Scientific Research Publishing

Open Journal of Applied Sciences, 2025, 15(4), 1050-1057
https://www.scirp.org/journal/ojapps
ISSN Online: 2165-3925
ISSN Print: 2165-3917

Human Red Blood Cell Stretching Using Optical Tweezers

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How to cite this paper: Yale, P., N'guessan, A.A.-B., Konin, J.M.E., Kouacou, M.A. and Zoueu, J.T. (2025) Human Red Blood Cell Stretching Using Optical Tweezers. *Open Journal of Applied Sciences*, **15**, 1050-1057.
https://doi.org/10.4236/ojapps.2025.154073

Received: March 10, 2025
Accepted: April 20, 2025
Published: April 23, 2025

Abstract

Studies of the deformation characteristics of living cells can offer insights into the connections among mechanical state, chemical and biological responses and the onset, progression of diseases. Deformation imposed by optical tweezers provides a useful means for the study of single cell mechanics under a variety of well-controlled stress-states. In this paper, the mechanics of the human red blood cell (erythrocyte) are subjected to deformation by optical tweezers. We then present new experimental and elastic properties of human red blood cells using a trapped silica bead in optical tweezers. The mean values of the properties obtained, in particular the elastic stiffness and the shear stiffness, were Eh = (10.82 ± 0.24) μN/m and Gh = (4.10 ± 0.89) μN/m. These results show that this approach to stretch the red blood cell can be used as an over method for RBC study.

Keywords

Optical Tweezers, Human Red Blood Cells, Stretch, Shear Modulus

1. Introduction

The red blood cell is an essential component of the blood, and its presence has an impact on its circulation. At between 6 and 8 μm in size, and generally biconcave in shape, the red blood cell must be flexible enough to pass through smaller, constricted capillaries [1] [2]. This is what enables it to perform its main function of transporting oxygen and carbon dioxide via the bloodstream. The elasticity of the red blood cell is provided by its membrane, and can be used as an indicator to test whether blood is suitable for transfusion to patients, or to give an idea of its phys-

iological state. Certain diseases, such as malaria, cancer and diabetes, can cause alterations in red cell elasticity. The characterization of red blood cells is therefore of the utmost importance. With the advent of technological innovations in the field of nano manipulation, optical microscopy using optical tweezers offers many solutions. It behaves like a trap, and is capable of capturing and non-invasively manipulating a single living cell [3]-[5].

High sensitivity, simple control of laser light intensity to adjust the force applied with precision down to piconewtons, and no risk of damage are the advantages of using optical tweezers (OT). Since then, a great deal of work has been carried out. The use of a scanning optical tweezer system incorporating an acousto-optic deflector (AOD) has made it possible to study the mechanical properties of the red blood cell from the stretching point of view. This was made possible by two microbeads bonded diametrically to the red blood cell. During this procedure, one of the beads remained stuck to the bottom of the sample thanks to non-specific adhesion, and the other was trapped and driven by the optical trap along a predefined trajectory [6]. A double trap was used to trap two silica microbeads attached diametrically to the cell. In this case, the cell is stretched by applying forces to the two microbeads [7]. Despite the vast amount of work that has been done, some aspects of these studies may not be well elucidated. For this reason, it is important to pursue the development of other approaches to address them. In the work we have carried out, we laterally stretched the red blood cell circulating freely in the fluid using a single trapped microbead adhering to its membrane.

2. Materials and Methods

2.1. Experimental Setup

In this section, we describe in detail the equipment and controls used for the experiment. **Figure 1** shows the experimental setup. The setup is based on the modular Thorlabs OT kit (OTKB, Thorlabs Inc., Newton, NJ, USA). The OT setup consisted of a Diode Laser (PL980P330J) at a wavelength of 980 nm with an output power of up to 330 mW. A large numerical aperture (NA 1.25) Nikon 100× oil immersion objective (MRP01902, Nikon, Tokyo, Japan) was used to focus a laser beam and form an optical trap. A white light-emitting diode (LED) source was mounted above the optical trap in order to illuminate a sample with light in the visible part of the electromagnetic spectrum. The forward-scattered light transmitted from the sample was collected by a Nikon 10× air condenser. The sample was mounted on a 3-axis piezo translation stage (MAX301) with strain gauge feedback. The particles used in these experiments were silica beads with diameters of 4.5 μm (Bangs Laboratories, Inc., Fishers, IN, USA) and human RBCs. In order to prepare a sample for our experiment, 0.5 μl of blood was immediately suspended into 5 ml phosphate-buffered saline (PBS) and this solution was incubated with beads concentration of 4.5 μm diameter. The images of the RBCs and silica beads were captured using a CCD camera and recorded on a videotape. The video images were then downloaded onto a computer and digitized for image analysis. The

individual frames of the recorded movies were analyzed using the Image-J software (version 1.43, National Institutes of Health, Bethesda, MD, USA).

