

Red blood cell in the field of a beam of optical tweezers

P.B. Ermolinskiy, A.E. Lugovtsov, A.N. Semenov, A.V. Priezzhev

Abstract. We consider the effect of a tightly focused laser beam with a wavelength of 1064 nm and a power from 10 to 160 mW on red blood cells during their optical trapping with optical tweezers. It is found that the shape of a red blood cell, which alters after optical trapping, ceases to change when the trapping duration is less than 5 min and the laser beam power is less than 60 mW. At a beam power above 80 mW, the red blood cell begins to fold at a trapping duration of about 1 min, and at powers above 100–150 mW, the red blood cell membrane ruptures in 1–3 min after optical trapping. It is also found that with repeated short-term capture of a red blood cell in an optical trap, the deformation properties of the membrane change: it becomes more rigid. The obtained results are important both for understanding the mechanisms of interaction of a laser beam with red blood cells and for optimising the technique of optical experiments, especially for measuring the deformation properties of a membrane using optical tweezers.

Keywords: red blood cell, optical tweezers, optical trapping, Nd:YAG.

1. Introduction

Optical tweezers are an instrument or device that uses one or more tightly focused laser beams to trap microparticles, including living cells, with their subsequent manipulation in space and time [1, 2]. For the first time, the method of optical trapping of dielectric microparticles using optical tweezers was proposed by Arthur Ashkin in the 1970s [3]. In 2018, Arthur Ashkin was awarded the Nobel Prize in Physics for the invention of optical tweezers, which are widely used for studying biological systems [4]. Because the optical trapping and manipulation of microparticles is carried out without mechanical contact, optical tweezers have become an important tool for solving many physical and biological problems. For example, using optical tweezers at the level of single cells, spermatozoa [5], bacterial flagella [6], viruses [7], blood cells [8, 9] (including *in vivo* conditions [10]), and many other biological objects [1, 11] were studied. Optical tweezers are often

used together with other optical methods, for example, digital holography, phase-sensitive optical coherent microscopy [12] and Raman microscopy [13, 14]; sometimes femtosecond lasers are used for optical trapping, which make it possible to obtain a higher instantaneous trapping force than with cw lasers [15]. It is essential that optical traps are formed in the laser beam waist, into which microparticles can be trapped, and optical tweezers are an instrument with optical elements that allow an optical trap to be formed. In studying living cells using optical tweezers, it is critically important to take into account the effect of optical trapping on these cells (see, for example, [16]). In this work, we investigated the effect of red blood cell optical trapping by a beam of cw laser radiation with a wavelength of 1064 nm.

Erythrocytes are red blood cells that carry respiratory gases and metabolic products in organs and tissues and perform a number of other important functions for the body [17]. Red blood cells and their features have been studied and continue to be studied at the level of single cells using optical tweezers for their deformability [18–20], aggregation [21] and other properties [1]. There are a number of studies that consider the effect of optical trapping on a red blood cell and its properties. Thus, Zhu et al. [22] considered the thermal heating of a red blood cell in an optical trap, the authors of Refs [23, 24] studied the dynamics of the behaviour of a red blood cell in the field of a laser beam as a function the osmotic conditions of the environment, Kelley et al. [25] investigated the behaviour of a red blood cell in an optical trap at a high beam power, and works [26–30] examined the rotation of red blood cells in the field of a laser beam. However, to date, there is no complete understanding of how optical trapping affects the shape of a red blood cell, its internal contents (especially haemoglobin) and the elasticity of its membrane.

One of the results of the impact of optical trapping on the trapped living cells, including red blood cells, is their thermal heating [1]. In order to minimise the thermal effect of a laser on a cell, a wavelength of radiation is chosen at which its absorption by the cell is minimal. The minimum absorption of most living cells and tissues is in the near-IR range (600–1350 nm) [31]. If we focus on a red blood cell, then, since the share of haemoglobin accounts for about 95% of all cell proteins, the spectrum of its absorption mainly determines the thermal effect of an optical trap on the red blood cell. The absorption spectrum of haemoglobin, both in oxidised and nonoxidised form, shows that it is minimal in the near-IR range, where the absorption coefficient does not exceed units of cm^{-1} [32], which makes 800–1000-nm diode lasers or a 1064-nm Nd:YAG laser – the most widespread and widely used near-IR laser – very suitable for studying red blood cells using optical tweezers.

