 1)$ is satisfied. In
fact, the ray optics regime is the opposite of the Rayleigh regime since diffractive effect are neglected. It is used by considering a beam as many individual propagating rays. This last method being the simplest of all, it is not surprising to find people using it in the literature. It is also the technique that will be used throughout this document.

This was use extensively, with great results, by Ashkin & al. to calculate the total force applied on the center of mass of a spherical object trapped in an optical tweezers. This trap was build using a high numerical aperture ($NA$) lens to focus a beam. The trap itself resemble to Fig. 1.2.

![Fig. 1.2. Schematic representation of an optical tweezers trap.](image)

When the laser beam encounters a dielectric interface, in occurrence a bead or a cell, scattering forces are generated by the change in photon’s momentum [14] \( P = n_s E/c \), where \( n_s \) is the refractive index of the buffer medium, \( E \) is the beam energy and \( c \) the speed of light. We denote the momentum of the incident, transmitted and reflected rays by \( \vec{P}_i \), \( \vec{P}_t \) and \( \vec{P}_r \), and their directional unit vectors by \( \vec{a}_i \), \( \vec{a}_t \) and \( \vec{a}_r \) respectively. Then, according to the law of momentum conservation, \( \Delta \vec{P} = \vec{P}_i - (\vec{P}_r + \vec{P}_t) \), the force \( \vec{F} \) applied to the cell’s refractive surface is expressed as:

\[
\vec{F} = \frac{\Delta \vec{P}}{\Delta t} = \frac{\vec{P}_i - (\vec{P}_r + \vec{P}_t)}{\Delta t} = \frac{1}{c} \frac{E}{\Delta t} n_i (\vec{a}_i - (nT\vec{a}_i + R\vec{a}_r)) = \frac{P}{c} \nabla
\]

where \( \vec{P} \) is the laser beam power, \( n = n_2/n_1 \), with \( n_1 \) and \( n_2 \) being the index of the medium surrounding the cell and inside the cell, respectively, \( T \) and \( R \) are the Fresnel transmittance
and reflectance, respectively, and $\bar{Q}$ is the dimensionless momentum transfer vector defined in Eq. (1.1). When a ray impact on the cell, the reflectance is on the order of $10^{-3}$ at normal incidence since the refractive index of the cells buffer is around $n_1 = 1.33$, and the cells index is around $n_2 = 1.38$. The refractive index being higher inside the cell and the reflectance small, the total photon’s momentum increase as it is going inside. For the law of momentum conservation to be satisfied, a force must be given to the membrane in the opposite direction of propagation as shown in Fig. 1.3. Similarly, at the second interface the momentum decreases when going from $n_2$ to $n_1$ leading to a force in the direction of propagation.

![Momentum Transfer Diagram](image)

**Fig. 1.3** Momentum transfer and resulting forces on a dielectric interface at normal incidence

- **a)** Represent the momentum of incident light, transmitted and reflected light.
- **b)** The force varies between 0 and 300pN on the surfaces depending on how much power is used (maximum power of 800mW).
- **c)** The total force is directed toward the direction of propagation.

In fact, for 150mW $F_{\text{front}} = -0.8 \frac{P n_i}{c} \hat{a}_k$ and that $F_{\text{back}} = +0.9 \frac{P n_i}{c} \hat{a}_k$ where $\hat{a}_k$ is the direction of propagation so that the total force ($F_{\text{front}} + F_{\text{back}}$) is directed toward the direction of propagation. With the high NA trap, as the angle of incidence varies the intensity of the force also varies. This difference of the force with the incidence angle gives rise to a
restoring force. In fact, when the trapped object is displaced the total force becomes higher in the opposite direction of the displacement forcing the object to come back to the stable position.

However, the problem is that this single beam trap was not design to deform whole cells but rather to displace them. The power needed to deform a cell with this trap would be so high that the intensity at the focus would burn the cell. As great interest dwell in the elasticity of living cells, other tools were created.

2. Optical stretcher

In the optical stretcher a spherical cell is trapped in a dual-beam fiber-optic trap. The stability of the trap is assured by the two counter propagating beams that overlap the cell as shown in Fig. 2.1. The total momentum given to the cell from both the left and right beams is the same so that the cell is literally ambushed at the middle of the fiber-to-fibre end distance.

Fig. 2.1. Schematic representation of a dual beam trap. The laser light overlaps the cell which is forced to stay on the middle of the trap.

When the sample is displaced in a transverse direction, restoring forces coming from the Gaussian intensity distribution will tend to center the cell on the optical axis. The main advantage of the dual beam trap over the single beam trap lies in its divergence. It keeps the intensity low on the whole membrane allowing us to use more power without damaging the cells, as opposed to the high numerical aperture trap where the astounding intensity can literally burn them [23]. Also, it gives the trap a uniform stress distribution on the whole cell. Since, the effect of localised stress is substantially more complex to calculate it keeps the theory from becoming utterly complex.
2.1. Experimental setup

The experimental work done using the optical stretcher was conducted with the collaboration of Yang-Ming University in Taipei, Taiwan. I was able during a one-month visit to Taiwan to do some experimental work and familiarize with this part of the research. The optical trap-and-stretch of RBCs was built with a fiber optical dual-beam configuration similar to the one reported by Guck in 2001 [7]. A schematic diagram of the experimental setup is depicted in Fig. 2.2. An all-fiber optical system integrated with a fiber laser (wavelength $\lambda = 1064$nm) was used for laser beam delivery. A transparent micro-fluidic sample chamber (made of PDMS) with well aligned v-grooves was used to achieve automatic alignment of two single-mode fibers ($NA=0.11$, single mode fiber diameter $w_0=3.3\mu m$) to provide the co-axial counter-propagating trapping beams. In addition, the sample chamber, which is perpendicular to the v-grooves, was connected to a motor-driven syringe pump for controlled delivery of the RBC samples into the trapping region. The trapped sample was illuminated by an incoherent light source from the side and imaged by a long working-distance microscope objective (100x, $NA = 0.55$) onto a CCD camera which was linked to a pc for digital image storage and analysis. In a typical experiment, RBCs were injected into the trapping region by the syringe pump one at a time by controlling the speed of the motor with the computer. As a RBC was trapped in the counter-propagating dual-beam trap the syringe pump was stop to keep the other cells from entering the trap. The trapping power was increased from approximately 19mW to a few hundred mW in several discrete steps (in the range of 8mW to 12mW). The image of the trapped RBC at each trapping power was then recorded by the CCD camera after the stable equilibrium was reached and stored digitally for shape and size analysis. Photographs of the actual experimental setup are depicted in Fig. 2.3.
Fig. 2.2. A schematic illustration of the experimental setup for an all-fiber integrated counter-propagating dual-beam optical trap-and-stretch.

**Dual beam trapping system**

**Image system**

Fig. 2.3. Photographs of the actual experimental setup corresponding to the schematic illustration depicted in Fig. 2.2
2.2. Applied Stress

Using Eq. (1.1) it is possible to calculate the force applied. However, when the deformation of an object is of interest, the total force on the center of mass is not suitable for proper calculations. The force per unit area as a function of position on the object is needed; i.e. the stress as a function of position on the RBC membrane. Following the mathematics from Eq. (1.1) we can find the following equation.

\[
\bar{\sigma} = \frac{\Delta \bar{P}}{A \Delta t} = \frac{\bar{P}_f - (\bar{P}_f^2 + \bar{P}_f^3)}{A \Delta t} = \frac{1}{c} \frac{E_i}{A \Delta t} \eta_i \left( \bar{a}_k - \left( nT \bar{a}_r + R \bar{a}_r \right) \right) \equiv \frac{n_i}{c} \frac{P}{A} \bar{Q}
\]

(2.1)

where \( A \) is the area covered by the beam. The problem is reduced to find \( \bar{Q} \) and \( A \) at every given position on the membrane. For a stable trap [15], the ratio of the full width at half maximum (FWHM) of the laser beam \( w \) over the radius of the cell \( \rho \), \((w/\rho)>1\). In Ref.[7] the incident rays are assumed parallel to the x-axis. However, our calculation shows that this simplification gives rise to deviation in the stress calculation that can alter the deformation results. We thus consider here a divergent laser beam from a fiber having a given \( NA \) and hitting the front surface (left half) of the sphere. For a given distance from the fiber end to the cell center \( D \), there is a unique relationship between the incident point defined by polar angle \( \phi_i \) and the incident angle \( \varepsilon \). In fact, as shown in Fig. 2.4, \( \phi_i = \varepsilon - \delta \) with \( \delta = \tan^{-1}(\rho \sin \phi_i/(D - \rho \cos \phi_i)) \). The polar angle \( \phi_i \) is the incident angle when the beam is parallel.
Fig. 2.4 Incident, reflected and transmitted rays on a spherical object

The refraction angle $\beta$ is determined by the Snell’s law $n_1 \sin \varepsilon = n_2 \sin \beta$. After the first refraction, the transmitted ray hits the back surface (right-half) of the sphere from the inside of the cell at the point defined by polar angle $\phi_2 = 2\beta - \phi_1$. The angle of the reflected ray to the x-axis is $\pi - (3\beta - \phi_1)$, and that of the transmitted ray is $\varepsilon + \phi_2 - 2\beta$, as shown in Fig. 2.4. As the reflectance is in the order of $10^{-3}$ at normal incidence for the refractive index $n_1 = 1.33$ for the buffer, and $n_2 = 1.8$ for the cells, the third and subsequent reflections inside the cell would have relatively weak power and result in weak stress, which can be neglected. Once all angles are found, one can deduce $Q$. For the front surface we have:

$$Q_{\text{front } X} = \exp\left[-2(\rho \sin(\phi_1)/w)^2 \left[\cos(\delta) - nT(\varepsilon) \cos(\phi_1 - \beta) + R(\varepsilon) \cos(2\varepsilon)\right]\right] \quad (2.2)$$

$$Q_{\text{front } Y} = \exp\left[-2(\rho \sin(\phi_1)/w)^2 \left[\sin(\delta) + nT(\varepsilon) \sin(\phi_1 - \beta) - R(\varepsilon) \sin(2\varepsilon)\right]\right] \quad (2.3)$$

For the back surface we have:

$$Q_{\text{back } X} = \exp\left[-2(\rho \sin(\phi_1)/w)^2 \left[T(\varepsilon) \left[n \cos(\phi_1 - \beta) + nR(\beta) \cos(3\beta - \phi_1) - T(\beta) \cos(\varepsilon + \phi_1 - 2\beta)\right]\right]\right] \quad (2.4)$$

$$Q_{\text{back } Y} = \exp\left[-2(\rho \sin(\phi_1)/w)^2 \left[T(\varepsilon) \left[-n \sin(\phi_1 - \beta) + nR(\beta) \sin(3\beta - \phi_1) + T(\beta) \sin(\varepsilon + \phi_1 - 2\beta)\right]\right]\right] \quad (2.5)$$

where $\exp[-2 \rho^2 \sin^2(\phi_1)/w^2]$ is a Gaussian beam correction factor. One should note that we made no assumption thus far about the state of polarization, which can affect the reflectance and the transmittance.

It is interesting to prove that the scattering stress is always perpendicular to the spherical refraction surface regardless of the incident angle and no matter whether the rays hit the surface from outside or inside the sphere; i.e. both $\hat{Q}_{\text{front}}$ and $\hat{Q}_{\text{back}}$ are perpendicular to the spherical surface. In the Appendix A we analytically prove, using Eq. (2.1) and Eqs. (2.2) to (2.5), that:
\[
\arctan \frac{Q_{\text{front}}(\phi_1)}{Q_{\text{front}}(\phi_1)} = \phi_1 \quad \text{and} \quad \arctan \frac{Q_{\text{back}}(\phi_1)}{Q_{\text{back}}(\phi_1)} = 2\beta - \phi_1 = \phi_2
\] (2.6)

Note that this proof is independent of the Fresnel reflectance \( R \) and transmittance \( T \) and therefore of the incident beam polarization. With the proof of the orthogonality we can write:

\[
Q_{\text{front}} x = \exp \left[ -2 \left( \frac{\rho \sin(\phi_1)}{w} \right)^2 \right] Q_{\text{front}} \cos(\phi_1)
\]

\[
Q_{\text{front}} y = \exp \left[ -2 \left( \frac{\rho \sin(\phi_1)}{w} \right)^2 \right] Q_{\text{front}} \sin(\phi_1)
\]

\[
Q_{\text{back}} x = \exp \left[ -2 \rho^2 \sin^2(\phi_1) / w^2 \right] Q_{\text{back}} \cos(2\beta - \phi_1)
\]

\[
Q_{\text{back}} y = \exp \left[ -2 \rho^2 \sin^2(\phi_1) / w^2 \right] Q_{\text{back}} \sin(2\beta - \phi_1)
\] (2.7)

Equations (2.7) were given in Ref.[7] without Gaussian beam correction and have been implied without proof that the stress is perpendicular to the spherical surface. For the sake of simplicity, a random polarization is usually considered [2,14] and the average reflectance and transmittance of the parallel and perpendicular polarizations are used. In that case, no parameter in Eqs. (2.2) to (2.7) varies with the meridional angle, so that the three dimensional trapping system is rotationally symmetric around the \( x \)-axis and can be analyzed in the \( x-y \) plane only.

It is interesting to examine the output polar angle, \( \phi_2 = 2 \sin^{-1} \left( \frac{n_1}{n_2} \sin \varepsilon \right) - \varepsilon + \delta \), where \( n_1 < n_2 \), as a function of the incidence angle \( \varepsilon \). For small \( \varepsilon \), \( \phi_2 \) increases with \( \varepsilon \) monotonically. Then, the increase of \( \phi_2 \) is slowed down and finally \( \phi_2 \) decreases with increasing \( \varepsilon \) for \( \varepsilon > \tilde{\varepsilon} \), as shown in Fig. 2.5, where \( \tilde{\varepsilon} \), corresponding to a maximum output polar angle \( \phi_2(\tilde{\varepsilon}) \), can be computed by the derivative of \( \phi_2 \) with respect to \( \varepsilon \). Computing the derivative of \( \phi_2 \) we obtain
Consequently, there is an upper limit of the output polar angle $\phi_2$ that depends on the indices $n_1$ and $n_2$, the ratio $w/\rho$, the fiber NA and the cell radius $\rho$. As an example, for $NA=0.11$, $n_1=1.335$, $n_2=1.37$, $D=39.9\mu m$ ($w/\rho =1.1$) and $\rho =3\mu m$, this is $71^\circ$. There is no incident ray at the front surface, whose refracted ray can hit the back surface at a position of polar angle higher than the upper limit. Below the upper limit there is a range of $\phi_2$, where the same output position $\phi_2$ can be reached by two different incident angles, as shown in Fig. 2.5A. This range is $65^\circ \leq \phi_2 \leq 71^\circ$ in our example. In Fig. 2.5B two rays incident to the front surface at angles $\varepsilon=78.2^\circ$ and $84.3^\circ$ pass through the cell and then hit the back surface at the same point of polar angle of $\phi_2=70^\circ$.

\[
\cos(\vec{\varepsilon}) = \frac{1}{\sqrt{3}} \left[ \frac{\sqrt{n_2^2 - n_1^2}}{n_1} \right]
\]  

(2.8)

In the dual-beam optical stretcher the two counter-propagating laser beams generate two stress distributions, which are added up. In the example shown in Fig. 2.5B on the back surface and at the polar angle position $\phi_2=70^\circ$ the cell is hit by a third incident ray, which comes in the $-x$ direction. At that position, the contributions of the three rays should be added up. One can therefore separate both the front and back surfaces into 4 regions. In the first region, one incident ray from outside and one ray from inside the cell hit the same.
point on the surface. This region is limited by the polar angle between $0^\circ < \phi < 65^\circ$, in the example shown in Fig. 2.6. In the second region, three rays, one incident ray from outside and two rays from inside of the cell hit the same point at the surface. This region is limited by the polar angle between $65^\circ < \phi < 71^\circ$ in our example. In the third region only one incident ray exerts a stress on the cell surface, and no other ray can hit this point from inside the cell. The third region is limited by the polar angle $71^\circ < \phi < 87^\circ$ in our example. The fourth region is limited by the highest position that a ray can hit on the surface for a given fiber NA and fiber-to-cell distance $D$. Thus, no stress is applied for $\phi > 87^\circ$ in the example. In the first and second regions, to calculate the stress applied to the front surface at a position $\phi_1$, we need to find the incidence angle of the ray ($\varepsilon_2$) and $\phi_2(\varepsilon_2)$ coming in the $-x$ direction, which will make a polar angle of $\phi_1 = (2\beta - \phi_2)$ to the $-x$ direction when hitting the position $\phi_1$. In other words, we need to solve equation $2\beta - \phi_2(\varepsilon_2) = \phi_1(\varepsilon_1)$ to find $\phi_2$ for a given $\phi_1$.

Then we compute Eqs. (2.2) and (2.3) with $\phi_1$, Eqs. (2.4) and (2.5) with $\phi_2$ and we add the results using Eq. (2.9):

$$Q_{\text{tot}} = Q_{\text{front}} + Q_{\text{back}} = \sqrt{(Q_{\text{front}} \cdot (\phi))}^2 + (Q_{\text{front}} \cdot (\phi_1))^2 + \sqrt{(Q_{\text{back}} \cdot (\phi_2))}^2 + (Q_{\text{back}} \cdot (\phi_2))^2$$

(2.9)

A sketch of $Q_{\text{back}}$ and $Q_{\text{front}}$ is depicted in Fig. 2.6, where the front surface is denoted as the first surface and the back surface as the second for the incident beam in $+x$ direction, and vise versa for the incident beam propagating in $-x$ direction. Indeed, in the region $0^\circ < \phi < 65^\circ$ the stress is the addition of contributions of the two rays. In the region $65^\circ < \phi < 71^\circ$, the stress is the addition of contributions of the three rays and is thus much more intense. In the region $\phi > 71^\circ$ only the rays hitting the surface from outside of the cell contribute. Finally, in the region $\phi > 87^\circ$ the stress profile at the first surface is cut to zero.
Fig. 2.6. Stress profile as function of the polar angle. Thick line: at the second surface; Thin line: at the first surface. $NA=0.11$, $n_1=1.335$ and $n_2=1.37$, $D=39.9\mu m$ ($w/\rho=1.1$) and $\rho=3\mu m$.