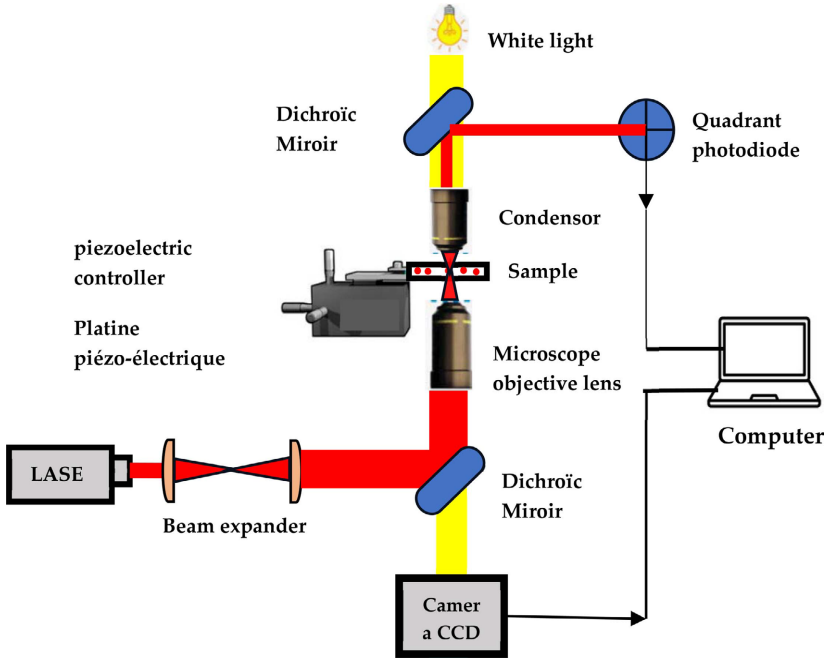

Figure 1. Scheme of the experimental setup.

2.2. Method and Force Calibration

The experimental approach is shown in Figure 2. The microbead and the biological cell circulate freely in the biological medium. A single-beam trap is used to trap the microbead. The stage is then moved horizontally to create contact between the microbead and the biological cell. Once contact has been made, we switch off the laser to release the bead from the optical trap, so that adhesion between the two is effective. A few minutes later, the stage is moved horizontally in the direction we want to separate the red blood cell from the trapped microbead. This causes the red cell to stretch each time the sample holder is moved.

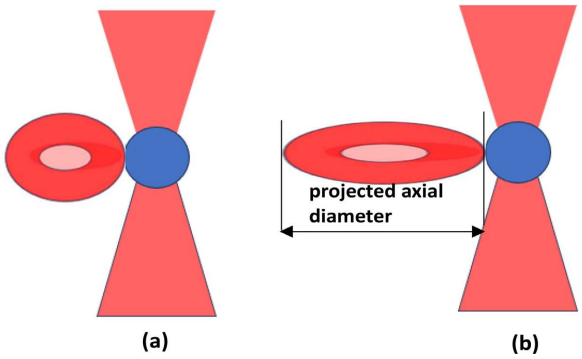

Figure 2. Illustration of an optical trap method for cell stretching: (a) Trapping of the microbead in contact with the red blood cell; (b) Stretching of the red blood cell.