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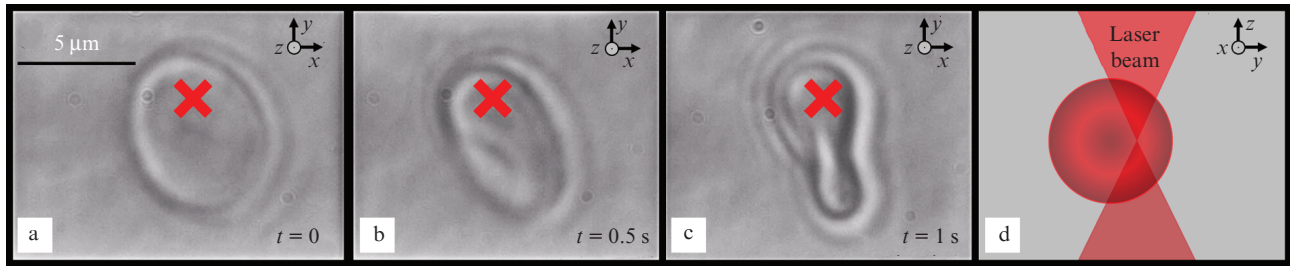


Figure 1. (Colour online) Process of the optical trapping of a red blood cell whose position is schematically shown with a red cross. (a) The red blood cell lies at the bottom of the cuvette at the time $t = 0$, (b) it begins to unfold and (c) is trapped in the optical trap ($t = 1$ s), as well as (d) a schematic image of the trapped red blood cell in a plane parallel to the incident beam. The radiation wavelength is 1064 nm, the power in the waist is 30 mW, and the radiation is linearly polarised.

The published-to-date results of some studies of the effect of an optical trap beam on red blood cells are somewhat contradictory. It was previously shown that for a laser radiation wavelength of 1064 nm at a radiation power of 55 mW in the waist of a focused Gaussian beam, there are no external changes in the red blood cell within 15 minutes after optical trapping: the shape of the red blood cell, which changes after optical trapping, does not undergo changes during this time [2]. It was also shown that the temperature difference inside the red blood cell and the environment at the same wavelength increases by about 1 °C for every 10 mW of radiation power [33]. Since most experiments on the optical trapping of red blood cells by optical tweezers are performed at room temperature, 20 °C, and their duration usually does not exceed several minutes, the red blood cell temperature in this case does not exceed the physiological temperature of 37 °C. It is important to note that these results are valid for a cw laser regime, and when a femtosecond laser is used, these ratios will change and will strongly depend on the average radiation power and pulse duration [34].

Using Raman spectroscopy, it was shown that the characteristic peaks in the Raman spectrum of haemoglobin do not change for 15 min at an incident radiation power of 20 mW at a wavelength of 1064 nm [35]. At the same time, the paper [36] argues that small changes in the Raman peaks can appear already 90 s after the trapping of a red blood cell even at a trap beam power of 5 mW; the reason for this is the haemoglobin denaturation and the formation of a photoproduct, which ultimately leads to photoinduced ‘haemoglobin aggregation’ inside the cell. Kelley et al. [25] demonstrated the possibility of ionisation of intracellular haemoglobin using optical trapping at a beam power of ~ 150 mW, which leads to the red blood cell ejection from the optical trap in a characteristic time of 10–20 s. In addition, it was shown that damage to the red blood cell cytoskeleton when it is heated to 50 °C leads to a decrease in membrane deformability [37].

Laser radiation also affects proteins located inside (under the membrane) of the red blood cell; the main protein that makes up the red blood cell is haemoglobin (95% of all proteins). Shirshin et al. [38] showed that irradiation of a red blood cell with a femtosecond (760 nm) or cw (375 nm) laser, even at low radiation powers, results in the formation of a photoproduct: haemoglobin changes its structure. To a certain extent, this should affect the viscosity of the intracellular contents and, consequently, the red blood cell deformability.