The total stress is the sum of those applied at the first and second surfaces, shown by the two curves in Fig. 2.6. The stress distribution is symmetric in each quadrant of Fig. 2.4, so that we have a stress distribution on the sphere as shown in Fig. 2.7. We can see the peaks in the stress distribution located at about $60^\circ$, $120^\circ$, $240^\circ$ and $300^\circ$ positions, which were not considered in the previous theory and will clearly influence the predicted cell deformation.
Fig. 2.7 Stress profile (Nm$^{-2}$) for different distances $D$ with $P=100$mW, $\rho=3\mu$m, $n_1=1.335$, $n_2=1.37$ and NA=0.11.

Fig. 2.7 shows the stress profiles for different distances $D$ from the fibers end to the cell center. At small incident angles, close to 0° and 180°, for a given cell radius $\rho$ the stress strength is proportional to the intensity of the input beam, which decreases as $1/D^2$. However the Gaussian beam correction factor $\exp[-2\rho^2\sin^2(\phi)/w^2]$ increases as the beam size $w$ or the distance $D$ increases. This is why at greater incident angles, close to 90° and 270°, the stress profiles are less sensible to the distance. We see in Fig. 2.7 that the surface region near 90°, where no stress is applied, gets smaller as the distance $D$ increases, and that the width of the peak stays almost the same at all distances.

In Fig. 2.8(b) we show the previously calculated stress distribution in Ref[7]. Both figures were calculated using the same physical parameters.
Fig. 2.8. The stress distribution (Nm$^{-2}$) on a cell is represented in circular coordinate system. a) Stress distribution as calculated by our method  b) Stress distribution as calculated by Guck. [7]. The same parameters were used; in both calculations, $\mathcal{P}=100$ mW, $\rho=3.30 \mu m$, $n_1=1.334$ and $n_2=1.378$ and $w/\rho=1.1$

We can clearly see the differences between the two distributions shown in Fig. 2.8(a) and (b), especially at 60°, 120°, 240° and 300° positions where peaks on the stress distribution are shown in our calculation..

Also of great interest, is the Matlab code used to calculate the stress distribution on the cells as a function of the experimental parameters. This code is given in Appendix B. With the stress distribution known on the whole surface of a spherical RBC, we can now calculate the resulting deformation as a function of power.

### 2.3. Deformation

To measure the elasticity of a cell with the dual-beam optical stretcher, the local stress distribution on the cell surface must be first calculated [1,7]. Then, the morphological deformation of the cell can be calculated from the stress distribution [1]. The parameters specifying the cellular elasticity are finally determined by comparing the experimentally measured cell’s deformation in optical stretcher with the theoretical cell deformation result. The local optical stress distribution on the cell surface has been calculated in the above section and in the literature [1,3,2] for spherical cells. The stress distribution in a dual-beam optical stretcher depends on the ratio of the laser beam-spot radius to the cell’s radius $w_0/R$. Guck et al have shown that when $w_0/R \sim 1.1$ the stress profile on a spherical cell can be approximated by

$$\sigma_r = \sigma_0 \cos^2(\phi)$$

Where $\sigma_0$ is the peak stress along the beam axis and $\phi$ is the polar angle with respect to beam axis [3]. A more general calculation taking into consideration the laser beam divergence and its Gaussian intensity profile has been reported recently [1] (see above section), by which the optical stress distribution over the cellular surface can be calculated.
for different distances between the cell and the fiber end-faces and different numerical apertures of the fibers with which the condition $w_0/R \sim 1.1$ is not fulfilled.

Deformation of the cell’s membrane by a given stress profile is usually computed with the principle of minimum energy [24] and the Euler-Lagrange equations, where the energy functional, describing the membrane energy and the work done by the stress, are solved for a given analytic expression of the stress distribution, such as Eq. (2.10) [7]. However, in general experimental conditions when $w/R \neq 1.1$ or when more precise calculation as presented in Ref. [1] is desirable, the stress distribution may no longer be expressed as Eq. (2.10) so that the equations for cell deformation used in [7] are no longer appropriate. One approach to circumvent this problem might be to fit the calculated numerical stress distribution with a polynomial and from each term in the polynomial find differential equations for the cell deformation. However, the differential equations derived from the Euler-Lagrange equations can be too complex, and the fitting of the stress profile to the polynomial would unavoidably introduce additional errors. In this part of the document, we present a numerical method to compute the cell’s deformation from the stress profile. The method is more general in the sense that it is applicable to various value of $w_0/R$ in the dual-beam optical stretcher and also to any other stress distribution, including the cases when the stress distribution can not be represented by analytical expressions. We apply this model to fit the experimental data for measurement of the deformation of a spherical human red blood cell trapped and stretched in a fiber-optical dual-beam stretcher with a single fitting parameter $Eh$ where $E$ is the Young’s modulus and $h$ is the thickness of the cell membrane.

One must remember that we consider a deformable spherical cell trapped in a dual-beam fiber-optical stretcher. An erythrocyte is modeled as spherical elastic membrane shell filled with homogeneous and isotropic fluid. The only elastic component in the RBC is the thin shell where the ratio of the membrane thickness $h$ on the cell radius $R$, $h/R \sim 0.01$. Novozilov showed [24] that the error introduced using the membrane theory of thin shell is on the order of $h/R$. This approximation comes from the inability of a perfectly thin membrane to sustain bending. For example a shell made of cloth would immediately lose its stability when compression forces are applied. Thus, neglecting the bending energy
when a shell has a small, but finite, flexural stiffness, results in a small error on the order of \( h/R \).

We consider only the cases where the cell’s deformation is small compared with the initial shape, so that the cell is not permanently deformed, and the linear elastic theory of membrane may be used. In general, a thin elastic shell supports an arbitrary external loading by means of internal forces, bending and twisting moments. Since we assume that RBC membrane cannot sustain bending or twisting moments, it may resist only tensile and compressive forces. For using the membrane of thin shell model, the applied stress must not generate bending forces or torques in any way and the deformed surface must stay smooth. Changes in curvature and in twist of the membrane must be very small. This model is valid to RBCs in the dual-beam optical stretcher, as the local photonics stress is normal to the membrane everywhere (see Appendix A) and observation of deformed cells clearly shows smooth cell surfaces.

We propose a numerical approach to the problem using the classical mechanical theory of the membrane of shell [24]. We first consider a differential membrane element isolated from the shell, which is in the static equilibrium state under external and internal forces and moments. The external surface loads are the photonics stress \((\sigma_r, \sigma_\theta, \sigma_\phi)\) and the internal forces are the membrane stresses \(N_\phi\) and \(N_\theta\), which form an orthogonal basis of forces with \(N_\phi\) tangent to the membrane element in the direction of the meridian and \(N_\theta\) is tangent to the membrane element in the direction of the parallel circle, as shown in Fig. 2.9. Our calculation for the cell’s deformation consists of three steps: First, compute the local stress profile according to experimental conditions such as the RBC size and refractive indices, fiber-to-fiber distance and laser power [1]. Second, compute the membrane stresses \(N_\phi\) and \(N_\theta\) from the photonics stress \((\sigma_r, \sigma_\theta, \sigma_\phi)\) using the Laplace equations. Finally, compute the cell’s strain and deformation using Hooke’s law.

In the linear elastic theory for membrane the Laplace equations [24] allow to calculate the internal membrane stresses \(N_\phi\) and \(N_\theta\), from the external surface stress load \((\sigma_r, \sigma_\theta, \sigma_\phi)\), where \(\sigma_r\) is the external stress normal to the membrane in the direction \(W\), \(\sigma_\theta\) is the external stress in the azimuthal angle direction parallel to \(V\) and \(\sigma_\phi\) is the external stress
along the meridional or polar angle direction parallel to \( U \), where \( V, U \) and \( W \) are local Cartesian coordinate system with the origin \( O \) in the center of the differential membrane element. \( V \) and \( U \) coincide with the directions of tangents curvilinear coordinate lines of the membrane and \( W \) points to the origin along the normal of the membrane element, as shown in Fig. 2.9. In the case of spherical membrane the Laplace equations are given as

\[
R \frac{\partial S}{\partial \theta} + \frac{\partial (N_\phi R \sin \phi)}{\partial \phi} - N_\phi R \cos \phi + R^2 \sin(\phi)\sigma_\phi = 0 \tag{2.11}
\]

\[
R \frac{\partial N_\theta}{\partial \theta} + \frac{1}{R \sin \theta} \frac{\partial (R^2 \sin^2(\phi)S)}{\partial \phi} + R^2 \sin \sigma_\theta = 0 \tag{2.12}
\]

\[
\frac{N_\phi + N_\theta}{R} + \sigma_r = 0 \tag{2.13}
\]

where \( R \) is the sphere radius, \( S \) is related to the shear strain with the relation \( S= Gh \gamma_{\phi\theta} \) where \( G \) the shear modulus, \( h \) is the cell thickness and \( \gamma_{\phi\theta} \) is the shear strain. In the dual-beam optical stretcher when the RBC is spherical as a shell of revolution around the z-axis, which is the laser beam axis, the whole system is rotational symmetric around the z-axis, so that all derivatives with respect to the azimuthal angle \( \theta \) would vanish in Eqs. (2.11) and (2.12). Moreover, the photonics stresses are normal to the membrane, and \( \sigma_r(\phi) \) has been calculated in Refs. [1], while \( \sigma_\theta=\sigma_\phi=0 \). Hence, Eq. (2.12) simply gives the shear strain \( S=0 \). We rewrite Eq. (2.13) as

\[
N_\theta = -\sigma_r(\phi)R - N_\phi \tag{2.14}
\]

Substituting Eq. (2.14) into Eq. (2.11) we obtain for \( N_\phi \) as

\[
N_\phi(\phi) = -\frac{R}{\sin^2(\phi)} \int_0^\phi \sin(\phi')\cos(\phi')\sigma_r(\phi') d\phi' \tag{2.15}
\]

where the upper limit of integration designate the position of a parallel where the value of \( N_\phi(\phi) \) need to be calculated.
Equation (2.15) can be computed by numerical integration such as Gauss Quadrature method, when the real stress distribution $\sigma_r(\phi)$ is known. In our case, we used Lobatto Quadrature.

![Coordinate system for a spherical cell](image)

Fig. 2.9. Coordinate system for a spherical cell. Two laser beams propagate in the $\pm Z$ direction.

Once the membrane stresses $N_\phi$ and $N_\theta$ are computed by Eqs. (2.14) and (2.15) from the photonics stress profile $\sigma_r(\phi)$, one may now calculate the strains of the differential membrane element, which are related to the membrane stresses $N_\phi$ and $N_\theta$ by Hooke’s law as

$$
\varepsilon_\phi = \frac{1}{E}(N_\phi - \nu N_\theta)
$$

$$
\varepsilon_\theta = \frac{1}{E}(N_\theta - \nu N_\phi)
$$

(2.16)

where the strains $\varepsilon_\phi$ and $\varepsilon_\theta$ are the total deformation per unit length of a segment on the differential membrane element in the meridional and azimuthal direction, respectively, $E$ is Young’s module and $\nu$ is the Poisson coefficient. It represents the membrane change of volume due to the deformation. It is usually admitted that the volume of the membrane is constant when small deformation occurs, then we use $\nu=0.5$.

In order to establish relationships between the strains and displacements of the differential membrane element we consider a segment $AB$ of length $Rd\phi$ on the meridian in the unstrained membrane element. After the straining, $AB$ is displaced to position $A'B'$. In
the small deformation approximation the high-order infinitesimal terms are neglected, then one can write the meridional strain as

$$\varepsilon_\theta = \frac{1}{R} \frac{\partial u}{\partial \theta} - \frac{w}{R} = \frac{1}{Eh} \left( N_\theta - \nu N_\varphi \right)$$ \hspace{1cm} (2.17)

where \( w, u, \nu \) represent the local displacement of the membrane in the radius direction, tangent directions to the meridian and to the parallel circle, respectively. Similarly, one can consider a segment on the parallel circle in the unstrained membrane element and compute its deformation. That gives the azimuthal strain as

$$\varepsilon_\varphi = \frac{1}{R} (u \cot \varphi - w) = \frac{1}{Eh} \left( N_\theta - \nu N_\varphi \right)$$ \hspace{1cm} (2.18)

Finally, the similar relation of displacements between \( u \) and \( v \) with the shear strain is given to preserve generality.

$$\sin \varphi \frac{\partial}{\partial \varphi} \left( \frac{v}{R \sin \varphi} \right) + \frac{1}{R \sin \varphi} \frac{\partial u}{\partial \theta} - \frac{S}{Gh} = 0$$ \hspace{1cm} (2.19)

Solving Eqs (2.17) and (2.18) to eliminate \( w \), one obtains

$$\frac{du}{d\phi} = u \cos \phi + R(1+\nu)(N_\theta - N_\varphi)$$ \hspace{1cm} (2.20)

The solution of this differential equation is

$$u(\phi) = \sin \phi \int_0^\phi \frac{R(1+\nu)(N_\theta - N_\varphi)}{Eh \sin \phi} d\phi + C \sin \phi$$ \hspace{1cm} (2.21)

where \( C \) is a constant determined from the boundary condition. It is easy to see that \( u(0) = 0 \). The displacement of the membrane in the radius direction \( w \) is a measurable deformation parameter as the change in radius of the cell and can be computed with Eq. (2.18) which gives
The deformation $u$ and $w$ can be calculated from Eqs. (2.21) and (2.22) with a numerical methods for any given stress profile [1]. In our case, we used Runge-Kutta of order 4 with error control. This approach allows us to compute the deformation in any general experimental conditions, provided that the stress profile can calculated by the method reported earlier [1]. No fitting of the stress profile to an analytical equation is required. In the special case when $\phi=90^\circ$, Eq. (2.22) becomes Eq. (2.23), and when $\phi=0^\circ$, Eqs. (2.22) and (2.21) give Eq. (2.24).

\[
\begin{align*}
\theta_{90} &= \frac{R}{Eh} \left( vN_{\theta}(90^\circ) - N_{\theta}(90^\circ) \right) \\
\theta_0 &= \frac{R}{Eh} \left( vN_{\theta}(0^\circ) - N_{\theta}(0^\circ) \right)
\end{align*}
\]

The deformation of the RBC in the directions along the optical axis and normal to optical axis (i.e., at $\phi=0^\circ$ and $\phi=90^\circ$) are easy to measure experimentally. Their theoretical values can be calculated from Eqs. (2.23) and (2.24), with the solutions for the membrane stress $N_{\phi}$ and $N_{\theta}$ obtained from Eqs. (2.14) and (2.15). We verified that in the special case when the stress distribution is given by $\sigma_r = \sigma_0 \cos^2(\phi)$, the deformation obtained by the numerical method is identical to that obtained from the analytical solutions [7].

Note that here again, the Matlab code used to calculate the deformation is available in Appendix C. This code is to be used with the Matlab code of Appendix B.

2.4. Comparison with experimental results

The theoretical deformations of RBC calculated using the above formulas was compared with the experimental observations in order to determine the Young modulus of the cell’s membrane. Within the linear elastic membrane approximation for small deformations of the RBC, Eqs (2.23) and (2.24) show that the deformations $\theta_{90}$ and $\theta_0$ are a linear function of the laser beam power because both the internal stresses $N_{\theta}$ and $N_{\phi}$ depend linearly on the photonics stress $\sigma_r$ according to Eqs. (2.14) and (2.15), and $\sigma_r$ is proportional to the laser...
power. To determine the Young modulus of the cell’s membrane we plot the observed normalized deformations, namely $w_0/R$ and $w_{90}/R$, along the optical axis and normal to the optical axis, respectively, as a function of the laser power. The best fit of Eqs. (2.23) and (2.24); i.e. the two deformation curves $w_{90}/R$ and $w_0/R$, to the experimental data give the value $Eh$, where $E$ is the Young modulus and $h$ the cell membrane thickness, as illustrated in Fig. 2.10 (b).

The experiments have been performed in a fiber-optical counter-propagating dual-beam stretcher with laser wavelength $\lambda=1064$ nm, fiber numerical aperture $NA=0.14$ and the distance between two fiber end-faces $d=150\ \mu m$. The human RBC sample has been osmotically swollen into spherical shape with the radius $R=3.3\ \mu m$. The refractive index of RBC was $n_2=1.378$ and that of the buffer was $n_1=1.335$. Micrographs of spherical human RBC trapped and stretched at different optical power are depicted in Fig. 2.10(a), with the laser power varied from 19mW to 230mW. The corresponding cell’s relative deformations in the length of the elongated sample along the major axis and the minor axis as a function of the optical power are plotted in Fig. 2.10 (b). Each data point represents the statistical average over 10 RBC samples, with the root-mean-square ($rms$) standard deviation indicated by the error bars.

In Fig. 2.10(b) the solid lines are the theoretical fits obtained from the computational method described above with a single fitting parameter $Eh$ where $E$ is the Young’s modulus and $h$ is the thickness of the cell membrane. Deviation of the experimental data from the straight lines in the high power regime is expected since our calculation is applicable only for small deformation in the linear regime associated with small stretching power. In this specific example, the deviations pronounce when the laser power is higher than 150-175mW and the relative deformation exceeds 10%. Those data points were neglected.

(a)
Fig. 2.10. (a) Micrographs of spherical human RBC trapped and stretched at different optical power; (b) Experimental data (black squares) and the calculated values (blue lines) of the fractional change in length of the elongated RBC: along the major axis (positive deformation) and the minor axis (negative deformation).

