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Measurement of interaction forces between red blood cells in aggregates by optical tweezers

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Abstract. **We have fabricated double-beam optical tweezers and demonstrated the possibility of their use for measuring the interaction forces between red blood cells (erythrocytes). It has been established experimentally that prolonged trapping of red blood cells in a tightly focused laser beam does not cause any visible changes in their shape or size. We have measured the interaction between red blood cells in the aggregate, deformed by optical tweezers.**

Keywords: *optical trap, optical tweezers, red blood cell, aggregate, interaction force between red blood cells.*

1. Introduction

A significant achievement of laser physics in the last decades is the creation of the optical tweezers (originally called 'single-beam gradient force trap') – a device that allows one to control the movement of small particles without mechanical contact with them. The action of optical tweezers is based on the phenomenon of light pressure, provided the prevalence of the gradient component of the force over the repulsive force. Such conditions arise, in particular, by focusing the laser beam. Single-beam gradient force trapping was demonstrated for the first time in the late twentieth century [1]. Experimental and theoretical aspects of optical trapping were discussed elsewhere (see, for example, [2–6]). It was shown that optical tweezers provide a highly accurate positioning of the particles ($\sim 10^{-10}$ m) and the possibility of measuring ultralow forces ($\sim 10^{-12}$ N). Currently, optical tweezers are widely used in various fields of science and technology, including biology and medicine.

Application of optical tweezers is also promising in such a field as blood rheology. In studies of human circulatory system, much attention is placed on microrheological parameters of blood, such as deformability and aggregation ability of red blood cells. It was established that these parameters significantly affect the blood microcirculation, and therefore have a direct bearing on the diagnostics and treatment of some diseases. To date, many aspects of the behavior of biological cells, including red blood cells, in an optical trap have been investigated. Khokhlova et al. [7] used double-beam optical tweezers to study features of the aggregation of red blood cells. They measured the interaction force between two red blood cells as a function of the distance between their centres. They also found that the rates of aggregation of red blood cells from healthy donors and patients suffering from various diseases differ significantly from each other. Papers [8–11] reported the observation of diffraction patterns upon scattering of the laser beam on individual red blood cells and other microscopic particles trapped by the optical trap. The optical trap in combination with the microflow technique allows automatic sorting of cells [12].

Optical tweezers make it possible to study the shape of the surface of red blood cells [13], as well as features of the interaction of cells in the process of coagulation [14]. Guck et al. [15] measured the deformability of a red blood cell using an optical trap with two counterpropagating beams. This trap does not require tight focusing of radiation; therefore, the power density of the radiation incident on the trapped particles is lower, which is important when working with living cells. Optical tweezers were used to measure the coefficient of elasticity of the red blood cell membrane [16], as well as the deformability of red blood cells by direct extension of cells [17]. In these experiments, the maximum tensile strength reached 400 pN. Ghosh et al. [18] observed a significant change in the shape of red blood cells, and their rotation under the action of the light field of the optical trap. The optical trap allows one to measure such parameters as elasticity and viscosity of red blood cell membranes [19], as well as the thickness of the double layer of the charge around the cells in the electrolytic solution [20]. In work [21] the optical trap was used to study the properties of the cytoskeleton of red blood cells. It was established [22, 23] that the orientation of the red blood cell in the optical trap depends on the laser beam polarisation and the ionic composition of the medium surrounding the cell. Bronkhorst et al. [24] used optical trapping to study the mechanisms of aggregation and disaggregation of red blood cells.

Nevertheless, there are still many fundamental problems of cell biophysics, which are difficult to solve without optical traps. These problems include, in particular, the problem of aggregation of red blood cells in norm and various pathological conditions. Currently, this issue is intensively studied by the methods of light scattering by suspensions of red blood cells [25]. However, the results obtained characterise large ensembles of particles, and their individual characteristics are hidden. Thus, it remains unclear why some cells do not par-

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ticipate in the formation of aggregates or lead to the formation of aggregates of specific forms, such as branched linear or lumpy aggregates. To answer this question we must be able to measure the interaction forces between individual cells and aggregates at rest and in flow.

The purpose of this paper is the fabrication of double-beam optical tweezers to study the interaction of red blood cells, in particular, to measure the interaction forces between red blood cells in the aggregate.