Optical trapping affects the red blood cell shape and orientation [39]. Upon trapping, the red blood cell, initially resting at the bottom of the cuvette, gradually turns its larger plane along the axis of the laser beam of the optical trap

(Fig. 1c) and takes an apparent pear-shaped form in about 1 s. In what follows, by the change in the red blood cell shape is meant the change in the already formed pear shape. The red blood cell, due to the incomplete symmetry of its shape, as a rule, is trapped in one optical trap not in the centre, but at its side (Fig. 1d), since the position of the optical trap in the centre of the cell corresponds to a nonequilibrium trapping state. Sometimes such manipulation is carried out in experiments using one or several microspheres attached to the membrane [40, 41]. In this case, the red blood cells are manipulated by moving the microspheres trapped in one optical trap. The disadvantage of this method is the presence of mechanical contact between the red blood cell membrane and microspheres, which can affect the properties of the red blood cell. Further in this work, we consider the trapping of a red blood cell by one or two optical traps without the use of microspheres.

Until now, the reason for the formation of a pear-shaped red blood cell in the optical trap remains not fully understood. Two assumptions can be made: either the red blood cell folds in the optical trapping field so that from above we see the projection of the red blood cell in this form [29], or part of the haemoglobin flows from one part of the cell to another until the optical trap forces and forces from the membrane balance each other. Perhaps both of these phenomena occur simultaneously. Numerical simulation [24] shows that when a red blood cell is trapped by two beams, it folds and deforms so that its sections near the laser beam waist ‘swell’, that is, take a pear-shaped form. It is important to note that in optical trapping, it is necessary to take into account the polarisation of light [1]. In the case of linearly polarised light, the red blood cell turns with its larger axis oriented along the vector of the electric field strength [29].

It is known that when a red blood cell is trapped by circularly polarised light [42] or when a cell with a pronounced inhomogeneity and asymmetry of shape (observed in echinocytes – a variety of red blood cells, whose surface is covered with asymmetric outgrowths) is trapped by linearly polarised light [23, 26], the trapped object rotates. Moreover, this rotation depends on the power of the incident light, its polarisation and the osmolarity of the medium [30].

The aim of this work is to study the effect of a tightly focused linearly polarised laser beam with a wavelength of 1064 nm and a power of 10 to 160 mW on trapped red blood cells outside the human body (*in vitro*).

2. Materials and methods

In the work, we used two different installations of optical tweezers and obtained identical results. A simplified scheme

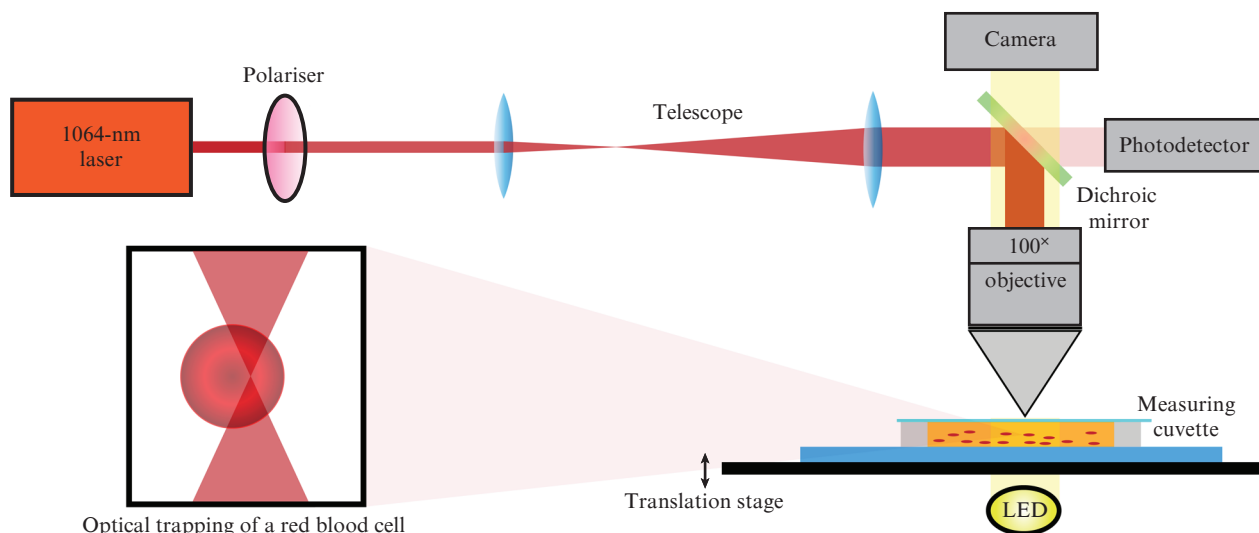


Figure 2. (Colour online) Simplified scheme of the optical tweezers used in the work. A Gaussian laser beam passes through a polariser, is expanded with a telescope, is incident on the lens and focused, and an optical trap is formed in the beam waist.

of the optical tweezers is shown in Fig. 2. The optical tweezers in question are based on diode-pumped cw Nd:YAG lasers generating TEM₀₀ mode radiation; the wavelength is 1064 nm, and the output power reaches 1 W. The power of the beams is controlled using polarisers. The error in determining the laser power is ± 1 mW, and the stability of its radiation in time after heating is ± 2 –3 mW.