From the best fit, calculated by taking into account only the data points with the relative deformation smaller than 10% we obtain $Eh = (20 \pm 2) \mu$Nm$^{-1}$. The corresponding shear modulus $Gh = Eh / 2(1 + \nu) = (6.67 \pm 0.07) \mu$Nm$^{-1}$ which is comparable to the results reported earlier: $8.5 \mu$Nm$^{-1}$ by M. Dao et al. [25], $13 \mu$Nm$^{-1}$ by J. Guck et al. [7] and $2.5 \mu$Nm$^{-1}$ by Hénon et al. [10].

The theory presented in this chapter allows obtaining, in a general optical stretcher experiment which can present different $NA$, cell radius $R$, trapping distance $d$, refraction indexes or wavelength, an accurate value for the elasticity of RBCs. The theory does not get more complex, in fact, it gets simpler as the increased flexibility allows one to build a universal code that can be used by anyone with basic knowledge of matlab.

After 10% deformation, the cell enters in a non linear deformation state and thus the linear theory described in this chapter does not hold. In fact, the non linear regime gives rise to permanent deformation of the membrane and can be seen as an invasive deformation that could damage the samples. It should then be avoided in any experiments especially if trying to test cell elasticity after multiple deformations.
3. Coupling coefficient

In this chapter we study the coupling from one fiber to another by a deformable object trapped in the dual beam optical stretcher. That is a RBC captured by the optical stretcher will couple light in both optical fibers according to the shape of the erythrocytes; i.e. their deformation. We computed the photonics force distribution on the cell’s surface using the ray optics, and the deformation of the cell using the mechanics of the linear membrane theory of thin shell. However, instead of measuring the cells deformation with an imaging system, we model the deformed cell as an ellipsoidal bull lens and compute the coupling coefficient between the two fibers. This technique could allow obtaining the elasticity of a large number of cells by rapidly measuring the light coupling efficiency as a function of the laser beam power and the fiber end-to-fiber end separation. The basic principle of this idea is represented in Fig. 3.1.

The deformed cell acts as a coupling lens between the two fibers. We assume that the laser beams from the two fibers have an equal power, so that the trapped cell is at the equal distances to the two fibers, and the system is symmetric with respect to the center of the cell. Moreover, as the two Gaussian beams are well aligned on the common optical axis, the deformation of the trapped cell is axially symmetric having a shape of revolution with respect to the optical axis. In addition, the deformation of the cell is also symmetrical with respect to the center.

![Fig. 3.1. Schematic representation of the dual beam optical stretcher. The trapped object is at equal distances from both optical fibers. The Gaussian beam of the trap is slightly divergent $N.A=0.11$. The small and the big black arrows represent the coupled light and the incident light respectively.](image-url)
3.1. **Defocus**

Using the paraxial optics and without considering aberrations for now, the deformed cell is modelled as a thick lens. Its focal length $f$ is calculated with paraxial optics as

$$f = \left[ (n_2 - n_1) \left( \frac{2}{R} - \frac{2b(n_2 - n_1)}{n_2 R^2} \right) \right]^{-1} \tag{3.1}$$

where $n_1$ and $n_2$ is the index of the surrounding medium and the cell, respectively, $b$ is the length of the major semi-axis of the ellipsoidal shaped cell. Here for the sake of simplicity, we approximate the radius of curvature in the two extremities of the ellipsoidal shaped surfaces by the radius of the non-deformed cell $R$ in computing the focal length. This approximation is valid for small deformation of the cell. In this configuration the ideal fiber coupler could be a 4-\(f\) imaging setup, which images one fiber end face to another and vice-versa with a unit image magnification. Thus, for a spherical red blood cell with the indices $n_2=1.378$ and $n_1=1.33$, for instance, when the deformation is small $b \sim R$ Eq. (3.1) gives $f \sim 10R$ and the ideal fiber-to-fiber distance of $40R$.

In the experiments with the dual-beam optical stretcher, when one takes most care of the stretching property rather than the coupling efficiency, the object distance is usually chosen arbitrarily with the only limitation for the trap stability condition $w/R > 1$. In this case, the object distance is determined by the experiment, and the image distance is determined by the lens equation from the lens focal length $f$ and the object distance $d_o$. However, the true image plane is the end face of the receiving fiber and the true image distance always equal to the object distance $d_i = d_o$, because of the symmetry of the optical system with respect to the center, imposed by the balance of the two axial scattering forces of the two beams on the cell. Then, the dual beam stretcher is considered as a fiber coupler and the receiving fiber end face is not in the image plane, resulting in a defocus aberration. The defocus aberration is estimated as

$$W_{20}(r) = \frac{r^2}{8} \left( \frac{1}{f} - \frac{1}{d_o} - \frac{1}{d_i} \right) = \frac{r^2}{8} \left( \frac{1}{f} - \frac{2}{d_o} \right) \tag{3.2}$$

where $r$ is the height of the ray in the pupil plane. The object and image distances, $d_o$ and $d_i$ are defined from the principal planes of the lens, so that $d_o = D - b + h$, where $D$ is the distance...
from the fiber end face to the center of the cell, and the distance \( h \) from the vertexes of right or left of the ellipsoidal cell surface to the principal planes is given by

\[
h = \frac{2bf(n_2 - n_1)}{n_2R}
\]

(3.3)

where the radius of curvature of the non-deformed cell \( R \) is again used approximately to compute \( h \). The defocus is usually used to balance the spherical aberration in the optical fiber coupler using the ball lens [26]. In the optical dual-beam stretcher experiments the choice of object distance \( d_o \) changes the defocus significantly and can be used in the experiments to increase the coupling efficiency. Our calculation shows that the cell’s deformation with parameter \( 2b \) has little effect on the defocus, when the deformation is small so that the linear elastic theory of membrane can be used and the radius of curvature of the ellipsoid can be approximated by \( R \) in Eqs. (1) and (3).

3.2. Spherical Aberration

The spherical wavefront aberration \( W_{40}(r) \) is calculated as the optical path length difference between the chief ray from the object point \( O \) to the paraxial image point \( O' \) with both points on the optical axis and a ray of height \( r \), As shown in Fig. 3.2, \( w_{40} = \left[ OPSO' \right] - \left[ OQUO' \right] \) where the deformed ellipsoidal cell is of semi-major axis \( b \) and semi-minor axis \( a \). \( P \) and \( S \) are the surface vertex of the first and second surface, respectively. An incident ray \( OQ \) of height \( r = y_1 \) is refracted at the first surface and intersected with the second surface at point \( U \) with height \( r = y_2 \).

![Fig. 3.2. Representation of the image point (O') of an ellipse shaped lens from the object point O, the center C is situated at a distance z=b from P and S.](image.png)
The position of the paraxial image point $O'$ can be calculated by solving for $SO' = S_{i2}$, the image distance for the second surface in the equations of paraxial refraction by the two single surfaces [29]

$$\frac{n_1}{S_{oi}} + \frac{n_2}{S_{i1}} = \frac{n_2 - n_1}{R}$$

$$\frac{n_1}{S_{oi}} + \frac{n_1}{S_{i2}} = \left(\frac{n_2 - n_1}{R} + \frac{n_2}{(S_{i1} - 2b)S_{i1}}\right)^2$$  \(3.4\)

where we still approximate the radius of curvature of the ellipsoidal shape at the first and second surfaces as the radius of the non-deformed cell $R$, $S_{oi}$ and $S_{i1}$ are the object and image distance for the first surface. Then, we can calculate $[OPO']$ by

$$[OPO'] = n_1(0P + SO') + n_2PS = n_1(D - b + S_{i2}) + n_2(2b)$$  \(3.5\)

In Fig. 3.2, the point $Q(z_1, y_1)$ is the intersection of the marginal ray with the first surface with the maximum height $y_1$. Thus, the plane $z=z_1$ is the entrance-pupil. To calculate the optical path $[OQUO']$, we have $z_1 = -b\sqrt{1 - y_1^2/a^2}$, from the ellipse equation, then the length of optical path $[OQ] = n_1\sqrt{(D + z_1)^2 + y_1^2}$. We have also $[QU] = n_2\sqrt{(y_1 - y_2)^2 + (z_1 - z_2)^2}$ and $[UO'] = n_1\sqrt{y_2^2 + (S_{i2} - (b - z_2))^2}$. The optical path length of the segment $[OQUO']$ is then represented by

$$[OQUO'] = n_1\sqrt{(D + z_1)^2 + y_1^2} + n_2\sqrt{(y_1 - y_2)^2 + (z_1 - z_2)^2} + n_1\sqrt{y_2^2 + (S_{i2} - (b - z_2))^2}$$  \(3.6\)

At the incident point $Q(z_1, y_1)$, tangent angle to the surface is calculated by the derivative $m = \arctan(dy/dz)$ of the ellipse equation and the normal to the surface has the angle $n=90^\circ - m$ as defined in Fig. 3.2. Then, the incident angle is $n+\theta$, where $\theta$ is the angle of the incident ray with respect to optical axis. The refracted angle is $\arcsin(n_1 \sin(n + \theta)/n_2)$ according to the Snell law and $(\arcsin(n_1 \sin(n + \theta)/n_2) - n)$ represents the angle made by the refracted ray $QU$ with the $z$-axis, so that the height of the output ray $y_2$ is calculated.
Equation (3.7) combined with the ellipse equation allows solving the values of both $y_2$ and $z_2$. The precedent calculation can be applied to an arbitrary incident ray for the spherical aberration $w_{40}(r)$. However, the incident point $Q$ at the first surface is no longer in the entrance-pupil. The height $r<y_1$ in the entrance-pupil and the height $r'$ in the exit-pupil of the ray should be defined by the projection of the point Q on the entrance and exit pupil.

For a given laser beam power in the optical dual-beam stretcher which traps a spherical RBC of radius $R=3.3\mu m$ and the fiber end face-to-fiber end face distance $d=150\mu m$ and numerical aperture of the beams $NA=0.11$ at $\lambda=1.064\text{nm}$ we computed the photonics stress distribution on the RBC surface using the method presented in Ref. [1], and the resultant RBC deformation, namely the lengths of the semi-major and semi-minor axes of the ellipse, using the method presented in Ref. [7]. Then we computed the spherical aberration $w_{40}$ of the marginal ray as a function of the laser power with $n_1=1.335$, $n_2=1.378$. We can see in Fig. 3.3 that the spherical aberration increases approximately linearly with laser power and Fig. 3.4 shows the spherical aberration as a function of the entrance-pupil diameter.
Fig. 3.3. Variation of the spherical aberration at maximum pupil diameter \((r=2a)\) in rad as a function of power in mW for an erythrocytes in an optical stretcher where the fiber-to-fiber distance was \(d=150\mu\text{m}, \ R=3.3\mu\text{m}, \ n_1=1.335, \ n_2=1.378\) and the numerical aperture of the beam is \(NA=0.11\) at \(\lambda=1.064\text{nm}\).

Fig. 3.4. Variation of the spherical aberration in rad as a function of pupil diameter for an erythrocytes in an optical stretcher where the power is 800mW, the fiber-to-fiber distance was \(d=150\mu\text{m}, \ R=3.3\mu\text{m}, \ n_1=1.335, \ n_2=1.378\) and the numerical aperture of the beam is \(NA=0.11\) at \(\lambda=1.064\text{nm}\).

Note that it is frequent to find in the literature [27] a variation of the spherical aberration in \(r^4\); i.e. \(W_{40}=Ar^4\). In fact, this value of spherical aberration is valid for a single dioptre only so that more calculation must be made to take into account the thick lens. Even then, a residual paraxial approximation is made to have an analytical function. The curve presented here is different and more precise, since we have performed a direct ray tracing of the thick elliptical lens.
3.3. Coupling coefficient

The coupling coefficient between two fibers is calculated by an overlap integral of the two mode field distributions at any convenient plane [28]. To calculate such field, the plane where the overlap integral is performed must be chosen to minimize the computation time. That plane is situated at the right of the cell at the exit-pupil plane of the deformed cell lens; i.e. at the tangent to the ellipsoidal cell lens at the right vertex $S$, as shown in Fig. 3.1. Then we can consider the left fiber as the source fiber and the right fiber as the receiving fiber.

Therefore, the coupling coefficient $T$ is computed using the overlap integral

$$T = \left| \int_S \psi_s L \psi_R dS \right|^2$$

where $\psi_s$ is the Gaussian field distribution emerging from the source fiber, which is propagated from the source fiber end-face to the left vertex $P$ of the cell lens and then through the thick and ellipsoidal shape cell lens to the exit plane $S$, $\psi_R$ is the far field distribution of the receiving fiber in the plane $S$. Both the $\psi_s$ and $\psi_R$ can be calculated by the Gaussian beam propagation. The term $L = \exp(ikW(r))$ describes the wave-front aberrations from plane $P$ to plane $S$ introduced by the cell lens, where $W(r) = W_{40}(r) + W_{20}(r)$ and $r$ is the position vector in the exit plane $S$.

For the Gaussian modes from the single mode fibers, the far field distributions in the exit plane $S$ can be expressed as

$$\psi_s = \frac{2}{\pi w_s} \exp \left( -\frac{r^2}{w_s^2} \right)$$

$$\psi_r = \frac{2}{\pi w_r} \exp \left( -\frac{r^2}{w_r^2} \right)$$

where $w_s$ and $w_r$ is the beam radius of the source and the receiving far field distributions respectively in the plane $S$ where the overlap integral is computed. Note that the wavefront radiuses of curvature of the Gaussian beam distribution are omitted since the difference in
wavefront is considered in the defocus aberration. In order to compute \( w_s \), we consider the thick and ellipsoidal shaped lens as two dioptrés in cascade and separated by a distance \( 2b \). Consider the Gaussian beam emerging from the source fiber. The beam radius at the distance \( D-b \) from the source fiber end face to the vertex \( P \) of the ellipsoidal shaped cell lens is calculated as

\[
w_1 = w_0 \sqrt{1 + \left( \frac{\lambda(D-b)}{\pi w_0^2} \right)^2}
\]

(3.10)

where \( w_0 \) is the beam radius of the single mode pattern in the source fiber end-face. The wavefront radius of curvature of the Gaussian beam at the vertex \( P \) of the first dioptré is calculated as

\[
R_1 = (D-b) + \frac{\left( \frac{\pi w_0^2}{\lambda} \right)^2}{(D-b)}
\]

(3.11)

Note that the focal length of the first dioptré \( f = R_{n1}/(n_2 - n_1) \) where the radius of curvature of the dioptré is approximated by the radius of the non-deformed cell \( R \). Also, because of the symmetry, \( R_1 = R_r \). Then, we use the imaging equation

\[
\frac{1}{f} = \frac{1}{R_1} - \frac{1}{R_2}
\]

(3.12)

to determine the wavefront radius of curvature \( R_2 \) of the transmitted wave through the first dioptré. We assume here that the transmitted beam radius \( w_t \) is not limited by the cell lens even though the beam width is greater than the cell radius, \( w/r > 1 \). In fact, we assume that the inside field distribution is not affected by the limited height of the cell, since the outside field will be cut from the overlap integral any way. Then, the convergent beam transmitted from the first dioptré is propagated by a distance \( 2b \) to the second dioptré at the vertex \( S \) of the cell lens, where its beam radius can be calculated by

\[
w_s = w_1 \left[ \left( 1 + \frac{(2b)^2}{R_2} \right)^2 + \left( \frac{\lambda(2b)}{\pi w_1^2} \right)^2 \right]^{1/2}
\]

(3.13)

The beam radius \( w_s \) does not change when passing through the second dioptré. However, its wavefront radius \( (R_s) \) of curvature is changed according to Eq. (3.12) where
the focal \( f = -\frac{Rn_2}{(n_1 - n_2)} \). The beam radius of the receiving far field distributions \( w_r \) can be calculated by using Eq. (3.10). This allowed us to verify the integrity of the method by comparing with the focus position calculated in Eq. (3.4). According to the Gaussian beam method, the position of the focus \( O' \), at a distance \( SO' = S_{i2} \), should correspond with the position of the minimum waist where the radius of curvature should be infinite. We then calculated the value of \( R_s \) from the focus \( O' \) with Eqs. (3.4) and (3.11) and from \( R_2 \) with the equations of Gaussian beam propagation and verified that the results matched.

The coupling coefficient can thus be calculated with the following equation

\[
T = \left[ \frac{2}{\pi w_y w_r} \int_0^{y_{\text{max}}} \exp \left[ -\left( \frac{1}{w_y^2} + \frac{1}{w_r^2} \right) r^2 \right] \exp \left[ -i \frac{2\pi}{\lambda} \left( W_{40}(r) + W_{20}(r) \right) \right] 2\pi rdr \right]^2
\]  

(3.14)

where \( y_{\text{max}} = y_2 \approx y_1 \) since it represents the exit pupil of the ellipse shaped lens. One should note that there is still an approximation in the calculation of the spherical aberration and the defocus. In fact, the integral is made in the exit pupil plane so that the optical transfer function \( L \) should be a function of the exit pupil plane. However, in this paper \( L \) is a function of the entrance pupil plane because \( W_{20} = W_{20}(r) \) where \( r \) represent a position in the entrance pupil plane and \( W_{40} \) is calculated using the value \( y_1 = r \) which is again a position in the entrance pupil plane. Nevertheless, it should be emphasised that because the index \( n_1 \) and \( n_2 \) are close, the refraction of the rays is small so that the entrance height \( y_1 \) is also close to the exit height \( y_2 \).

The integral in Eq. (3.14) was made using Lobatto Quadrature. The result for the coupling efficiency variation as a function of power is shown in Fig. 3.5.
Fig. 3.5. Coupling coefficient as a function of power in mW for a ratio \( w/R = 1.12 \). The fiber-to-fiber distance was \( d = 90 \mu \text{m} \), \( R = 3.3 \mu \text{m} \), \( n_1 = 1.335 \), \( n_2 = 1.378 \), the numerical aperture of the beam is \( NA = 0.11 \) at \( \lambda = 1.064 \text{nm} \) and the mode field diameter is 3.3\( \mu \text{m} \).