2. Experimental setup

To manipulate two or more red blood cells it is needed to create double-beam tweezers with independent control of each of the beams. The experimental setup is shown in Fig. 1. The radiation sources were a 1064-nm cw, TEM_{00}-mode, diode-pumped Nd : YAG laser with an output power of 300 mW, as well as a 810-nm semiconductor laser with an output power of 500 mW. The use of a dichroic mirror made it possible to direct both laser beams into a water immersion objective (Olympus LUMPlanFl 100 ×) with a numerical aperture NA = 1.00. The image formed by the objective was focused by a lens on the DCC1645C camera (Thorlabs). Overexposure to the laser light is cut off by the blue-green filter. The laser beams in the plane of the objective lens was controlled by rotating the telescope (a magnification of 1 : 3) in the arm of the semiconductor laser and the telescope (1 : 2) in the arm of the Nd : YAG laser. The centres of the telescopes are located in the plane, which is conjugate with the plane of the entrance aperture of the objective. Thus, at small rotational displacements, the beams remain within the entrance aperture of the objective.

3. Functional check of the system

To align the system and detect the region of optical trapping, use was made of red blood cells, placed in saline at a ratio of 1 : 500. The maximum output power after the objective was 55 mW for the Nd : YAG laser and 60 mW for the semiconductor laser. The minimum size of the focal spot (estimated on the surface of the cover glass with respect to the size of the test polystyrene microspheres) was 2.5 μm for the Nd : YAG-laser beam and 3.5 μm for the semiconductor laser beam. Therefore, the intensity of laser radiation in the beam focal waist was 1 MW cm^{-2}.

Red blood cell heating by the focused laser beam can be estimated by the formula $\Delta T = P\delta/(4\pi\chi)$, where ΔT is the red blood cell temperature increment; P is the laser beam power in the focal waist; δ is the light absorption coefficient by the particle material; χ is the thermal conductivity of water. Assuming P = 60 mW, δ = 800 m^{-1}, χ = 0.6 W m^{-1} K^{-1}, we obtain $\Delta T \approx 6$ K. This estimate demonstrates that the heating of a cell by laser radiation is in the physiological range. Our experiments showed that when a red blood cell was trapped in each of the beams of the optical tweezers for 15 min, we observed no visible changes in it. The corresponding images of red blood cells are presented in Fig. 2.

4. Calibration of the optical tweezers

To measure the interaction forces between red blood cells in the aggregate, we used the following technique. Using double-beam optical tweezers, the aggregate was stretched from two opposite end-sides. The power of one laser beam (from a semiconductor laser) remained constant, while that of the sec-