The laser beam (Fig. 2) passes through a polariser, which controls the optical beam power, and then is expanded using a telescope. This makes it possible to increase the trapping efficiency, which depends on the intensity and gradient of the radiation intensity; the tighter the focusing of radiation, the greater the intensity gradient [43]. Then the beam reflected by a dichroic mirror is incident on the lens and is focused; the size of the beam waist is about a few micrometers, and it forms an optical trap.

Individual red blood cells were obtained by centrifugation of whole blood taken from the cubital vein of healthy volunteers who gave informed consent to participate as blood donors in the experiment. Ethylenediaminetetraacetic acid (EDTA) was used as an anticoagulant to prevent blood clotting. Whole blood was centrifuged at 180g for 10 min at room temperature; then, the upper layer of blood plasma with platelets and white cells was separated and centrifuged at 3000g for 10 min to obtain platelet-depleted plasma. Red blood cells sedimented at the bottom of the tube were separated from the surrounding fluid and diluted with platelet-depleted plasma or phosphate buffer solution (PBS) in a ratio of 1:1000. Then the suspension with red blood cells was placed in a measuring cuvette, consisting of a glass slide and a cover slip, separated by a gap of about 160 μm . Drying of the sample was prevented by sealing the cuvette with petroleum jelly. Red blood cells were trapped inside the measuring cuvette. The experiments were performed at room temperature (20–22°C) within 5 hours from the moment of blood sampling. The obtained numerical data were processed using the Origin 2018 program.

3. Results and discussion

Figure 3 shows micrographs of a red blood cell in the optical trap field at a radiation power in the beam waist from 10 to

157 mW (an intensity of 10^5 – 10^6 W cm⁻²) 30 s after the beginning of the trapping. The minimum radiation power at which optical trapping is possible is approximately 2 mW. At low powers (less than 10 mW), the asymmetry of the red blood cell shape is not observed even at a long trapping duration. With an increase in the trapping power (from 10 to 60 mW), the shape of the red blood cell captured in the optical trap becomes asymmetric, that is, pear-shaped (its transverse size increases in the place where the optical trap is located, as the beam power increases) and does not change for about 5 min after trapping. At a power above 80 mW, the shape of the red blood cell begins to differ from the pear-shaped in about 30 s from the moment of optical trapping. At radiation powers of more than 100–150 mW, the red blood cell membrane is damaged 1–3 min after trapping, and almost all intracellular haemoglobin flows out. It is important to note that in conventional measurements of the interaction of red blood cells with each other or measurements of the red blood cell deformability, the optical trapping time does not exceed 2–3 min at powers of no more than 30 mW [44]; therefore, the above-described changes in the red blood cell shape do not affect the results of such measurements.

Demonstration No. 1 [45] presents 5 times accelerated videos in which the incident radiation power was gradually increased from 10 to 160 mW for red blood cells in PBS and blood plasma. It was found that the diluting liquid (PBS or blood plasma) does not affect the shape of the red blood cells in the optical trap at a fixed power. It is also important to note that in demonstration No. 1 the red blood cell exhibited only an increase in the asymmetry of the pear-shaped form until the moment of membrane damage, while the folding of the red blood cell (shown in Fig. 3 at powers of more than 81 mW) was not observed, which is explained by the continuous monotonic increase in the radiation power over time, in the difference from the effect of power at a constant level for a certain period of time. It is also important to note that we did not observe the ejection of red blood cells from the optical trap at high powers (160 mW), described in paper [25].

Demonstration No. 2 [45] (20 times accelerated video) shows an example of a long-term presence of the red blood cell in the optical trap (40 min) at a fixed power of 40 mW (Fig. 4).