We can see that the coupling greatly varies with power. In fact, the normalized curve allows seeing that there is almost 50% less coupling when the power reaches 300mW which is a really large variation and a measurable quantity. That means, the sensibility of the method with the deformation is enough to be measurable; i.e. the coupling is very sensitive to the shape of the cell. Also, the second curve shows that there would be enough coupling, so that it would be detected by a simple apparatus measuring power. In fact, the absolute coupling coefficient is around 30% which is sufficient to be detected easily. That means the experiment would not need to have very sensitive apparatus to detect the coupling allowing the costs to stay low.

Furthermore, it is possible to change the value of absolute coupling and the shape of the curve by changing the fiber-to-fiber distance. By increasing the distance, the width of the beam to the cell radius ratio \( w/R \) increases. Then, the light is more divergent and more power is lost so that the coupling efficiency should decrease rapidly. However, as we can see in Fig. 3.6, the coupling is still high. This illustrates the impact of aberrations in the coupling efficiency.
Fig. 3.6. Coupling coefficient as a function of power in mW for a ratio \( w/R = 1.8 \). The fiber-to-fiber distance was \( d = 150 \mu m \), \( R = 3.3 \mu m \), \( n_1 = 1.335 \), \( n_2 = 1.378 \), the numerical aperture of the beam is \( NA = 0.11 \) at \( \lambda = 1.064 \text{nm} \) and the mode field diameter is 3.3\( \mu m \).

In fact, at \( d = 150 \mu m \), the beam is more divergent but the spherical aberrations are well balanced so that the overall coupling is good. The absolute coupling coefficient is around 10\% and the sensibility with power is still very good as there is 60\% less coupling at 300mW.

We can also see the effect of aberration by comparing Fig. 3.5 and Fig. 3.6. Fig. 3.6 decreases slowly at weak power and then tends to be quite linear but Fig. 3.5 is almost stable until 50mW where a linear decrease begins. These two phenomenons come from the defocus and the spherical aberration. At different distances, the defocus and the spherical aberration compensate differently from each other. In fact, we can see in Fig. 3.3 that the spherical aberration at maximum pupil diameter is around \(-1.4(2\pi)\) and we calculated the defocus at maximum pupil diameter is around \(-0.009(2\pi)\) so that the total aberration is \(-1.41(2\pi)\). The total aberration is then close to \(-3\pi\) which would give half wavelength aberration. The defocus and spherical aberration can be played with by changing the fiber-to-fiber distance to make the coupling coefficient optimum.
Also, every modification to the experiment that helps keep the beams focused can help increase the coupling even more. It is then interesting to see what happens if we do not limit the exit pupil diameter; i.e. we consider that all the light coming from one fiber can be couple inside the second fiber so that the integral in Eq. (3.14) goes from 0 to infinity. Doing the calculation gives a coupling coefficient on the order of 0.9 for $d=150\mu m$ and 10mW of power. Note that it mathematically represents a cell that is infinitely big, but it gives an idea of how much light is wasted because it does not focalise enough. The rest of the coupling lost is mainly due to the spherical and defocus aberrations which, as we saw, are also very important.

The experimental setup of such an experiment has not been attempted during my master degrees but would definitely worth trying if accurate equipment can be found. The Matlab code used to calculate the coupling coefficient, the aberrations, the pupil height and all the other parameters explained in this section are available in Appendix F.
4. Oscillating tweezers

4.1. Experimental setup

Another tool to deform cells was built in collaboration with Yang-Ming University in Taiwan where I was able to do some experimental work during the month I went there. A schematic diagram of the experimental setup is illustrated in Fig. 4.1. An expanded and collimated infrared laser beam (wavelength $\lambda = 1064$nm, 300mW from a cw Nd:YVO laser), strongly focused by an oil-immersion objective lens (100x, $NA = 1.25$) was used for optical trapping of the RBC samples suspended in buffer solution in a sample chamber. Controlled oscillation of the focal spot of the trapping beam with amplitude on the order of a few microns and oscillation frequency from a few Hz to a few kHz was achieved with the aid of an acoustic-optical modulator (AOM, Isomet 1201E 2). A set of 4-f telescopic imaging optics was used to image the output plane of the AOM to the entrance aperture of the objective lens such that the angular scan of the output laser beam from the AOM was mapped to a lateral scan of the focal spot of the trapping beam without any beam walk-off at entrance aperture of the objective lens. An additional probing beam (633nm from a HeNe laser) projected an image of the trapped RBC sample onto a quadrant photodiode (QPD) to track the transverse position of the sample with a sampling rate $\sim 10$ kHz and a spatial resolution of approximately 20nm. In addition, an incoherent light was used to illuminate the sample for incoherent imaging via a CCD camera and to monitor the shape, and the size of the RBC. Calibration with a Ronchi Ruling (5000lp per inch) indicated that the image scale on the CCD was approximately 50nm per pixel. The CCD camera was linked to a pc for digital image storage and analysis.
In a typical experiment, we trapped an RBC sample with stationary optical tweezers (i.e., without scanning the trapping beam) first and then applied an appropriate voltage to the AOM to scan the focal spot of the trapping beam with a selected oscillation amplitude increased step-by-step such that the distance between the two focal points varied from 4μm to 9.4μm in 0.9μm step. The frequency can be chosen in the range of a few Hz to a few kHz. When changing the amplitude and frequency, the output power is measured to make sure the same power reach the sample. At relatively low scanning frequency on the order of a few Hz, the trapped RBC moved in response to the scanning spot, with a certain phase delay due to viscous damping. At relatively high scanning frequency (on the order of a few hundred Hz to a few kHz) the trapped RBC could not respond fast enough to track the rapidly scanning focal spot. Instead of an oscillatory motion, the RBC deformed (i.e., elongated) as we will explain in what follows. In the experiment performed in Taiwan, the oscillatory motion of the RBC sample at slow scanning frequency was tracked by the QPD whereas the change in shape of the RBC sample at high scanning frequency was imaged and measured by the pre-calibrated CCD camera. Using the oscillatory optical tweezers
described above we have trapped and stretched different samples including RBCs (in either spherical shape or in bi-concave shape) as well as spherical liposomes. We observed that, in comparison with cases where the trapping beam was scanned continuously by applying a sinusoidal voltage to the AOL, stretching of the sample was more pronounced when a square-wave voltage was applied to the AOL to scan the focal spot of the trapping beam discretely between two fixed points. Then, only the case where the beam scan discretely between two points will be studied here since it gives better results and was the main subject of the collaboration. The side-view of the elongated profile of the discotic RBC in each step as was imaged by the microscope objective lens on the CCD camera was shown in Fig. 4.2, and the stretched length as a function of the scan distance is depicted in Fig. 4.11. Also note that when the distance between the two scan focal spots was increased beyond 9.4μm, the stretched length saturated at about 8.5μm and even decreases, the cells often escaped from the trap and also the output power level becomes impossible to maintain at the same level and unavoidable decreases.

Fig. 4.2. The side-view of a discotic RBC trapped and stretched by an oscillatory optical stretcher where the focal spot (with optical power = 12 mW) was discretely scanned between two points at f=100Hz. From (1) to (6), the distance between the two points was increased from 4.0μm to 9.4μm in steps of 0.9μm.
4.2. Applied stress

We assume that the RBC’s response time to the scan of the focused laser beam is much slower compared with the beam scan frequency of 100 Hz, so that the cell sees two simultaneous laser beams focused at \( y = \pm D \). In the estimation for the RBC’s deformation, we use a model of the 3D shape represented in Fig. 4.3(b), which shows a circle of radius \( \rho \) in view from the top, and an ellipse in view from the side. This shape is an approximation to the biconcave model of the RBC, shown in Fig. 4.3(a), the curvature is null at a certain point leading to a radius of curvature infinite. This renders the numerical calculations tedious and was of no interest here.

![Fig. 4.3. Biconcave shape of RBC (a) and the shape used in the calculations (b).](image)

We consider the RBCs as a thin shell because the ratio of the membrane thickness \( h \) on the cell radius \( \rho \), \( h/\rho \sim 0.01 \). Here, we present an approximate theory on the RBC’s deformation under the photonics radiation pressure by the optical oscillating focused beam. In first place, we calculate the local force distribution on the cell surface. Evans [21] showed that the RBC has a biconcave disk shape, as shown in Fig. 4.6. Assume that the biconcave disk lies down in the \( x-y \) plane. In view from the top along the \(-z\) direction, the disk is represented by its largest circumference in the \( x-y \) plane, as shown by the red circle in Fig. 4.4. The focused laser beam propagating in the \(-x\) direction is shifted along the \( y\)-axis by the oscillation, such that its focus is located on the \( y\)-axis at the points \( y = \pm D \) alternatively. We now calculate the stress distribution on the circle in the \( x-y \) plane using the ray optics.
When an optical ray is incident to an interface, the momentum of the incident, transmitted and reflected rays are denoted by $\vec{P}_i$, $\vec{P}_t$ and $\vec{P}_r$, and their directional unit vectors by $\vec{a}_i$, $\vec{a}_t$ and $\vec{a}_r$, respectively. According to the law of momentum conservation, $\Delta \vec{P} = \vec{P}_i - \left( \vec{P}_t + \vec{P}_r \right)$, the photonics radiation pressure stress $\vec{\sigma}$ applied to the interface is expressed as:

$$\vec{\sigma} = \frac{\Delta \vec{P}}{A \Delta t} = \frac{\vec{P}_i - \left( \vec{P}_t + \vec{P}_r \right)}{A \Delta t} = \frac{1}{c} \frac{E_i}{A \Delta t} n_1 \left( \vec{a}_k - \left( n T \vec{a}_i + R \vec{a}_r \right) \right) \equiv \frac{n_1}{c} \frac{P}{A} \vec{Q}$$

(4.1)

where $E$ is the beam energy, $c$ is the speed of light, $P$ is the laser beam power, $A$ the area covered by the beam, $n = n_2/n_1$, with $n_1$ and $n_2$ being the index of the medium surrounding the cell and inside the cell respectively, $T$ and $R$ are the Fresnel transmittance and reflectance, respectively, and $\vec{Q}$ is the dimensionless momentum transfer vector. Thus, the applied stress is proportional to the beam power and inversely proportional to the beam area on the interface.

Fig. 4.4. Ray tracing in the red plane of the RBC as a function of distance $D$ from the center and angle $\phi$. 
Consider now one of the optical rays in the $x$-$y$ plane in the Gaussian laser beam, which is incident to the circle toward the focal point $x=0, \ y=D$, as shown in Fig. 4.4. The components $Q_x$ and $Q_y$ can be written as

\[ Q_y = \exp\left[- \left( \rho \cos(\phi) - D \right)^2 / w_0^2 \right] \cos(\delta + \phi) - nT(\delta)\cos(\beta + \phi) + R(\delta)\cos(\phi - \delta) \]

\[ Q_x = \exp\left[- \left( \rho \cos(\phi) - D \right)^2 / w_0^2 \right] \sin(\delta + \phi) + nT(\delta)\sin(\beta + \phi) - R(\delta)\sin(\phi - \delta) \]  

(4.2)

where the angle of the incident ray with respect to the $x$-axis is $\alpha \in [-\alpha_{0\ max}, \alpha_{0\ max}]$ with $\alpha_{0\ max}$ determined by the numerical aperture of the focused beam of numerical aperture $NA = n_t \sin(\alpha_{0\ max})$. The incident point of this ray on the circle is defined by the azimuthal angle $\delta = 90^\circ - (\phi + \alpha)$. The incident angle $\delta$ to the circle normal depends on the beam displacement $D$ by the relation $\rho \sin(\delta) = D \sin(\gamma)$ with the red circle radius $\rho$ and $\gamma = 90 + \alpha$. We model the focused laser beam as a bundle of optical rays with the given $NA$, while the intensity distribution in the cross section of the beam is Gaussian. In Eq. (4.2), the Gaussian distribution, $\exp\left[- \left( \rho \cos(\phi) - D \right)^2 / w_0^2 \right]$, modulates intensities of the rays in the beam, where $w_0 = 2 \rho \sin(\phi) \tan(\alpha_{0\ max})$ is the width of the Gaussian beam, intersected by a plane, which is parallel to the $x$-$y$ plane at the incident point. In our beam model each incident ray has its own incident point on the circle, defined by the azimuthal angle $\phi$, and its nominal intensity useful for computing the local photonic force according to the Gaussian intensity factor in Eq. (4.2), which varies with $\phi$. Note that the focused beam width varies significantly in $x$ as a function of position of the incident point, so that the nominal beam width $w$ in Eq. (4.2) is highly dependant of the position of the incident point on the circle, at which the beam is cut by a plane parallel to the $y$-axis. As the beam oscillation amplitude increases, the incident rays having the same direction $\alpha$ with respect to the $x$-axis have their incident point closer to the $y$-axis, with a decreased $\phi$ and decreased nominal beam width $w$, so that its nominal intensity increases significantly. On the contrary, a ray in the same beam but in the direction $-\alpha$, for instance as shown in Fig. 4.4, would have its incident point on the circle farer from the $y$-axis and with a higher $\phi$ and larger $w$. Its nominal intensity and thus its force applied would be therefore much lower. Moreover, it has been proven that the photonic force is perpendicular to the circular refracting surface,
independently on the angle and position of the incident ray [1]. The computed stress distribution on the circle for a set of displacement $D$ from the center by the focused beam of $NA=1.25$ is shown in Fig. 4.5.

As the difference between the refractive index of the buffer $n_1=1.33$ and that of the RBC $n_2=1.37$ is small, we neglected in this calculation the focusing power of the cell; i.e. we did consider the refraction when calculating the stress distribution on the front interface ($x>0$) with Eq. (4.2), but for the sake of simplicity, when calculating the stress on the rear interface ($x<0$) we treated the incident ray as it passes straight the front interface to the focus and continues propagating until refracted by the rear interface from the inside of the cell. Under this approximation, the stress distribution in the rear surface is symmetric to that in the front surface with respect to the y-axis as shown in Fig. 4.5.

![Stress Distribution](image)

Fig. 4.5. Stress $\sigma(\phi)$ Nm$^{-1}$ distribution on the circumference of the RBC as a function of the beam shift from the center $D=1\mu$m (red), $2\mu$m (green), $3\mu$m (black) and $3.3\mu$m (blue) for the RBC long axis $\rho = 4.01\mu$m. a) is a polar representation and b) is a simple quadrant representation.

As we can see in Fig. 4.5, despite of the high $NA$ of the focused beam, the significant photonics force is distributed only in a limited range of angle $\phi$, and when the beam oscillation amplitude $2D$ increases, the values of forces increase significantly, the force angular distribution becomes narrower and the mean direction of the forces becomes closer to the $\pm y$ axis with smaller angle $\phi$. This is because the effect of the nominal intensities of
optical rays which vary significantly with the ray’s propagation direction and the beam shift $D$ in our Gaussian beam model. Recall that the force is always perpendicular to the surface, thus not only the values of the force increases with the decrease of angle $\varphi$, its component along the $\pm y$-axis increases even more. This explains why the stretch of the RBC increases along the $y$-axis with the increase of the beam scan amplitude $2D$, for a constant laser beam power 12mW.

The red circle in Fig. 4.4 is the largest circumference of the RBC biconcave disk, which lies down on the $x$-$y$ plane. The stress distribution would vary when the circle moves up or down in $\pm z$ directions. In order to estimate the stress distribution on the parallel circles and for the sake of simplicity, we model the RBC’s as of an ellipsoidal disk shape instead of the biconcave shape, as shown in Fig. 4.6, which is a revolution about the $z$-axis of an ellipse in the $y$-$z$ plane with the semi-major axis of $\rho$, which is the radius of the largest circumference of the RBC disk in the $x$-$y$ plane, and the semi-minor axis of $\chi$, where $2\chi$ is the thickness of the RBC in the $z$-direction.

The optical axis of the shifted laser beam lies down in a plane which is parallel to the $x$-$z$ plane at the position of the beam focus $y=\pm D$. This plane cuts the cell ellipsoidal disk resulting in a yellow ellipse on the cell surface, as shown in Fig. 4.6. The shape of the yellow ellipse depends on the beam position $D$ as the beam center defines the $y$ position of the ellipse. Its semi-major axis is $a = \rho \sin \kappa$ with $\kappa = \arccos(D/\rho)$, as can be seen in Fig. 4.4, and its semi-minor axis is $b = (\chi/\rho)\sqrt{\rho^2 - D^2}$, as calculated in the ellipse of the cell disk cut by the $y$-$z$ plane.
Fig. 4.6. A three dimensional representation of the oscillating beam trap. $\phi$ is the angle on the XY plane, $\theta$ is the polar angle and $\psi$ represents the angle on the yellow plane (see Fig. 4.7).

Fig. 4.7 Ray tracing in the yellow plane of the RBC as a function of distance D from the center and angle $\psi$.

With the equation of the yellow ellipse $\frac{x^2}{a^2} + \frac{y^2}{b^2} = 1$ we can trace the ray and calculate the stress distribution on the yellow ellipse. Consider an incident ray in the focused laser beam. The ray makes an angle $\alpha$ with respect to the beam axis $\alpha$. The maximum of $\alpha$ is limited by the NA of the focusing beam. The incident angle at the yellow ellipse is $\delta = \eta - \alpha$ with $\eta = 90 - m$, and $m$ is the tangent angle to the ellipse at the incident point, as shown in Fig. 4.7. Hence, we have Eqs. (4.3) for calculating the stress profile.
\[
Q_x = \exp\left[-\frac{(R(\psi)\sin(\psi))^2}{w_0^2}\right] \cdot \cos(\psi) + nT(\delta)\cos(\beta + \psi) - R(\delta)\cos(\psi + 2\delta)
\]
\[
Q_z = \exp\left[-\frac{(R(\psi)\sin(\psi))^2}{w_0^2}\right] \cdot \sin(\psi) + nT(\delta)\sin(\beta + \psi) - R(\delta)\sin(\psi + 2\delta)
\]  
(4.3)

where refracted angle \(\beta\) is calculated by the Snell law \(n_1 \sin \delta = n_2 \sin \beta\) and the focused beam width in the Gaussian beam intensity correction factor, \(\exp\left[-\frac{(R(\psi)\sin(\psi))^2}{w_0^2}\right]\), is calculated with \(w_0 = R(\psi)\cos(\psi)\tan(\psi_{\text{max}})\) where \(R(\psi) = a b / \sqrt{a^2 \sin^2(\psi) + b^2 \cos^2(\psi)}\), as shown in Fig. 4.7.