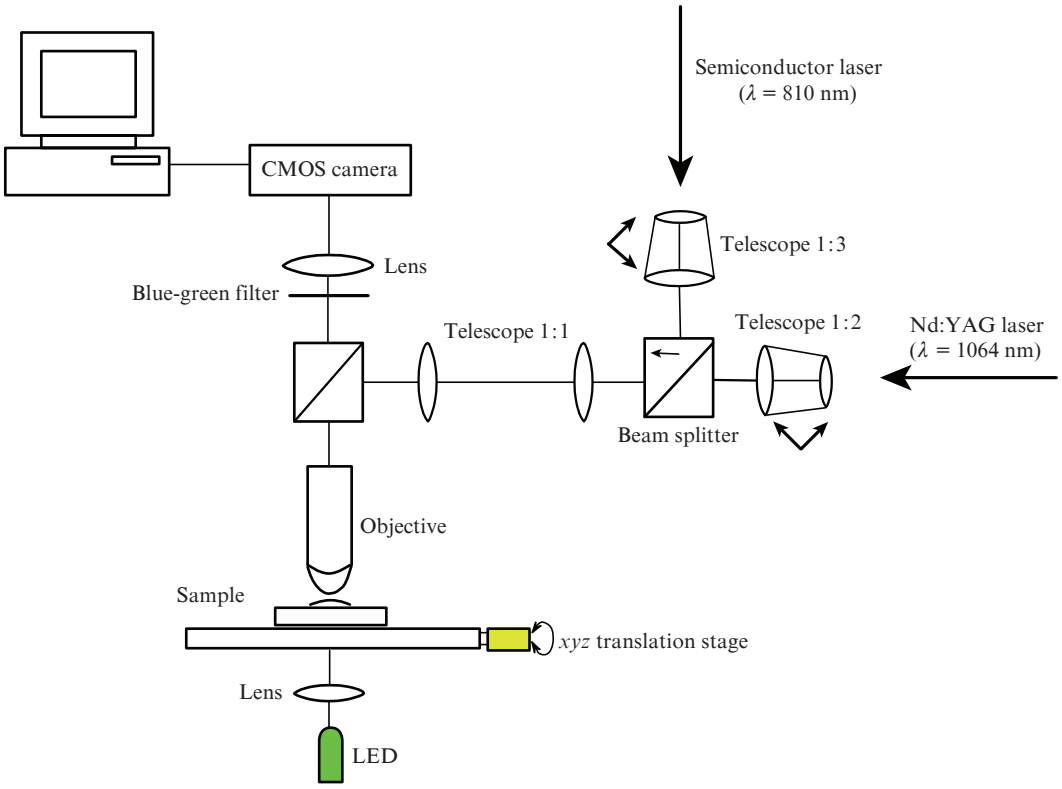

Figure 1. Schematic diagram of the double-beam optical tweezers.

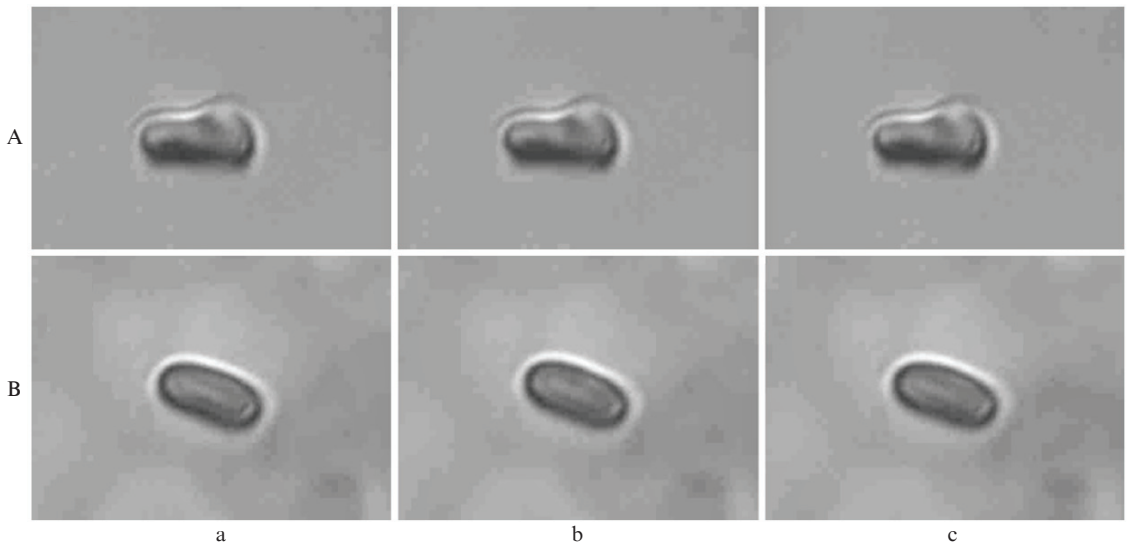

Figure 2. Images of the red blood cell immediately after trapping (a) and after 10-min (b) and 15-min (c) trapping. The images A correspond to a channel created by the Nd:YAG laser, B – by the semiconductor laser.

ond one (from the Nd:YAG laser) was decreased until the force of interaction between the cells exceeded the force of optical trapping and the end of the aggregate was pulled out of the trap. To perform quantitative measurements using optical tweezers, they must be calibrated.

Calibration was carried out by the method based on the use of viscous forces acting on the particle by the fluid flow. The fluid flowed due to the movement of the object table with the cuvette relative to the trapped red blood cell. The moment of rip-out of the red blood cell was recorded by a video camera. Then, by using the ImageJ code (http://rsbweb.nih.gov/ij/) we determined the rate of the fluid flow with the help of the video recording. After that, the force pulling the red blood cell out of the trap was calculated by Stokes' formula:

$$F_{\text{vis}} = \gamma v = 6\pi\eta r v,$$

where η is the coefficient of the fluid viscosity; r is the effective radius of the red blood cell (radius of the sphere whose volume equals the volume of the red blood cell); v is the velocity of the fluid flow.