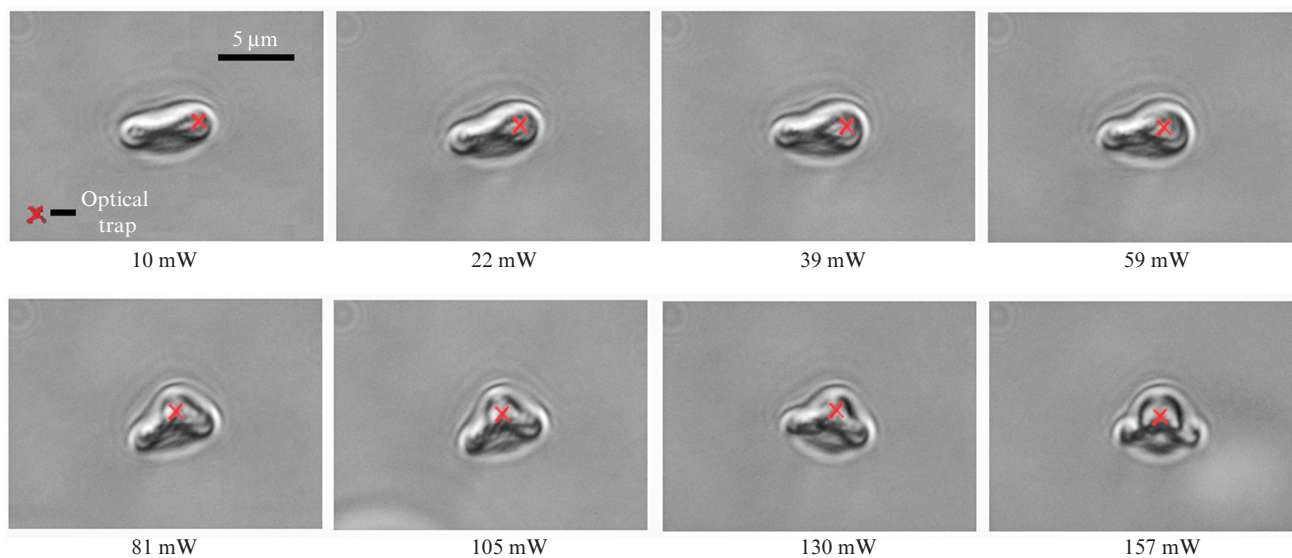


Figure 3. (Colour online) Change in the shape of a red blood cell in the optical trap field (its position is schematically shown by a red cross) 30 s after trapping with a variation in the radiation power in the waist from 10 to 157 mW. The radiation is linearly polarised.