Using these equations one can compute the stress distribution \(\sigma_m(\psi)\) as shown in Fig. 4.8. This stress distribution is normalized by the stress on the largest circumference of the red disk in Fig. 4.4; i.e. it is normalized by the stress at \(\psi = 0\). As \(\psi\) increases, the thickness of the yellow ellipse decreases so that the intensity of the rays increases. In addition, the angle of the rays with the normal of the membrane does not increase a lot because of the high NA of the lens. These two factors together make \(\sigma_m(\psi)\) increases as \(\psi\) increases as shown in Fig. 4.9. Our calculation shows that \(\sigma_m(\psi)\) does not vary with the position \(D\) of the beam focus on the corresponding yellow ellipse plane. However, for a given distance \(D\), \(\sigma_m(\psi)\) would vary as we consider subsequent yellow planes parallel to one another. For the sake of simplicity we represent the full three-dimensional stress profile on the cell’s surface approximately as \(\sigma(\phi, \psi) = \sigma(\phi)\sigma_m(\psi)\). Here again, we consider the stress distributions on the front \((x<0)\) and rear \((x>0)\) surfaces symmetrical with respect to the \(y\)-axis, as we did when calculate the distribution in Fig. 4.5.
Fig. 4.8. Normalized stress distribution $\sigma_m(\psi)$ in the yellow plane of Fig. 4.7 as a function of $\psi$ for any distance $D$ from the center of the cell. a) is a polar representation and b) is a simple quadrant representation.

Fig. 4.9. 3D stress profile $\sigma(\phi, \psi)$ for a) $D=3.5\mu$m and b) $D=0\mu$m. Top view of the stress distributor for c) $D=3.5\mu$m and d) $D=0\mu$m. RBC disk radius $\rho = 4.01\mu$m, beam $NA=1.25$, $n_1=1.33$ for buffer and $n_2=1.37$ for RBC.

From Fig. 4.9 we can see that, when the beam shift $D=3.5\mu$m the stress is more than 15 times stronger than when $D=0\mu$m and the area on which the stress applies is almost the same. Then, as $D$ increases the sum of stress applied on every differential element of the sphere increases, resulting in larger deformation of the RBCs. Also this force is always normal to the membrane has we can always draw a sphere passing by any point on the membrane which as the same curvature at this point, thus the proof in [1] still hold. As $D$ increases, the angle $\phi$ at which the force is non-zero increases, then as $D$ increases the force
in the direction parallel to the oscillating beam increase leading to a deformation in the same direction.

However, one should note that this simple model is not appropriate to calculate the deformation in the axis perpendicular to the oscillating beam. In fact, the problem comes from the ellipsoidal shape instead of the biconcave shape. The ellipsoidal shape allows the stress distribution in the yellow plane to increase as $\phi$ increases which would lead to a specific deformation in the direction perpendicular to the oscillating beam. However, if taking into account the biconcave shape, the stress would be unimportant in the middle of the RBC as the concave part would be shaded by the rest of the cell or the angle of incidence would be grazing in this region. This would lead to a completely different deformation, since the stress would be very small in the middle.

As the computed deformation in the direction parallel to the oscillating beam is less dependant to the stress profile in the perpendicular yellow plane, we can approximate that the deformation will mostly depends on the stress varying with $D$; i.e. the red plane. We then approximate that the three dimensional stress becomes two-dimensional and symmetric with $\theta$; i.e. that for a given $\phi$ the stress is computed and its value is the same at every $\theta$ position. According to the calculations for the yellow plane, making this approximation will makes us under evaluate the intensity of the stress as $\psi$ increases but we will over evaluate it at the center where the hallow is situated so that the overall stress is, in first approximation, well represented.

Then, it gives a stress distribution that varies with $D$, rotationally symmetric with respect to $\theta$, with a stress profile well represented by Fig. 4.5 and two-dimensional which will ease the computation of the resulting deformation.

The Matlab code to calculate the fully three dimensional stress distribution is available in Appendix D. This code also explains how to use this stress distribution to calculate the deformation that we explain in the following section. Note that the deformation codes are given in Appendix E.
4.3. Deformation

To measure the elasticity of a cell with the oscillating tweezers, the local stress distribution on the cell surface is first calculated, and the morphological deformation of the cell is calculated from the stress distribution as we did in section 2.3. The parameters specifying the cellular elasticity are finally determined by comparing the experimentally measured cell’s deformation in optical stretcher with the theoretical result. The local optical stress distribution on the cell surface has been calculated in the above section for an approximate cell shape given in Fig. 4.3(b). The stress distribution in an oscillating tweezers depends on the scanning distance \( D \) where the power is held constant throughout the experiment.

The approximations made in the previous section allow calculating the deformation in the red plane of Fig. 4.6 with a simple 2D model instead of the more complex 3D model. We can then proceed as we did in the section 2.3 to calculate the deformation for different distance of beam scan \( \pm D \). We use the linear theory of thin shells to calculate the deformation since only the initial shaped changed; i.e. the conditions to use this theory does not depends on the initial shape. In fact, the applied stress is always perpendicular and smooth and the deformed RBC has a smooth surface as seen in Fig. 4.2, so that any bending energy can be neglected. Also, we consider only the cases where the cell’s deformation is small compared with the initial shape, so that the cell is not severely and irrecoverably deformed.

However, the shape of the initial RBC is different here so that we need to modify the equation of section 2.3 to adapt this difference. The initial shape will be considered to be as represented in Fig. 4.3(b) since the real biconcave shape is tedious to use within a numerical calculation. The fact is, the hollow in the center give rise to a change in curvature sign so that the curvature is 0 somewhere near the middle. A curvature of 0 means that the radius of curvature is equal to infinity at this point rendering the calculations much more difficult. The initial shape of an object is taken into account by defining the two principal radius of curvature. In the previous case of a sphere, these radius were equal and constant everywhere on the surface, however we clearly see in Fig. 4.3 or Fig. 4.6 that the two principal radius of curvature will depends on the angle \( \theta \) and \( \phi \). The equations used in section 2.3 are then a simplification of what is used here. More specifically, Eqs. (2.11) to
(2.13) becomes Eqs. (4.4) where $R_1$ and $R_2$ are the principal radius of curvature of the Smarties-like RBC.

These Laplace equations [24] allow us to calculate the internal membrane stresses $N_\phi$ and $N_\theta$, from the external surface stress load $(\sigma_r, \sigma_\theta, \sigma_\phi)$, where $\sigma_r$ is the external stress normal to the membrane in the direction $W$, $\sigma_\theta$ is the external stress in the azimuthal angle direction parallel to $V$ and $\sigma_\phi$ is the external stress along the meridional or polar angle direction parallel to $U$; as shown in Fig. 1, $V$, $U$, and $W$ are local Cartesian coordinate system with the origin $O$ at the center of the differential membrane element. $V$ and $U$ coincide with the directions of tangential curvilinear coordinate lines of the membrane and $W$ points to the origin along the normal of the membrane element. In this case, the Laplace equations can be expressed as

$$R_1 \frac{\partial S}{\partial \theta} + \frac{\partial (N_\phi R_2 \sin \phi)}{\partial \phi} - N_\theta R_1 \cos \phi + R_2 R_1 \sin (\phi) \sigma_\phi = 0$$

$$R_1 \frac{\partial N_\theta}{\partial \theta} + \frac{1}{R_2 \sin \theta} \frac{\partial (R_2^2 \sin^2 (\phi) S)}{\partial \phi} + R_2 R_1 \sin \phi \sigma_\theta = 0$$

$$\frac{N_\phi}{R_1} + \frac{N_\theta}{R_2} + \sigma_r = 0 \quad (4.4)$$

The nomenclature is the same here than in section 2.3 so that Fig. 4.10 applies with the same definition as above. However, note that the angle $\phi$ is defined at $\pi/2$ from that of Fig. 4.6.
As we explained in section 4.2, the stress distribution can be considered, in first approximation, symmetric with respect to $\theta$. Then the stress calculated on the $XY$ plane is symmetric around the $y$-axis leading to a 2D stress distribution. The derivative with respect to $\theta$ in Eqs. (4.4) will then vanish. The second equation gives $S=0$. It is useful to use the change of variable $\zeta = N_\phi R_2 \sin^2 \phi$ to further simplify the first and the third equations to the following

$$\frac{d\zeta}{d\phi} = -R_1 R_2 \sin \phi \cos \phi \sigma_r (\phi) \quad (4.5)$$

$$N_\phi = -R_2 \sigma_r (\phi) - \frac{\zeta}{\sin^2 \phi} \frac{1}{R_1} \quad (4.6)$$

Then using a numerical method for differential equation we can find the membrane stresses $N_\phi$ and $N_\theta$. We use a Runge-Kutta method of order 4 with error control to solve Eqs. (4.5) and (4.6), then the change of variable give $N_\phi$. To solve Eq. (4.5) one needs an initial condition, note that $\zeta(0)=0$.

The displacements equations which are related to the membrane stresses $N_\phi$ and $N_\theta$ by Hooke’s law are given, for the case where the two principal radius of curvature are a function of $\theta$ and $\phi$, as
The first relation is found by considering the displacement of a differential membrane element AB of a given length on the meridian in the unstrained membrane element. Similarly, one can consider a segment on the parallel circle in the unstrained membrane element and compute its deformation to obtain the second relation. The last relation is found by considering the effect of the shear strain to the membrane if the membrane moves in the direction to a parallel circle.

The derivative with respect to \( \theta \) vanishes in Eqs. (4.7) giving \( v=0 \). Then, simplifying the first and second equation gives

\[
\frac{du}{d\phi} = u \cot(\phi) + \frac{R_1}{Eh} (N_\phi - \nu N_\phi) - \frac{R_2}{Eh} (N_\phi - \nu N_\phi)
\]

\[w = u \cot \phi - \frac{R_2}{Eh} (N_\phi - \nu N_\phi)\]

A change of variable can be use to further simplify the Eqs. (4.8) and (4.9). In fact, we know from Fig. 4.10 that \( u(0)=0 \) because of the symmetry. Then if we pose \( \Omega = \frac{u}{\sin \phi} \) with \( \Omega(0)=0 \), we have Eqs. (4.8) and (4.9) that simplifies to
\[
\frac{d\Omega}{d\phi} = \frac{1}{Eh \sin \phi} \left[ R_1 \left( N_\phi - \nu N_\phi \right) - R_2 \left( N_\theta - \nu N_\theta \right) \right]
\]
(4.10)

\[
w = \Omega \cos \phi - \frac{R_2}{Eh} \left( N_\theta - \nu N_\theta \right)
\]
(4.11)

As the experiment done in Taiwan only take into account the elongation of the cell; i.e. the deformation at \( \phi = 90^\circ \), we have

\[
w_{90^\circ} = -\frac{R_2}{Eh} \left( N_\theta - \nu N_\theta \right)
\]
(4.12)

The elongation is then easy to calculate as we only need to solve for \( N_\phi \) and \( N_\theta \) with Eqs. (4.5) and (4.6). However, one still needs to define the shape of the initial RBC by calculating the principal radius of curvature \( R_1 \) and \( R_2 \). These can be calculated by the following relations if the parametric equation \( \vec{r} = X\hat{i} + Y\hat{j} + Z\hat{k} \) of the shape is known and represented in the coordinate system of Fig. 4.10.

\[
R_1 = \frac{a_{11}}{b_{11}}
\]
(4.13)

\[
R_2 = \frac{a_{22}}{b_{22}}
\]
(4.14)

where \( a_{11}, a_{22} \) and \( a_{12} \) are given by,
\[ a_{11} = \frac{d}{d\phi} \vec{r} \cdot \frac{d}{d\phi} \vec{r} \]  
(4.15)

\[ a_{22} = \frac{d}{d\theta} \vec{r} \cdot \frac{d}{d\theta} \vec{r} \]  
(4.16)

\[ a_{12} = \frac{d}{d\phi} \vec{r} \cdot \frac{d}{d\theta} \vec{r} \]

\[ b_{11} \text{ and } b_{22} \text{ are given by.} \]

\[ b_{11} = \frac{1}{\sqrt{a_{11}a_{22} - a_{12}^2}} \frac{1}{\frac{\partial^2 X}{\partial \phi^2} \frac{\partial^2 Y}{\partial \theta^2} \frac{\partial^2 Z}{\partial \phi \partial \theta} - \frac{\partial^2 X}{\partial \phi \partial \theta} \frac{\partial^2 Y}{\partial \phi \partial \theta} \frac{\partial^2 Z}{\partial \theta^2}} \]  
(4.17)

\[ b_{22} = \frac{1}{\sqrt{a_{11}a_{22} - a_{12}^2}} \frac{1}{\frac{\partial^2 X}{\partial \theta^2} \frac{\partial^2 Y}{\partial \theta^2} \frac{\partial^2 Z}{\partial \theta^2} - \frac{\partial^2 X}{\partial \theta \partial \phi} \frac{\partial^2 Y}{\partial \theta \partial \phi} \frac{\partial^2 Z}{\partial \phi \partial \theta}} \]  
(4.18)

Note that \( a_{12} = 0 \) here since we used a spherical coordinate system that is orthogonal. Also, to complete the calculation, one needs to know the parametric equation of the RBC. The approximate RBC shape representing a circle as seen from the top and an ellipse as seen from the optical axis can be expressed as follows in the coordinate system of Fig. 4.10

\[ X = \rho \sin \phi \cos \theta \]
\[ Y = \rho \sin \phi \sin \theta \]
\[ Z = \chi \cos \phi \]  
(4.19)

where \( \chi \) represents the semi-minor axis of the RBC and \( \rho \) the maximum radius of the cell.

Using Eqs. (4.13) to (4.19) gives the following relation for \( R_1 \) and \( R_2 \)
\[ R_1 = -\frac{(\rho^2 \cos^2 \phi + \chi^2 - \chi^2 \cos^2 \phi)^{3/2}}{\rho \chi} \]  \quad (4.20)

\[ R_2 = -\frac{\rho(\rho^2 \cos^2 \phi + \chi^2 - \chi^2 \cos^2 \phi)^{1/2}}{\chi} \]  \quad (4.21)

The Matlab code used to define the differential equations is given in Appendix E under the name of EQD.m.
4.4. Comparison with experimental results

The theoretical deformations of erythrocytes calculated using Eq. (4.12) can be compared with experimental results. To determine the Young modulus of the cell’s membrane we plot the observed length of the cell normal to the optical axis, namely \( w_{90^+}\rho \), in the oscillating tweezers as a function of the scanning distance \( D \). The best fit of Eqs (4.12) to the experimental data gave the parameter \( Eh \) of the deformation curve \( w_{90^+}\rho \), where \( E \) is the Young modulus and \( h \) the cell membrane thickness, as illustrated in Fig. 4.11.

We found a Young modulus between \( Eh=29\mu\text{Nm}^{-1} \) and \( 11\mu\text{Nm}^{-1} \) depending on the RBC sample from mice (d) or human blood (a-c). This value of the Young modulus is well within the expected values if we compare with other experiments on RBCs. As the usual value of elasticity is given as the shear modulus in the literature, we can transform our result with \( Gh=Eh/2(1+\nu) \). It gives for Fig. 4.11 a) \( Gh=9.67\mu\text{Nm}^{-1} \), b) \( Gh=6.67\mu\text{Nm}^{-1} \) in b) \( Gh=8.33\mu\text{Nm}^{-1} \) in c) and \( Gh=3.67\mu\text{Nm}^{-1} \) in d) which compare to \( Gh=8.5\mu\text{Nm}^{-1} \) for M. Dao et al. [25], \( Gh=13\mu\text{Nm}^{-1} \) for J. Guck et al. [7] and \( Gh=2.5\mu\text{Nm}^{-1} \) for Hénon et al. [10].
Fig. 4.11 Comparison between the measured deformation of RBC and the calculation. The Young modulus that fits the results is a) $E_h=(29\pm4)\ \text{uNm}^{-1}$ b) $E_h=(20\pm3)\ \text{uNm}^{-1}$ c) $E_h=(25\pm5)\ \text{uNm}^{-1}$ d) $E_h=(11\pm1)\ \text{uNm}^{-1}$

Note that there is a large variation in shear modulus for RBCs in the literature since almost no one uses the same method of preparation and deformation which can directly lead to a different stress response of the cells. However, the variation in the values is always on the same order. More experiment made with the same deformation tool, the same preparation method and with much better statistic would lead to much more constant erythrocytes elasticity values.
Conclusion

In the case of the dual-beam optical stretch and trap, neglecting the subsequent reflections inside the spherical cell, we have expressed the scattering stress distribution as a function of the fiber-to-cell distance, which is directly measurable in experiments for a given fiber NA. We have shown that the focusing power of the spherical cell concentrate the refracted rays to a smaller area for the second refraction, resulting in peaks on the stress distribution around certain angular positions. In addition, we have demonstrated that the scattering stress is perpendicular to the spherical surface independently on the incident angle, polarization of the incident beam, reflectance and transmittance at the cell surface. We have also demonstrated that this method of calculation gives accurate results when compared with experiments.

In fact, we report a numerical method to calculate the deformation of a spherical RBC from an arbitrary smooth stress distribution over the cell membrane. In the special case when the stress distribution can be approximated analytically by \( \sigma_r = \sigma_0 \cos^2(\phi) \), our results reduce to those reported earlier for this special case [7]. We applied the model to fit the experimental data for the deformation of a red blood cell in a fiber-optical dual-beam trap. From the best fit, the shear modulus of the red blood cell was determined to be \((6.67\pm0.07) \ \mu Nm^{-1}\) which is comparable with the results reported earlier [7, 10, 25]. The theory does not get more complex, in fact, it gets simpler as the increased flexibility allows one to build a universal code, like the one given in Appendix, that can be used by anyone with basic knowledge of matlab.