Another parameter needed to calculate elasticity is the cell's stretching force. The microbead initially in contact with the cell is trapped in a stable equilibrium position. As a stable equilibrium corresponds to a potential well, the Boltzmann statistics method can be used to calculate the induced forces. At equilibrium, the potential well of the trapped microbead is reconstructed with a potential written as: $U_1 = k_1(\Delta r_1)^2 / 2$. The displacement of the cell, favoring its stretching will then disturb the first equilibrium position of the trapped microbead. The trapped microbead will have a new equilibrium position in this new phase, with a potential well: $U_1' = \frac{k_1'(\Delta r_1')^2}{2}$ with k_1, k_1' et r_1, r_1' are respectively the trap stiffness and the position of the trapped bead before and after the stretching. Since it is the trapped bead that we can master, then the energy communicated to the trapped bead by the RBC can be written [8]:

$$U = U_1 - U_1' = \frac{k_1(\Delta r_1)^2}{2} - \frac{k_1'(\Delta r_1')^2}{2} \tag{1}$$

As the force derives from the potential: $F_s = gradU$, the stretching force can be written according to [8]:

Video analysis is used to locate the silica microbead center of mass for each frame, and reconstructs the silica bead path. Image-J software has been used to reconstruct the xyz-path of the silica microbead from a video recording of its Brownian movement. There are several independent methods that have been used to determine the trapping force based on the Brownian motion of a trapped particle [9] [10]. However, in this work we used the Boltzmann statistics to obtain the trap stiffness. Using Boltzmann statistics, the optical potential reconstruction can be used to calculate any continuous trapping landscape in the accessible region by thermal agitation [10]. In equilibrium, the probability density $p(x)$ of the 1D particle position is given by

$$p(x)dx = Ce^{\frac{-E(x)}{K_B T}} \tag{2}$$

with C a normalization constant and $E(x)$ the trap potential. The shape of $E(x)$ can be determined from the normalized histogram of the trapped bead positions as

$$\begin{aligned} E(x) &= \frac{1}{2}k_{trap}(x)^2 = -k_B T \ln\left(p(x)\right) \\ k_{trap} &= \frac{-2k_B T}{x(t)^2}\ln\left(p(x)\right). \end{aligned} \tag{3}$$

In the case of the commonly used TEM_{00} Gaussian trapping beam, which results in a harmonic trapping potential, one can fit a parabola $y = ax^2 + b$ to the data in the central region of the potential to extract the trap stiffness and check for possible deviations from the perfect harmonic shape. The trap stiffness $k_{trap} = 2a/k_B T$ obtained in such manner is more accurate than (Equation (3)). Another advantage of such calibration is that it also gives the information about the potential in the region away from the trap center where the optical potential is non-harmonic [10].

2.3. Elastic Stiffness and Shear Stiffness Calculation

The deformation of the red blood cell is observed using a microscope attached to a CCD camera. In addition, the trapping force is measured from the displacement of the trapped microbead. The Young's modulus of the cell is evaluated using a Hertzian contact model. Assuming that the cell is a uniform sphere and that the microbeads are rigid, we can calculate the Young's modulus E by *S.* Sakuma *et al.* [11] as Equation (4):

$$E = \frac{3\left(1-\nu^2\right)}{D^2\sqrt{\varepsilon_x^3}} F_s, \varepsilon_x = \frac{D}{D_0} \tag{4}$$

with ε_x the deformation ratio. The shear modulus G is calculated taking into account the flexural modulus B, based on an axisymmetric theory valid for large deformations.

$$G = \sqrt{\frac{1}{125rB}\left(\frac{F_s}{\varepsilon}\right)^3} \tag{5}$$

where F_s is the applied force, r is the radius of the undeformed red blood cell, B is the flexural modulus, ε is the extension ratio expressed as

$$\varepsilon = \frac{D - D_0}{D_0} \tag{6}$$

where D_0 and D are the diameter of the undeformed red blood cell and the diameter of the deformed red blood cell, respectively. In the literature, values of B range from 1.8×10^{-19} to 7×10^{-19} N·m [12] [13]. The preferred value adopted by Evans is 2×10^{-19} N·m [14]. In this study, we used the fish coefficient $\nu = 0.43$ [15].

3. Results and Discussion

We used a silica bead with a diameter of 4.5 μm and a red blood cell with a diameter of 7.9 μm. This bead was trapped with a force of 11 pN. The stretching force applied is given for each variation in the contact diameter. The various images resulting from the videos recorded with each measurement are presented in **Figure 3**.