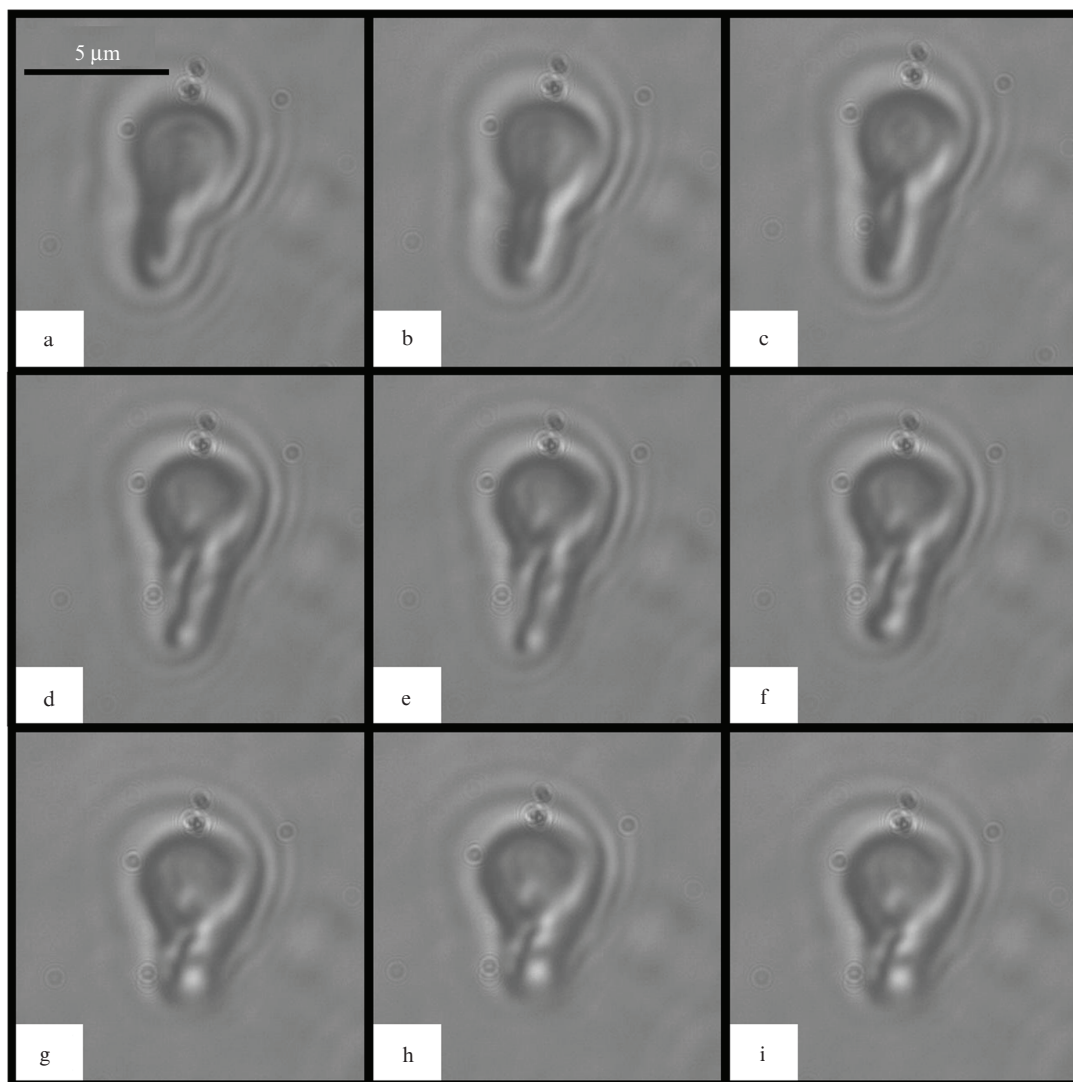


Figure 4. Change in the shape of the red blood cell in the optical trap at a fixed power of 40 mW and retention time of (a) 0, (b) 5, (c) 10, (d) 15, (e) 20, (f) 25, (g) 30, (h) 35 and (i) 40 min.

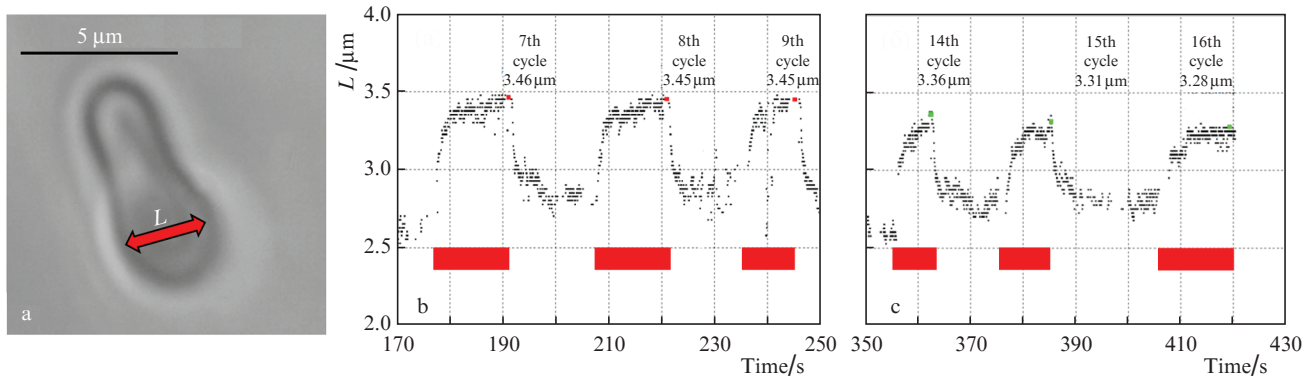


Figure 5. (Colour online) Red blood cell in the optical trap field with a power of 22 mW. (a) The red arrow shows the largest transverse size L in the trapping area, as well as (b–c) the change in L when the optical trap is switched on and off for a short time (the moments when the laser is turned on are indicated by red stripes) at (b) the 7th–9th cycles and (c) the 14th–16 cycles of the relaxation process of changes in the red blood cell shape.

One can see that, in this case, external changes begin to appear already 10 min after trapping (Figs 4a and 4c). Note that, at a radiation power of 22 mW, changes in the shape are not observed even after 20 min. With cyclic trapping (see below) and a radiation power of 22 mW, a change in the pear-shaped form is observed already at the 7th minute.