For the same trap, we constructed a theoretical model to calculate the cells deformation without the need to record the image of the RBCs. This model based on the variation of the coupling efficiency as the cells deform shows that this method is sensible enough to detect the change in deformation. In fact, we found that, in the linear regime of deformation, the change in coupling efficiency is almost 50% in certain configuration. The absolute coupling efficiency is also high enough to be detected. In fact, at a fiber-to-fiber distance of \( d=90\mu m \) the coupling could be as high as 30%. The theory also gives insight as
what should be done to increase the absolute coupling efficiency if too low for certain configurations.

The measure of coupling has the advantage of being incredibly fast to record compare to an image analysis. The number of cells to be processed in a given time could then be increased considerably. The optical stretcher used with this method could become an exceedingly precise cell sorter that could help diagnose many different diseases with a blood sample. In fact, as we increase the number of cells being deformed, the elasticity value becomes insensible to statistical variations inside a given population. That leads to an increase sensibility when looking at the variation between different populations, which traduces in an increase ability to discriminate among different sickness.

We demonstrated a novel method to optically trap and stretch a non swollen discotic RBC and formulated a simple theoretical model to analyze the associated force distribution on the cell as well as the resulting morphological deformation of the cell in response to the optical stretch. From the theoretical fit to the experimental data, the in-plane elastic constant of a discotic RBC along the platelet diameter is estimated to be between 20uNm$^{-1}$ and 29uNm$^{-1}$ for human RBC and around 11uNm$^{-1}$ for mice RBC.

With a two-dimensional AOM, the applications of this method can be easily extended to three-dimensional orientation manipulation of RBC as in optically driven motors [31] as well as to the probing of mechanical properties of other micron-size soft materials [32]. A three-dimensional AOM could have the potential to be used to create any required stress distribution. To use the RBCs as a marker for diseases or as a tool to understand the diseases themselves, it makes sense to use the non-swollen shape as it is the real shape. However, we saw that describing the scattering of light on such a complex shape is not easy and that the calculation of the resulting deformation was even more tedious. In light of the results given in this paper, we could use a three-dimensional AOM or two two-dimensional AOM to create a specific stress distribution with a certain symmetry that would greatly reduce the mathematical effort needed to describe and thus enhanced the precision and the flexibility.
The first utility of these tools all together could be to help understand blood related diseases such as diabetes or to use the deformation as a marker to detect non related diseases such as cancer formation [33].
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Appendix A

For an arbitrary ray incident to the surface at a point of polar angle $\phi$ and having an incident angle $\varepsilon$, we can rotate the $x$-$y$ axes by $\delta = \varepsilon - \phi$ such that the incident ray is parallel to the new $x$-axis, as shown in Fig. 2.4. Thus, the incident angle is equal to the polar angle $\varepsilon = \phi$ with the new axes. We consider $G = Q_{\text{front}} y / Q_{\text{front}} x$. Using the Snell’s law $n = n_2 / n_1 = \sin(\phi) / \sin(\beta)$ and redistributing the parentheses, we obtain

$$G = \frac{\sin(\phi) [\sin(\phi) \cos(\beta) - \cos(\phi) \sin(\beta) + 2 \cos(\phi) \sin(\beta) \sin(\phi)] - 2 \cos(\phi) \sin(\beta) \sin(\phi)}{-T \sin(\phi) \cos(\phi) \cos(\beta) + [-T \sin^2(\phi) \sin(\beta) + T \sin(\beta) - 2T \cos^2(\phi) \sin(\beta)] + 2 \cos^2(\phi) \sin(\beta)} \quad (A1)$$

where the bracketed term in the denominator reduce to $-T \cos^2(\phi) \sin(\beta)$. Equation A1 can then be simplified to obtain A2:

$$G = \frac{\sin(\phi)}{-\cos(\phi)} \frac{T [\sin(\phi) \cos(\beta) + \cos(\phi) \sin(\beta)] - 2 \cos(\phi) \sin(\beta)}{-\cos(\phi) \cos(\beta) + \cos(\phi) \sin(\beta)} = -\frac{\sin(\phi)}{\cos(\phi)} \quad (A2)$$

therefore $\arctan(G) = -\phi$, where the negative sign means the stress is in $+y$ direction and $-x$ direction for $0 < \phi < \pi/2$, i.e. the stress is directed outside the cell, and thus stretching the cell.

For the proof of the second Eq. (2.6) we consider $G = Q_{\text{back}} y / Q_{\text{back}} x$ and we use $\phi_2 = 2\beta - \phi$ to express the angles. We have:

$$G = \frac{-n \sin(\beta - \phi_2) + n(1-T) \sin(\beta + \phi_2) + T \sin(\phi - \phi_2)}{n \cos(\beta - \phi_2) + n(1-T) \cos(\beta + \phi_2) - T \cos(\phi - \phi_2)} \quad (A3)$$

Using the sine and cosine laws, we rewrite:

$$G = \frac{2n \cos(\beta) \sin(\phi_2) - nT [\sin(\beta) \cos(\phi_2) + \cos(\beta) \sin(\phi_2)] + T \sin(\phi) \cos(\phi_2) - T \cos(\phi) \sin(\phi_2)}{2n \cos(\beta) \cos(\phi_2) + nT [\cos(\beta) \cos(\phi_2) - \sin(\phi) \sin(\phi_2)] - T \cos(\phi) \cos(\phi_2) + T \sin(\phi) \sin(\phi_2)} \quad (A4)$$

and using the Snell’s law, A4 reduce to $G = \sin(\phi_2) / \cos(\phi_2)$. Again, $\arctan(G) = \phi_2 = 2\beta - \phi$. The positive sign meaning the stress is in $+y$ direction and $+x$ direction for $0 < \phi < \pi/2$. Thus, the local scattering force is perpendicular to the spherical refraction surface.
Appendix B

This matlab code was built to calculate the stress distribution on the surface of RBCs for the case of the optical stretcher.

```matlab
%Calculate the normalized load (Q) applied on the surface of the cells
%---------------------------------------------------------------
% Name: load.m
% Date: 15/5/2006
% Autor: Paul Brûlé Bareil
% % Required files: None
% 
% n1     Buffer refraction index
% n2     Cells refraction index
% R      Cell radius
% NA     Numerical aperture of the optical fibers
% d      Distance from a fiber end to the center of the trap.
% angle  Polar angle where the stress is calculated
% #---------------------------------------------------------------

function Q=load(n1,n2,R,NA,d,angle)

% Dummy variable
%---------------
dd=0;            % Dummy variable
bb=0;            % Dummy variable
g=0;             % Needed for code startup
#---------------------------------------------------------------

% Experimental values reformating
%---------------------------------
n=n2/n1;         % Ratio of the refraction index
kk=angle;        % Reformating the angle definition
#---------------------------------------------------------------

% Gaussian Beam value
%---------------------
w=d*NA/n1;        % Beam width at trap center
wrm=w/R;          % Normalized beam width
#---------------------------------------------------------------

% CODE START UP
%---------------------------------------------------------------
```

%Solving for the polar angle on the first surface \( x \), that will give ... 
%an incidence at the polar angle named \( \text{ANGLE} \) at the second surface
\[
f=@(x)2\times\text{asin}(n1\times\text{sin}(\text{atan}(R\times\text{sin}(x)/(d-R\times\text{cos}(x)))+x)/n2)-x-\text{angle};
g = \text{fzero}(f,kk); \quad \%\text{Becomes polar angle of second ray}
\]

if \( g \geq 0 \)

\[
a=\text{angle}; \quad \%\text{Polar angle}
delta=\text{atan}(R\times\text{sin}(a)/(d-R\times\text{cos}(a))); \quad \%\text{Angle from x-axis}
a1=\delta+a; \quad \%\text{Incidence angle}
deltag=\text{atan}(R\times\text{sin}(g)/(d-R\times\text{cos}(g))); \quad \%\text{Angle from x-axis of second ray}
\]

%%Two rays coming from other side?
\[
g2=\text{fzero}(f,\pi/2); \quad \%\text{Polar angle of third ray}
deltag2=\text{atan}(R\times\text{sin}(g2)/(d-R\times\text{cos}(g2))); \quad \%\text{Angle from x-axis of third ray}
\]

\[
b=\text{asin}(n1\times\text{sin}(a)/n2); \quad \%\text{Beta angle}
\]

%Reflection and transmission coefficients calculation
\[
\text{R1pe}=(n1\times\text{cos}(ai)-n2\times\text{cos}(b))^2/(n2\times\text{cos}(b)+n1\times\text{cos}(ai))^2; \\
\text{R1pa}=(n2\times\text{cos}(ai)-n1\times\text{cos}(b))^2/(n2\times\text{cos}(ai)+n1\times\text{cos}(b))^2; \\
\text{R1}=(\text{R1pe}+\text{R1pa})/2; \\
\text{Tpe}=1-\text{R1pe}; \\
\text{Tpa}=1-\text{R1pa}; \\
\text{T1}=(\text{Tpe}+\text{Tpa})/2; \\
\]

%Normalized stress in the x and y direction for the ray touching... 
%the first surface
\[
\text{Qapar}=(\text{cos}(\delta)-n\times\text{T1}\times\text{cos}(a-b)+\text{R1}\times\text{cos}(2\times ai-\delta)); \\
\text{Qaper}=(\text{sin}(\delta)+n\times\text{T1}\times\text{sin}(a-b)-\text{R1}\times\text{sin}(2\times ai-\delta)); \\
\]

\[
b2=\text{asin}(n1\times\text{sin}(g+deltag)/n2); \quad \%\text{Beta angle of second ray}
b22=\text{asin}(n1\times\text{sin}(g2+deltag2)/n2); \quad \%\text{Beta angle of third ray}
\]

%Reflection and transmission coefficients calculation of second ray
\[
\text{R2pe}=(n2\times\text{cos}(b2)-n1\times\text{cos}(g+deltag))^2/(n1\times\text{cos}(g+deltag)+n2\times\text{cos}(b2))^2; \\
\text{R2pa}=(n1\times\text{cos}(b2)-n2\times\text{cos}(g+deltag))^2/(n1\times\text{cos}(b2)+n2\times\text{cos}(g+deltag))^2; \\
\text{R2}=(\text{R2pe}+\text{R2pa})/2; \\
\text{T2pe}=1-\text{R2pe}; \\
\text{T2pa}=1-\text{R2pa}; \\
\text{T2}=(\text{T2pe}+\text{T2pa})/2; \\
\]

%Reflection and transmission coefficients calculation of third ray
\[
\text{R3pe}=(n2\times\text{cos}(b22)-n1\times\text{cos}(g2+deltag2))^2/(n1\times\text{cos}(g2+deltag2)+n2\times\text{cos}(b22))^2; \\
R3pa = (n1*\cos(b22) - n2*\cos(g2+deltag2)^2)/(n1*\cos(b22) + n2*\cos(g2+deltag2))^2; 
R3 = (R3pe+R3pa)/2; 
T3pe = 1-R3pe; 
T3pa = 1-R3pa; 
T3 = (T3pe+T3pa)/2; 

%Normalized stress in the x and y direction for the ray(s) touching %the second surface %----------------------------------------------- 
Qarpar = T2*(n*cos(g-b2) + n*R2*cos(3*b2-g) - T2*cos(2*g+deltag-2*b2)); 
Qarpar2 = T3*(n*cos(g2-b22) + n*R3*cos(3*b22-g2) - T3*cos(2*g2+deltag2-2*b22)); 
Qarper = T2*(-n*sin(g-b2) + n*R2*sin(3*b2-g) + T2*sin(2*g+deltag-2*b2)); 
Qarper2 = T3*(-n*sin(g2-b22) + n*R3*sin(3*b22-g2) + T3*sin(2*g2+deltag2-2*b22)); 

%Value of the stress due to the second, third and first ray %respectively %----------------------------------------------- 
ar = exp(-2*sin(g)^2/wrm^2)*sqrt(Qarper^2+Qarpar^2); 
ar2 = exp(-2*sin(g2)^2/wrm^2)*sqrt(Qarper2^2+Qarpar2^2); 
av = exp(-2*sin(a)^2/wrm^2)*sqrt(Qaper^2+Qapar^2); 

%This section determines is there is only, one, two or three rays %applying a stress at the point defined by ANGLE. %----------------------------------------------- 
if g2<0 
ar2 = 0; 
end 
if g2==g 
ar2 = 0; 
end 
if g2>pi/2 
ar2 = 0; 
end 
if g2+deltag2>pi/2 
ar2 = 0; 
end 
if g<0; 
ar = 0; 
end 
if g>pi/2; 
ar = 0; 
end 
if g+deltag>pi/2; 
ar = 0; 
end 

%-------------------------------------------------------------------- 
%ANWSER%-------------------------------------------------------------------- 
Q = ar+av+ar2; 
%--------------------------------------------------------------------
% Vector form for further analysis
%--------------------------------
if g>=0
    bb=bb+1;
    resultat(bb,2)=Q;
    resultat(bb,1)=a;
end

% If first part of code is not able to solve for a value of g>=0, it
% means that Matlab is not searching at the right interval. This part
% of code does the same thing but assures that an answer for g is
% always found for any value of ANGLE.
else

    c=angle;                           % Polar angle
    delta=atan(R*sin(c)/(d-R*cos(c)))); % Angle from x-axis
    ci=delta+c;                        % Incident angle

    % Solving for the polar angle on the first surface x,
    % that will give an incidence at the polar angle named ANGLE at the
    % second surface
    %--------------------------------
    f=@(x)2*asin(n1*sin(atan(R*sin(x)/(d-R*cos(x))) +x)/n2)-x-c;  %
    g = fzero(f,1.3);
    deltag=atan(R*sin(g)/(d-R*cos(g))); % Angle from x-axis of second ray
    g2=fzero(f,pi/2-c);                 % Angle from x-axis
    deltag2=atan(R*sin(g2)/(d-R*cos(g2))); % Angle from x-axis of third ray
    b=asin(n1*sin(ci)/n2);              % Beta angle

    % Reflection and transmission coefficients calculation of first ray
    %--------------------------------
    R1pe=(n1*cos(ci)-n2*cos(b))^2/(n2*cos(b)+n1*cos(ci))^2;
    R1pa=(n2*cos(ci)-n1*cos(b))^2/(n1*cos(ci)+n2*cos(b))^2;
    R1=(R1pe+R1pa)/2;
    Tpe=1-R1pe;
    Tpa=1-R1pa;
    T1=(Tpe+Tpa)/2;

    % Normalized stress in the x and y direction for the ray touching...
    % the first surface
    %--------------------------------
    Qapar=(cos(delta)-n*T1*cos(c-b)+R1*cos(2*ci-delta));
    Qaper=(sin(delta)+n*T1*sin(c-b)-R1*sin(2*ci-delta));
    b2=asin(n1*sin(g+deltag)/n2);       % Beta angle of second ray
    b22=asin(n1*sin(g2+deltag2)/n2);    % Beta angle of third ray

    % Reflection and transmission coefficients calculation of second ray
    %--------------------------------
    R2pe=(n2*cos(b2)-n1*cos(g+deltag))^2/(n1*cos(g+deltag)+n2*cos(b2))^2;
    R2pa=(n1*cos(b2)-n2*cos(g+deltag))^2/(n1*cos(b2)+n2*cos(g+deltag))^2;
    R2=(R2pe+R2pa)/2;
\[ T_{2pe} = 1 - R_{2pe}; \]
\[ T_{2pa} = 1 - R_{2pa}; \]
\[ T_2 = \frac{(T_{2pe} + T_{2pa})}{2}; \]

% Reflection and transmission coefficients calculation of third ray
%---------------------------------------------------------------
\[ R_{3pe} = \frac{(n_2 \cos(b_{22}) - n_1 \cos(g_2 + \Delta g_2))^2}{(n_1 \cos(g_2 + \Delta g_2) + n_2 \cos(b_{22}))^2}; \]
\[ R_{3pa} = \frac{(n_1 \cos(b_{22}) - n_2 \cos(g_2 + \Delta g_2))^2}{(n_1 \cos(b_{22}) + n_2 \cos(g_2 + \Delta g_2))^2}; \]
\[ R_3 = \frac{(R_{3pe} + R_{3pa})}{2}; \]
\[ T_{3pe} = 1 - R_{3pe}; \]
\[ T_{3pa} = 1 - R_{3pa}; \]
\[ T_3 = \frac{(T_{3pe} + T_{3pa})}{2}; \]

% Normalized stress in the x and y direction for the ray(s) touching the second surface
%----------------------------------------------------------------
\[ Q_{arpar} = T_2 \left( n \cos(g - b_2) + n R_2 \cos(3b_2 - g) - T_2 \cos(2g + \Delta g - 2b_2) \right); \]
\[ Q_{arpar} = T_3 \left( n \cos(g_2 - b_{22}) + n R_3 \cos(3b_{22} - g_2) - T_3 \cos(2g_2 + \Delta g_2 - 2b_{22}) \right); \]
\[ Q_{arper} = T_2 \left( -n \sin(g - b_2) + n R_2 \sin(3b_2 - g) + T_2 \sin(2g + \Delta g - 2b_2) \right); \]
\[ Q_{arper} = T_3 \left( -n \sin(g_2 - b_{22}) + n R_3 \sin(3b_{22} - g_2) + T_3 \sin(2g_2 + \Delta g_2 - 2b_{22}) \right); \]

% Value of the stress due to the second, third and first ray respectively
%----------------------------------------------------------------
\[ a_r = \exp(-2 \sin(g)^2/w^2) \sqrt{Q_{arper}^2 + Q_{arpar}^2}; \]
\[ a_{r2} = \exp(-2 \sin(g_2)^2/w^2) \sqrt{Q_{arper_2}^2 + Q_{arpar_2}^2}; \]
\[ a_v = \exp(-2 \sin(c)^2/w^2) \sqrt{Q_{aper}^2 + Q_{apar}^2}; \]