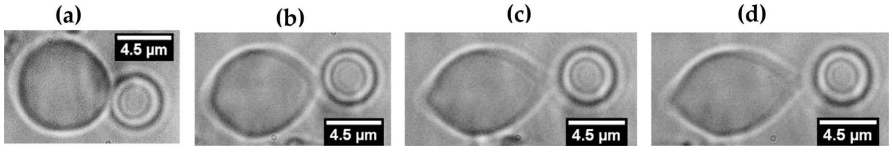

Figure 3. Images of the red cell being stretched from 0 pN to 5 pN: (a) 0 pN; (b) 3.89 pN; (c) 4.35 pN; (d) 5 pN.

Figure 3 shows a sequence of optical images revealing deformation response of the red blood cell at different stretching forces. At 5 pN, the axial diameter of the cell increases by 41.5%. The mean values of RBC properties obtained, in particular

the elastic stiffness and the shear stiffness, were E_h = 10.82 ± 0.20 μN/m and G_h = 4.10 ± 0.90 μN/m.

Table 1 presents all the measurements of the elasticity modulus and the shear modulus obtained from RBC.

Table 1. Mechanical properties values of the studied RBC.

Measures	Shear stiffness (μN/m)	Elastic stiffness (μN/m)
1	4.48	10.03
2	3.67	10.68
3	3.52	9.53
Mean	**4.10 ± 0.90**	**10.82 ± 0.20**

The red blood cell is responsible for regulating oxygen in the body. This function depends on its structure and the dynamics of its membrane. The deformability of the red blood cell influences its properties. Shear modulus and modulus of elasticity are intrinsic parameters for characterizing the elasticity of the red cell membrane [13]. The study of the mechanical properties of red blood cells using a single-beam optical tweezer has been developed. A microbead and a red blood cell circulating freely in the fluid were held in interaction. Contact between the two was maintained thanks to a strong adhesion. This strong adhesion may depend on the surface state of the membrane, which is related to the density of proteins adsorbed on the red cell membrane. Protein density increases with the age of the cell [14]. The mean value of the shear modulus is close to that obtained by the optical tweezer red blood cell stretching method using two beads adhering to the red cell membrane, which is (2.5 ± 0.4) μN/m [7] [15]. It falls within the range 4 < Gh < 10 μN/m obtained by the suction method in a micropipette [16] [17]. However, this value is lower than that obtained by Guck, J *et al.*, which is 13 μN/m [4]. Moreover, the shear modulus appears to be the most intrinsic parameter for characterizing the elasticity of the RBC membrane. Young's modulus, meanwhile, is lower than that obtained by Paul Brûlé Bareil [18], which is (20 ± 2) μN/m. However, it is of the same order as that obtained by Yalé *et al.*, which is 11.08 μN/m [19]. We explain this difference by the population selection of different RBCs according to their affinity with glass. Aspiration experiments with non-glass micropipettes do not function for membranes that adhere to micropipettes. Measurement is thus performed on RBCs that adhere to glass. In certain cases of OT, a category of RBC that has a great affinity with glass is used. With this technique, two beads of glass adhere spontaneously to the RBC membrane. This technique could circumvent some of the experimental difficulties associated with the use of two or more microbeads, or with studying the deformation characteristics of malaria-infected cells using micropipette aspiration, where cell rigidity and increased cell membrane adhesion to the glass surface can lead to significant uncertainty and scatter in experimental data.

4. Conclusion

We have demonstrated, both in this paper and in our recent works [20] [21], that optical tweezers can be used to investigate systematically the deformation characteristics of human blood cell in direct indentation or stretch. In this study, we used a simple setup to measure red cell deformation using a single trapped microbead. Red blood cells could be stretched with small forces (<10 pN). The hertz model was evaluated and used to calculate the mechanical properties of the red blood cell. The use of this stretching technique made it possible to manipulate the cell without it coming into contact with the laser. The optical tweezers are easy to set up and can thus help us to obtain the physical properties of red blood cells resulting from this type of stretching.

Author Contributions

P. Yale, J.-M. E. Konin, A. A.-B. N'guessan conceived and designed the experiments; P. Yale, J.-M. E. Konin, A. A.-B. N'guessan performed the experiments; P. Yale analyzed the data; P. Yale, J.-M. E. Konin, M. A. Kouacou and J. T. Zoueu wrote and revised the paper.

Funding

The research was financially supported by the International Science Programme (ISP) and The World Academy of Sciences (TWAS).

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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