In order to study the process of relaxation of the shape of the red blood cell, we performed an experiment on its multiple short-term optical trapping. The red blood cell was trapped in the optical trap at a fixed radiation power of 22 mW and was retained for ~ 10 s until it acquired an equilibrium pear-shaped form. Then the laser beam was blocked, the red blood cell relaxed to its original symmetric shape, after which it re-entered the optical trap and changed its shape. This cyclical procedure was repeated several times in succession. The recorded video was processed using the Matlab program, and the largest transverse size of the red blood cell L (Fig. 5a) was measured during its change in time. This parameter characterises the red blood cell deformability as a function of the elasticity of its membrane. The dependences of the periodic change in the parameter L are presented for the 7th–9th cycles (Fig. 5b) and for the 14th–16th cycles (Fig. 5c) of the observed process of relaxation changes in the red blood cell shape. One can see that with time the amplitude of the change in L slowly, by approximately $0.2 \mu\text{m}$, decreases (the difference in the L values between the 16th and 7th cycles), which indicates an increase in the rigidity of the membrane upon its multiple deformation and relaxation.

Because the camera registers the image of the red blood cell trapped in the optical trap from the side (see Fig. 1), it becomes difficult to determine the cause of the formation of its observed pear-shaped form. To see the real change in the red blood cell shape by microscopy, we performed the following experiment. The red blood cell in PBS was captured in the optical trap formed at a constant power of 60 mW. Then it moved to the microfluidic channel, through which a flow of glutaraldehyde with a concentration of 0.02% was passed. When the red blood cell was placed in a glutaraldehyde medium, its membrane became rigid (fixed), which prevented its deformation [46]. Thus, the red blood cell was first trapped in the optical trap. Then its membrane was fixed in a pear-shaped state, and finally the optical trap was turned off and the red blood cell unfolding was observed (see demonstration No. 3 [45] and Fig. 6). It follows from the video and Fig. 6 that the change in the shape of the trapped red blood cell is accompanied by both its folding and simultaneously ‘swelling’ of the part that was in the optical trap, which is explained by the overflow of part of the haemoglobin into the optical trap area until the moment when the forces from both the trap and the deformed membrane balance each other.

4. Conclusions

A red blood cell is optically trapped by its side with a radiation power of less than 60 mW and takes a pear-shaped form. This shape does not change with a trapping duration of less

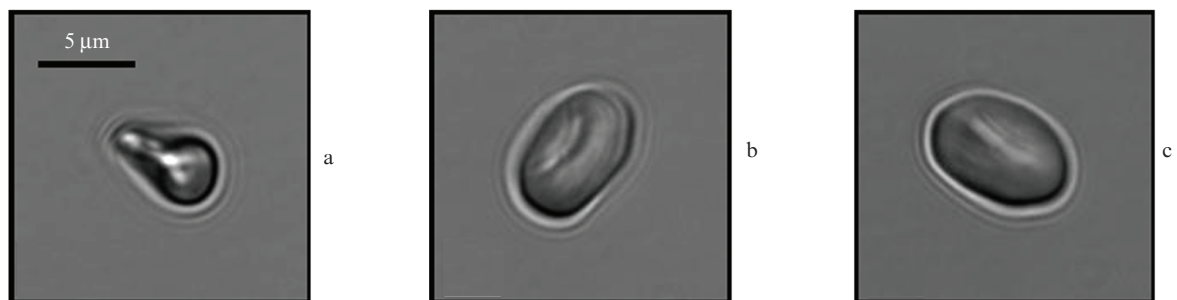


Figure 6. (a) Optically trapped red blood cell with a rigid membrane and (b, c) other projections of the red blood cell with a rigid membrane when the optical trap is turned off.

than 5 min. When the beam power exceeds 80 mW, the red blood cell begins to fold at a trapping duration of about 1 min. At radiation powers exceeding 100–150 mW, the red blood cell membrane is damaged 1–3 min after optical trapping. The deformation properties of the red blood cell change when the optical trap is periodically turned on/off even at low (22 mW) radiation power. The pear-shaped geometry of the red blood cell in the optical trap is caused by two processes: folding of the red blood cell and the overflow of part of the haemoglobin into the trapping area.

The obtained results are important for understanding the mechanisms of interaction of a laser beam with trapped red blood cells, as well as for optimising the technique for conducting optical experiments with them, especially for measuring the deformation properties of membranes.

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