% This section determines is there is only, one, two or three rays applying
% a stress at the point defined by ANGLE.
%----------------------------------------------------------------
if \( g_2 < 0 \)
    \( a_{r2} = 0; \)
end
if \( g_2 > \pi/2 \)
    \( a_{r2} = 0; \)
end
if \( g_2 = g \)
    \( a_{r2} = 0; \)
end
if \( g_2 + \Delta g_2 > \pi/2 \)
    \( a_{r2} = 0; \)
end
if \( g < 0 \)
    \( a_r = 0; \)
end
if \( g > \pi/2 \)
    \( a_r = 0; \)
end
if \( g + \Delta g > \pi/2 \)
    \( a_r = 0; \)
end
if ci>pi/2
  av=0;
end
%--------------------------------------------------------------------
%ANWSER-----------------------------------------------------------------
Q=ar+av+ar2;
%--------------------------------------------------------------------
%--------------------------------------------------------------------
end
%///////////////////////////////////////////////////////////////////////
%                           END
%///////////////////////////////////////////////////////////////////////
Appendix C

We present here a Matlab code built to calculate the deformation of the RBCs trapped in the optical stretcher according to the stress distribution calculated on Appendix B. This code is the most general method to use as it directly solve by numerical calculations the differential equations. First, the differential equations are defined in the function named `EQDgeneral`.

```matlab
% Generate the two differential equations to solve
%-------------------------------------------------
% Name:    EQDgeneral.m
% Date:    15/12/2006
% Author:  Paul Brûlé Bareil
%
% Required files: None
%
% t     Angle phi
% y     Vector representing both N1=[Y(xx,1)] and U=[Y(xx,2)]
%-------------------------------------------------
function dy=EQDgeneral(t,y)

% Experimental values reformating
%-------------------------------
Eh=1;       %Value of Young Modulus for purpose of calculation
P=1;        %Value of power for purpose of calculation
% We need to multiply by P and divide by Eh to get the total elongation

d=0.5*150e-6;   %Distance from fiber end to trap center
n1=1.335;       %Buffer index
n2=1.378;       %Cells index
NA=0.11;        %Numerical aperture
R=3.3e-6;       %Cells radius
w=d*NA/n1;      %Gaussian beam width
wr=w/R;         %Normalized gaussian beam width

I=2*P/(pi*(R*wr)^2);  %Intensity value
S=n1*I/3e8;
v=0.5;                  %Poisson coefficient

% CODE START UP

dy = zeros(2,1);
```
\%Values of radius of curvature
R1=@(t)R;
R2=@(t)R;
R21=@(t)0;
N2=@(t)(-R2(t)*-S*load(n1,n2,R,NA,d,t)-y(1));
e1=@(t)((y(1)-v*N2(t))/Eh); \%Epsilon one
e2=@(t)((N2(t)-v*y(1))/Eh); \%Epsilon two

dy(1)=(2*y(1)+R1(t)*-S*load(n1,n2,R,NA,d,t))*(-cot(t))-y(1)*R21(t)/R2(t);
\%First differential equation
dy(2)=( R1(t)*e1(t)-R2(t)*e2(t)+y(2)*cot(t) ); \%Second differential equation
\%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
\%                           END
\%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%

Then the following code will get the value of the slopes for the long axis and the short axis.

%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
\%Calculate the value of the slope of deformation in the case of the optical stretcher
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
\%    Name:    solutionEQDgeneral.m
\%    Date:    15/12/2006
\%    Autor:   Paul Brûlé Bareil
\%    Required files: EQDgeneral.m
\%
\%n1     Buffer refraction index
\%n2     Cells refraction index
\%R     Cell radius
\%NA     Numerical aperture of the lens
\%D     Distance from center of cell
\%t     Angle phi
\%teta  Angle teta
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%

\%Initialisation
%-----------------
clear
clc

%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
\%Experimental values reformatting
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
Eh=1; \%Value of Young Modulus for purpose of calculation
P=1; \%Value of power for purpose of calculation
\%We need to multiply by P and divise by Eh to get the total elongation
d=0.5*150e-6;   %Distance from fiber end to trap center
n1=1.335;       %Buffer index
n2=1.378;       %Cells index
NA=0.11;        %Numerical aperture
R=3.3e-6;       %Cells radius
w=d*NA/n1;      %Gaussian beam width
wr=w/R;         %Normalized gaussian beam width
I=2*P/(pi*(R*wr)^2);    %Intensity value
S=n1*I/3e8;     %Poisson coefficient

% Numerical solution of the differential equations
%------------------------------------------------
options = odeset('RelTol',3e-14,'AbsTol',[1e-12 1e-12]);
[T,Y] = ode45(@EQDgeneral,[0.00000000000000000001 pi/2], [0 0], options);

% Numerical treatment of the solutions
%------------------------------------
plot(T,Y(:,1),'-o')     %Plot the graph of N1
hold all
figure                  %Add a figure
plot(T,Y(:,2),'-o')     %Plot the graph of U

% Values of radius of curvature
%-----------------------------
R1=@(t)R;
R2=@(t)R;
pp=size(Y);         %Define the size of the Y vector

% Calculation of w(xx,1) for every value of phi.
%-----------------------------------------------
for xx=1:1:pp(1,1)
    N2=(-R*-S*load(n1,n2,R,NA,d,T(xx,1))-Y(xx,1));
    e2=(N2-v*Y(xx,1))/Eh;
    w(xx,1)=cot(T(xx,1)).*Y(xx,2)-R2(T(xx,1))*e2;
end

% ANSWER--------------------------------------------
w0=w(1,1)           %Long axis slope
w12=w(pp(1,1),1)    %Small axis slope
%
Appendix D

In this appendix, we describe the matlab code built to calculate the 3D stress distribution on the surface of RBCs for the case of the oscillating tweezers. First of all, the function named load_tetafunc, was created to calculate the variation of the stress as a function of the angle \( \psi \) as defined at Fig. 4.7.

```matlab
%########################################################################
%Calculate the normalized transverse load (Q) applied on the surface of the cells
%------------------------------------------------------------------------
%    Name:    load_tetafunc.m
%    Date:    15/01/2007
%    Autor:   Paul Brûlé Bareil
%
%    Required files: None
%
%n1     Buffer refraction index
%n2     Cells refraction index
%NA     Numerical aperture of the lens
%D      Scanning distance from center of cell
%gamma  Angle of the beam according to the optical axis
%########################################################################
function Sm=load_tetafunc(n1,n2,NA,D,gamma)

%Experimental values reformating
%-----------------------------
P=1;                %Power for purpose of calculation
DD=8.02e-6;         %Maximum diametre max of the cell
R=DD/2;             %Maximum radius of the cell
amax=asin(NA/n1);   %Maximum aperture angle
n=n2/n1;            %Index ratio

% CODE START UP

%Angle and object definition
%-----------------------------
alpha=0;            %Angle from scanning axis
ff=acos(D/R);       %Angle between D and R
a=R*sin(ff);        %Semi-major axis of Fig. 26.
BB=R/3;             %Semi-minor axis of the whole cell.
B=1/R*sqrt(BB^2*R^2-D^2*BB^2);%Semi-minor axis of Fig. 26.
```
%------------------First time for normalisation--------------------------
%------------------------------------------------------------------------
Ra=a*B/sqrt(a^2*sin(alpha)^2+B^2*cos(alpha)^2); %Radius of curvature
y=a*tan(alpha)/sqrt(1+a^2/B^2*tan(alpha)^2); %Value of y

%Value of x
%--------
if alpha~=0
    x=y/tan(alpha);
else
    x=a;
end

%Value of m (tangent to surface)
%-------------------------------
if y~=0
    dydx=-x*B^2/(a^2*y);
    m=abs(atan(dydx));
else
    m=pi/2;
end

%Value reformatting
%------------------
eta=pi/2-m;
delta=eta-alpha;
b=asin(n1*sin(delta)/n2); %Beta angle
w2=Ra*cos(a)*tan(amax); %Gaussian beam width

%Reflection and trasmission coefficients calculation
%---------------------------------------------------
Rlpe=(n1*cos(delta)-n2*cos(b))^2/(n2*cos(b)+n1*cos(delta))^2;
Rlpa=(n2*cos(delta)-n1*cos(b))^2/(n2*cos(delta)+n1*cos(b))^2;
RR=(Rlpe+Rlpa)/2;
Tpe=1-Rlpe;
Tpa=1-Rlpa;
T=(Tpe+Tpa)/2;

%Normalized stress in the x and y direction
%------------------------------------------
Qfrontx=(-cos(delta)+n*T*cos(b+alpha)-RR*cos(alpha+2*delta));
Qfrontz=(-sin(delta)+n*T*sin(b+alpha)-RR*sin(alpha+2*delta));

I=2*P/(pi*(w2)^2); % Value of the intensity
S=n1*I/3e8; % Parameter S defined

%------------------Second time for normalisation------------------------

Q1=S*sqrt(Qfrontx^2+Qfrontz^2)*exp(-2*(Ra*sin(alpha))^2/w2^2);
alpha = abs(gamma); % Angle of the beam according to the optical axis

ff = acos(D/R); % Angle between D and R
a = R * sin(ff); % Semi-major axis of Fig. 26.
BB = R/3; % Semi-minor axis of the whole cell.
B = 1/R * sqrt(BB^2 + R^2 - D^2 + BB^2); % Semi-minor axis of Fig. 26.

Ra = a*B/sqrt(a^2*sin(alpha)^2+B^2*cos(alpha)^2); % Radius of curvature
y = a*tan(alpha)/sqrt(1+a^2/B^2*tan(alpha)^2); % Value of y

% Value of x
%-----
if alpha ~= 0
    x = y/tan(alpha);
else
    x = a;
end

% Value of m (tangent to surface)
%-------------------------------
if y ~= 0
    dydx = -x*B^2/(a^2*y);
    m = abs(atan(dydx));
else
    m = pi/2;
end

% Value reformatting
%------------------
eta = pi/2 - m;
delta = eta - alpha;

b = asin(n1*sin(delta)/n2); % Beta angle
w2 = Ra*cos(a)*tan(amax); % Gaussian beam width

% Reflection and transmission coefficients calculation
%------------------------------------------------------
R1pe = (n1*cos(delta) - n2*cos(b))^2/(n2*cos(b) + n1*cos(delta))^2;
R1pa = (n2*cos(delta) - n1*cos(b))^2/(n2*cos(delta) + n1*cos(b))^2;
RR = (R1pe + R1pa)/2;
Tpe = 1 - R1pe;
Tpa = 1 - R1pa;
T = (Tpe + Tpa)/2;

% Normalized stress in the x and y direction
%------------------------------------------
Qfrontx = (-cos(delta) + n*T*cos(b + alpha) - RR*cos(alpha + 2*delta));
Qfrontz = (-sin(delta) + n*T*sin(b + alpha) - RR*sin(alpha + 2*delta));

I = 2*P/(pi*(w2)^2); % Value of the intensity
S = n1*I/3e8; % Parameter S defined

%-----------------------------------------
% ANSWER----------------------------------
Then the function load3d was created to calculate the variation of the stress as a function of the angle $\phi$ as defined in Fig. 4.6.

```matlab
function Q=load3D(n1,n2,R,NA,D,t,teta)

%Experimental values reformatting
P=12e-3; %Power used in Watts
n=n2/n1; %Ratio of the refraction index
amax=asin(NA/n1); %Maximum angle
TT=t; %Angle reformatting

%Value of the angle between the optical axis and the ray.
if TT~=0
  a=atan((R*cos(TT)-D)/R/sin(TT));
else
  a=pi/2;
end
```
if abs(a) < amax;

% Angle definitions
%-----------------
gamma = pi/2 + a;
delta = asin(D*sin(gamma)/R);
phi = pi/2 - (delta + a);
b = asin(n1*sin(delta)/n2); % Beta Angle

w2 = R*sin(phi)*sin(amax); % Value of the Gaussian beam width

% Reflection and transmission coefficients calculation
%--------------------------------------------------
R1pe = (n1*cos(delta) - n2*cos(b))^2/(n2*cos(b) + n1*cos(delta))^2;
R1pa = (n2*cos(delta) - n1*cos(b))^2/(n2*cos(delta) + n1*cos(b))^2;
RR = (R1pe + R1pa)/2;
Tpe = 1 - R1pe;
Tpa = 1 - R1pa;
T = (Tpe + Tpa)/2;

% Normalized stress in the x and y direction
%------------------------------------------
Qfronty = (cos(delta + phi) - n*T*cos(b + phi) + RR*cos(phi - delta));
Qfrontx = (-sin(delta + phi) + n*T*sin(b + phi) - RR*sin(phi - delta));

I = 2*P/(pi*(w2)^2); % Value of the intensity
S = n1*I/3e8; % Parameter S defined

% ANWSER---------------------------------------------------------------
%---------------------------------------------------------------------
Q = S*sqrt(Qfronty^2 + Qfrontx^2)*exp(-2*(R*cos(phi) - D)^2/w2^2)*...
   load_tetafunc(n1, n2, NA, D, teta);
%---------------------------------------------------------------------
%---------------------------------------------------------------------
else
    Q = 0;
end

%///////////////////////////////////////////////////////////////////////
%                           END
%///////////////////////////////////////////////////////////////////////
Appendix E

We present here a matlab code built to calculate the deformation of the RBCs trapped in the oscillating tweezer experiment according to the stress distribution calculated in Appendix D. This code is the most general method to use as it directly solve by numerical calculations the differential equations. First, the differential equations are defined in the function named `EQD`.

```matlab
% Generate the two differential equations to solve
%------------------------------------------------
% Name: EQD.m
% Date: 25/02/2007
% Autor: Paul Brûlé Bareil
%
% t     Angle phi
% y     Vector representing both N1=[Y(:,1)./(R2(T(:,1)).*sin(T(:,1)).^2]
%      and U=[y(xx,2).*sin(T(:,1))]
%---------------------------------------------------------------
function dy=EQD(t,y)

% Experimental values reformating
%---------------------------------
Eh=1;       %Value of Young Modulus for purpose of calculation
P=1;        %Value of power for purpose of calculation
%We need to multiply by P and divise by Eh to get the total elongation
Eh=1;
P=1; %Il faut donc multiplier par P et diviser par Eh pour avoir l'elongation finale
n1=1.335;       %Buffer index
n2=1.378;       %Cells index
n=n2/n1;        %Index ratio
DD=8.02e-6;     %Maximum diametre of the cell
R=DD/2;         %Maximum radius of the cell
NA=1.2;         %Numerical aperture

% Discrete values of scanning distance
%---------------------------------------
D=3e-6;
D=0.87e-6;
D=1.23e-6;
D=1.59e-6;
D=1.95e-6;
D=2.31e-6;
D=2.67e-6;
```
D = 3.03e-6;
D = 3.39e-6;
D = 3.75e-6;

v = 0.5;

bb = 2.626/2*1e-6;    % Semi minor diameter of the whole cell
b = bb/2;             % Semi minor radius of the whole cell

% CODE START UP

cc = 0;    % Dummy variable

dy = zeros(2,1);

% Defines the radius of curvature
R1 = @(t) (-R.^2*cos(t).^2 + b.^2 - b.^2*cos(t).^2).^(3/2)/R./b;
R2 = @(t) (-R./b.*(R.^2*cos(t).^2 + b.^2 - b.^2*cos(t).^2).^(1/2));
R1R2 = @(t) ((R.^2*cos(t).^2 + b.^2 - b.^2*cos(t).^2).^2/b.^2);

% Defines the value of N2 and epsilon
N2 = @(t) (-R2(t)*load3D(n1,n2,R,NA,D,t,0) - R2(t)/R1(t)*y(1)/(R2(t).*sin(t).^2));
e1 = @(t) ((y(1)/(R2(t).*sin(t).^2)-v*N2(t))/Eh);
e2 = @(t) ((N2(t)-v*y(1)/(R2(t).*sin(t).^2))/Eh);

% Defines the two differential equations

dy(1) = -R1R2(t).* sin(t).* load3D(n1,n2,R,NA,D,t,0).* cos(t);
dy(2) = (R1(t)*e1(t)-R2(t)*e2(t))/sin(t);

% Solve the two differential equations

Then the following code will get the value of the slope for the long axis deformation.
%Initialisation
%------------------
clear
clc
hold all

Experimental values reformatting
%----------------------------------
Eh=1;       %Value of Young Modulus for purpose of calculation
P=1;        %Value of power for purpose of calculation
%We need to multiply by P and divide by Eh to get the total elongation
Eh=1;
P=1; %Il faut donc multiplier par P et diviser par Eh pour avoir l'élongation finale

n1=1.335;       %Buffer index
n2=1.378;       %Cells index
n=n2/n1;        %Index ratio
DD=8.02e-6;     %Maximum diameter of the cell
R=DD/2;         %Maximum radius of the cell
NA=1.2;         %Numerical aperture

Discrete values of scanning distance
%-----------------------------------

D=3e-6;
D=0.87e-6;
D=1.23e-6;
D=1.59e-6;
D=1.95e-6;
D=2.31e-6;
D=2.67e-6;
D=3.03e-6;
D=3.39e-6;
D=3.75e-6;

v=0.5;              %Poisson ratio
bb=2.626/2*1e-6;    %Semi minor diameter of the whole cell
b=bb/2;             %Semi minor radius of the whole cell

Numerical solution of the differential equations
%N1=[Y(:,1)./(R2(T(:,1)).*sin(T(:,1)).^2) and U=[y(xx,2).*sin(T(:,1)]

options = odeset('RelTol',3e-14,'AbsTol',[1e-12 1e-12]);
[T,Y] = ode45(@EQD,[1e-30 pi/2],[0 0],options);

% N.B. U=0 at 0° and 90°
%Values of radius of curvature
%----------------------------
R1 = @(t)(-(R.^2*cos(t).^2+b.^2-b.^2*cos(t).^2).^(3/2)/R./b);
R2 = @(t)(-R./b.*(R.^2*cos(t).^2+b.^2-b.^2*cos(t).^2).^(1/2));

%Numerical treatment of the solutions
%-------------------------------------
plot(T,Y(:,2).*sin(T(:,1)),'-o')    %Plot the graph of U
hold all
figure                              %Add a figure
plot(T,Y(:,1)./(R2(T(:,1)).*sin(T(:,1)).^2),'-o') %Plot the graph of N1

pp=size(Y);         %Define the size of the Y vector
cc=0;               %Dummy variable

%Calculation of w(xx,1) for every value of phi.
%----------------------------------------------
for xx=1:1:pp(1,1)
  cc=cc+1;
  N2=(-R2(T(xx,1))*-load3D(n1,n2,R,NA,D,T(xx,1),0)-R2(T(xx,1))/R1(T(xx,1))*Y(xx,1)./(R2(T(xx,1)).*sin(T(xx,1)).^2));
  e2=(N2-v*Y(xx,1)./(R2(T(xx,1)).*sin(T(xx,1)).^2))/Eh;
  w(xx)=cos(T(xx,1))*Y(xx,2).*sin(T(xx,1))-R2(T(xx,1))*e2;
end

%ANSWER--------------------------------------------------------------
%---------------------------------------------------------------
w0=w(pp(1,1))  %Long axis slope
%---------------------------------------------------------------
%--------------------------------------------------------------------
Appendix F

We present here a matlab code built to calculate the coupling efficiency in the optical fibers in the case of the optical stretcher experiment. This code may need Appendix B part to run properly for any experimental parameters. The integral made uses Lobatto Quadrature.

%########################################################################
%Calculate the coupling coefficient of the optical fibers
%--------------------------------------------------------
%    Name:     couplage.m
%    Date:    19/07/2007
%    Autor:  Paul Brûlé Bareil
%%    Required files: wrr.m, wss.m, maximumpupil.m, w200.m, w400.m, wrr.m,
%    wss.m, R11.m, R33.m
%%    N.B. The rate of deformation as calculated by solutionEQDgeneral.m
% must be change in every files when the distance is modified.
%n1     Buffer refraction index
%n2     Cells refraction index
%R      Cell radius
%NA     Numerical aperture of the optical fibers
%d      Distance from a fiber end to the center of the trap.
%Eh     Young Modulus of elasticity
%lambda Wavelength used
%w0     Rate of deformation of the long axis per unit of Watts/[Eh]
%w12    Rate of deformation of the short axis per unit of Watts/[Eh]
%P      Power in mW
%
%########################################################################
%Initialisation
%----------
clear
clc
format long

%Experimental values reformatting
%--------------------------------
n1=1.335;
n2=1.378;
n=n2/n1;
R=3.3e-6;
Eh=2.2e-5;

NA=0.11;
lambda=1064e-9;
%d=75e-6;
%w0=-5.20e-11;
%w12=4.53e-11;
d=45e-6;
w0=-1.445379580796321e-010; %to be calculate with solutionEQDgeneral.m
w12=7.781568896298622e-011; %to be calculate with solutionEQDgeneral.m

% Dummy variable
%-------------------
c=0; %Dummy variable

% CODE START UP

for P=0:5:300    %Variation of power
t=(R-w0/Eh*P*0.001)*2; %Cell long axis
b=t/2; %Ellipse parameter b
a=(R-w12/Eh*P*0.001); %1/2small axis of the cell
amax=maximumpupil(P,d); %Maximum entrance pupil diameter
w00=3.3e-6; %Gaussian beam width at fiber exit
di=d-b; %Distance between the fiber and the cell entrance

% Integral calculation
%----------------------
F2=@(r)exp(-r.^2/wss(P,d).^2-r.^2/wrr(P,d).^2).*exp(-i*2*pi/lambda.*
  (w400(P,r,d)+w200(P,r,d)))*2*pi.*r;
int2=quadl(F2,0,amax);
c=cc+1; %Dummy variable

% ANSWER--------------------------------------------------------------
%--------------------------------------------------------------------
reponse2(cc)=norm(int2*2/wss(P,d)/pi/wrr(P,d)); %Value of coupling
%--------------------------------------------------------------------
%--------------------------------------------------------------------
power(cc)=P;
end
% Post processing
%------------------
subplot(1,2,1)
plot(power(:,),reponse(:)/reponse(1))
xlabel('Power mW')
ylabel('Normalized coupling coefficient')

subplot(1,2,2)
plot(power(:,),reponse(:))
xlabel('Power mW')
The next code part is to be named maximumpupil.m. It calculates the maximum entrance pupil.

```matlab
function [maximum]=maximumpupil(P,d)

%Initialisation
%-----------------
format long

%Experimental values reformating
%------------------------------
  n1=1.335;
n2=1.378;
n=n2/n1;
R=3.3e-6;
```

`ylabel('Coupling coefficient')`

The next code part is to be named maximumpupil.m. It calculates the maximum entrance pupil.
Eh=2.2e-5;
NA=0.11;
lambda=1064e-9;
w0=-1.44537958076321e-010; %to be calculate with solutionEQDgeneral.m
w12=7.78156886298622e-011; %to be calculate with solutionEQDgeneral.m
%**************************************************************************
% CODE START UP
%**************************************************************************
t=(R-w0/Eh*P*0.001)*2;  %Cell long axis
b=t/2;                  %Ellipse parameter b
a=(R-w12/Eh*P*0.001);   %1/2small axis of the cell
inc=1e-9;               %Minimum step size
critere=0;              %To start code (maximum incidence angle admissible)
y1=0.5e-7;              %Starting height
while critere<=pi/2     %Run while critere<=pi/2
     y1=y1+inc;      %Increase pupil height by step
     z1=-b.*sqrt(1-y1.^2/a.^2);   %Calculate the corresponding value of z1
     m=abs(atan(-a^2/b^2*z1/y1));  %Tangente w/r horizontal
     n=pi/2-m;                %Angle of the normal w/r horizontal
     teta=atan(y1/(d+z1));    %Exit angle of the optical fiber
     critere=teta+n;          %Maximum incidence angle admissible on the
                              %surface
     if  critere>=pi/2       %to verify if critere is ok
         y1=y1-inc;          %The answer is the one before
         z1=-b.*sqrt(1-y1.^2/a.^2);   %Calculate the corresponding value
                              %of z1
         m=abs(atan(-a^2/b^2*z1/y1));  %Tangente w/r horizontal
         n=pi/2-m;                %Angle of the normal w/r horizontal
         teta=atan(y1/(d+z1));    %Exit angle of the optical fiber
         %Solutionning for z2 to see if allright
         %------------------------------------------------------
         fun=@(x)-x+b.*sqrt((1-(y1+tan(asin(n1.*sin(teta+n)/n2)-n)).*(x-z1)).^2./a^2);
         %y2={y1-(asin(n1*sin(teta+n)/n2))-n}*(z2-z1));
         options=optimset('NonlEqnAlgorithm','lm','TolFun',...
                          1e-015,'TolX',1e-015,'MaxFunEvals',1000,'MaxIter',1000);
         [z2]=fsolve(fun,2e-6,options); %can add [] 'Display','iter'
         %Display if error
         %-----------------
         if imag(z2)~=0
             display('NOMBRE IMAGINAIRE POUR Z2')
         end
         %--------------------------------------
The next code part is to be named w200.m. It calculates the defocus aberration as a function of power, pupil diameter and distance from one optical fiber to the trap center.

```matlab
function [w20]=w200(P,r,d)

% Initialisation
% ------------------
format long

% Experimental values reformating
%---------------------------------

% Buffer refraction index
n1=1.335;

% Cells refraction index
n2=1.378;

% Cell radius
R=3.3e-6;

% Numerical aperture of the optical fibers
NA=

% Distance from a fiber end to the center of the trap.
d=d;

% Young Modulus of elasticity
Eh=

% Wavelength used
lambda=

% Rate of deformation of the long axis per unit of Watts/[Eh]
w0=

% Rate of deformation of the short axis per unit of Watts/[Eh]
w12=

% Power in mW
P=

% Distance from one optical fiber to the trap center.
d=

% This code needs maximumpupil.m, w400.m, w200.m, wss.m and wrr.m to run.

% Calculates the defocus aberration as a function of power, pupil diameter and distance
%------------------------------------------------------------------------
%    Name:     w200.m
%    Date:    19/07/2007
%    Autor:  Paul Brûlé Bareil
%    Required files: None
%    N.B. The rate of deformation as calculated by solutionEQDgeneral.m must be change in every files when the distance is modified.
% %n1 Buffer refraction index
%n2 Cells refraction index
%R Cell radius
%NA Numerical aperture of the optical fibers
d Distance from a fiber end to the center of the trap.
%Eh Young Modulus of elasticity
%lambda Wavelenght used
%w0 Rate of deformation of the long axis per unit of Watts/[Eh]
%w12 Rate of deformation of the short axis per unit of Watts/[Eh]
%P Power in mW
%N.B. This code needs maximumpupil.m, w400.m, w200.m, wss.m and wrr.m to run.
```
Eh=2.2e-5;
NA=0.11;
lambda=1064e-9;
w0=-1.445379580796321e-010; %to be calculate with solutionEQDgeneral.m
w12=7.781568896298622e-011; %to be calculate with solutionEQDgeneral.m

% CODE START UP

t=(R-w0/Eh*P*0.001)*2;  %Cell long axis  
b=t/2;                  %Ellipse parameter b  
a=(R-w12/Eh*P*0.001);   %1/2small axis of the cell

f=1/((n2-n1)*(2/R-(n2-n1)/(n2*R^2)*t)); %Focal length of thick lens

% w20 Calculation
h=t*f*(n2-n1)/n2/R; %Distance between membrane and principal plane

di=d-t/2;           %Distance between membrane and optical fiber

dii=h+di;           %Distance between principal plane and optical fiber

% ANSWER

w20=1/8.*(2.*r).^2.*(1/f-2/dii); %defocus

The next code part is to be named w400.m. It calculates the spherical aberration as a function of power, pupil diameter and distance from one optical fiber to the trap center.

% Calculates the spherical aberration as a function of power, pupil diameter and distance
% Name:    w400.m
% Date:    19/07/2007
% Autor:   Paul Brûlé Bareil
% Required files: None
% N.B. The rate of deformation as calculated by solutionEQDgeneral.m
% must be change in every files when the distance is modified.
%
%n1 Buffer refraction index
%n2 Cells refraction index
%R Cell radius
%NA Numerical aperture of the optical fibers
%d Distance from a fiber end to the center of the trap.
%Eh Young Modulus of elasticity
%lambda Wavelenght used
%w0 Rate of deformation of the long axis per unit of Watts/[Eh]
%w12 Rate of deformation of the short axis per unit of Watts/[Eh]
%P Power in mW

%N.B. This code needs maximumpupil.m, w400.m, w200.m, wss.m and wrr.m to
%run.

function [w40]=w400(P,r,d)

%Initialisation
%-----------------

%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
%Experimental values reformating
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
n1=1.335;
n2=1.378;
n=n2/n1;
R=3.3e-6;
Eh=2.2e-5;
NA=0.11;
lambda=1064e-9;
w0=-1.445379580796321e-010; %to be calculate with solutionEQDgeneral.m
w12=7.781568896298622e-011; %to be calculate with solutionEQDgeneral.m

%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
% CODE START UP
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
t=(R-w0/Eh*P*0.001)*2; %Cell long axis
b=t/2; %Ellipse parameter b
a=(R-w12/Eh*P*0.001); %1/2small axis of the cell

f=1/((n2-n1)*(2/R-(n2-n1)/(n2*R^2)*t)); %Focal lenght of thick lens

%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
%Paraxial image distance calculation
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
h=t*f*(n2-n1)/n2/R; %Distance between membrane and principal plane
di=d-t/2; %Distance between membrane and optical fiber
dii=h+di; %Distance between principal plane and optical fiber
Sol=di; %Object distance from first surface
S1=1/((n2-n1)/R/n2-n1/n2/Sol); %Image distance from first surface
S2=1/(((n2-n1)*2/R+n2/(S1-2*b)/S1-n1/Sol)/n1); %Image distance from second surface.

%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
%Calculation of \[OPO'\]
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
OPO=n1*(d-b+S2)+n2*(2*b);

%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
%Calculation of \[OQRO'\]
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
y1=r; %Pupil height
z1=-b.*sqrt(1-y1.^2/a.^2); %Calculate the corresponding value of z1
m=abs(atan(-a^2/b^2*z1/y1)); %Tangente w/r horizontal
n=pi/2-m; %Angle of the normal w/r horizontal
theta=atan(y1/(d+z1)); %Exit angle of the optical fiber

%Solutionning for z2 to calculate \(w40\)
------------------------------------
fun=@(x)-x+b.*sqrt(1-(y1+tan(asin(n1.*sin(theta+n2)/n2)-n).*(x-z1)).^2./a^2);
y2=(y1-(asin(n1*sin(theta+n2)/n2)-n)*(z2-z1));
options=optimset('NonlEqnAlgorithm','lm','TolFun',... 
1e-015,'TolX',1e-015,'MaxFunEvals',1000,'MaxIter',1000);
[z2]=fsolve(fun,2e-6,options); %can add \[
\] 'Display','iter'
if imag(z2)~=0
    display('NOMBRE IMAGINAIRE POUR Z2')
end

y2=a*sqrt(1-z2^2/b^2); %Calculate the corresponding value of y2

OQRO=n1.*sqrt((d+z1).^2+y1.^2)+n2.*sqrt((y1-y2).^2+(z1-z2).^2)+n1.*sqrt(y2.^2+(S2-(b-z2)).^2);

%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
%Aberration spherique \(W40\)
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
%ANSWER------------------------------------------------------------
%---------------------------------------------------------------
w40=OPO-OQRO;
%-------------------------------------------------------------------
%END
%-------------------------------------------------------------------

The next code part is to be named wrm.m. It calculates the Gaussian beam width of the receiving beam as a function of power, pupil diameter and distance from one optical fiber to the trap center.
function [wr]=wrr(P,d)

%Initialisation
-------------
format long

%Experimental values reformating
-----------------------------
n1=1.335;
n2=1.378;
n=n2/n1;
R=3.3e-6;
Eh=2.2e-5;

NA=0.11;
lambda=1064e-9;
w0=-1.445379580796321e-010; %to be calculate with solutionEQDgeneral.m
w12=7.781568896298622e-011; %to be calculate with solutionEQDgeneral.m

% CODE START UP

% t=(R-w0/Eh*P*0.001)*2; %Cell long axis
b=t/2; %Ellipse parameter b
a=(R-w12/Eh*P*0.001); %1/2small axis of the cell
f=1/((n2-n1)*(2/R-(n2-n1)/(n2*R^2)*t)); %Focal lenght of thick lens

%wr calculation

w00=3.3e-6; %Gaussian beam width at fiber exit
di=d-b; %Distance between the fiber and the cell entrance
wr=w00*sqrt(1+(lambda*di/pi/w00^2)^2); %Gaussian beam width at cell entrance
M=f/(f-di); %M factor due to the cell.
ws=M*wr; %Gaussian beam width at cell exit

The next code part is to be named wss.m. It calculates the Gaussian beam width of the source beam as a function of power, pupil diameter and distance from one optical fiber to the trap center.

%Calculates the width of the source field
%-----------------------------------------
%    Name:    wss.m
%    Date:    19/07/2007
%    Autor:  Paul Brûlé Bareil
%    Required files: None
%    N.B. The rate of deformation as calculated by solutionEQDgeneral.m must be change in every files when the distance is modified.
%    %
%    %n1  Buffer refraction index
%    %n2  Cells refraction index
%    %R  Cell radius
%    %NA  Numerical aperture of the optical fibers
%    %d  Distance from a fiber end to the center of the trap.
%    %Eh Young Modulus of elasticity
%    %lambda Wavelenght used
%    %w0  Rate of deformation of the long axis per unit of Watts/[Eh]
%    %w12 Rate of deformation of the short axis per unit of Watts/[Eh]
%    %P  Power in mW
%    %N.B. This code needs maximumpupil.m, w400.m, w200.m, wss.m and wrr.m to run.
%    %%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%

function [ws]=wss(P,d)

%Initialisation
%-------------
format long

%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
%Experimental values reformatting
%-----------------------------------
n1=1.335;
n2=1.378;
n=n2/n1;
R=3.3e-6;
Eh=2.2e-5;

NA=0.11;
lambda=1064e-9;
w0=-1.445379580796321e-010; %to be calculate with solutionEQDgeneral.m
w12=7.781568896298622e-011; %to be calculate with solutionEQDgeneral.m

%###########################################END###########################################

% CODE START UP

% t=(R-w0/Eh*P*0.001)*2; %Cell long axis
b=t/2; % Ellipse parameter b
a=(R-w12/Eh*P*0.001); % 1/2 small axis of the cell

f=1/( (n2-n1)*(2/R-(n2-n1)/(n2*R^2)*t)); % Focal length of thick lens

% wr calculation
w00=3.3e-6; % Gaussian beam width at fiber exit
di=d-b; % Distance between the fiber and the cell entrance
wr=w00*sqrt(1+(lambda*di/pi/w00^2)^2); % Gaussian beam width at cell entrance
M=f/(f-di); % M factor due to the cell.
ws=M*wr; % Gaussian beam width at cell exit

% END

%_fluttering_width
w=(w00^2+(lambda*di/pi/w00^2)^2)^(-1/2); % Fluttering width

% w00 calculation
w00=3.3e-6; % Gaussian beam width at fiber exit
di=d-b; % Distance between the fiber and the cell entrance
wr=w00*sqrt(1+(lambda*di/pi/w00^2)^2); % Gaussian beam width at cell entrance
M=f/(f-di); % M factor due to the cell.
ws=M*wr; % Gaussian beam width at cell exit

% ///////////////////////////////////////////////////////////////////////////
% END
% ///////////////////////////////////////////////////////////////////////////