The red blood cell suspension was prepared by adding a drop of whole blood into saline at a ratio 1:1000. Thus, the viscosity of the fluid, where red blood cells resided, was considered equal to the viscosity of water, for which η = 0.00101 Pa s. The effective radius r of the red blood cell was chosen to be 2.7 μm, which corresponds to the red blood cell volume $V = 82\ \mu\text{m}^3$. The fluid flow velocity v was determined by five to seven measurements with different red blood cells.

Thus, by measuring (at different laser beam powers) the fluid flow velocity, which pulls the particle out of the optical trap, we can obtain the dependence of optical trapping force on laser power. The dependence obtained in our experiments is shown in Fig. 3. Note that it is close to linear. The maximum force of the red blood cell trapping, which is equal to 20 pN, is reached at an Nd:YAG-laser beam power of 50 mW.

Particle trapping efficiency of the optical trap can be characterised by the coefficient Q, defined by the formula $F = Qn_{\text{m}}P/c$. Here, F is the trapping power; P is the power of the laser beam incident on the particle; n_{m} is the refractive index of the medium surrounding the particle; c is the speed of light in vacuum. Evaluation by this formula shows that in our experiments the red blood cell trapping efficiency is $Q \approx 0.09$.

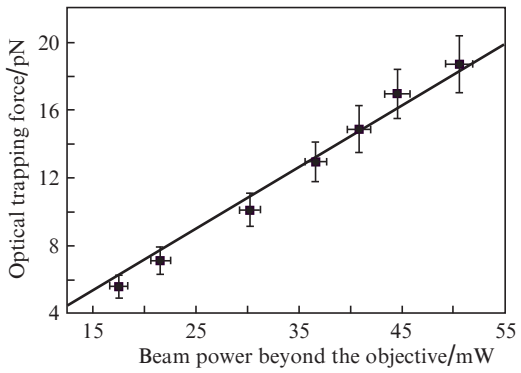

Figure 3. Experimentally measured dependence of the optical trapping force on the power of the laser beam incident on the red blood cell.

5. Measurement of interaction forces between red blood cells in the aggregate

To carry out the corresponding measurements, the red blood cell suspension was prepared in a special way. First, fresh whole blood was settled down for several hours until most of the red blood cells were settle. Then, using a micropipette we pumped out 1 mL of plasma from the top layer and added 5 μL of the settled red blood cells into it. Thus, the concentration of red blood cells was significantly reduced, making it possible to work with individual red blood cells and aggregates. After placing a drop of the sample on a glass slide we waited for 10–15 min, until all large particles (red blood cells, platelets, etc.) settle to the bottom of the cuvette. Otherwise, the work is complicated by the fact that foreign particles fall into the trap. In addition, during this time almost all the red cells merge into aggregates, and there are almost no cells in the free state (Fig. 4).

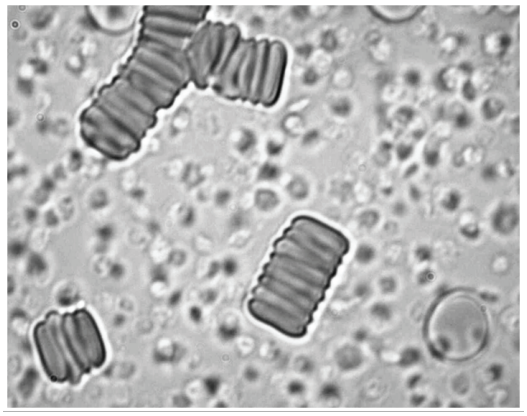

Figure 4. Images of the aggregates in the form of rouleaux and smaller particles (platelets) at the bottom of the cuvette.

The measurements were performed with linear aggregates consisting of several cells (2–9). First, using one trap the aggregate was raised from the bottom of the cuvette. Then, moving the sample stage, we made the fluid flow, which turned the aggregate in the horizontal plane, and the opposite end of the aggregate was captured by the second trap. After that, the laser beams were moved apart in opposite directions, which led to the destruction of the aggregate, i.e., disaggregation. All stages of the process are shown in Fig. 5.

In one of our experiments, a linear aggregate of red blood cells, the so-called rouleaux, was captured by the ends by the double-beam optical tweezers. Then, the laser beams were moved apart in opposite directions for some distance, which caused deformation (stretching) of the aggregate. After that, we started decreasing the Nd:YAG-laser beam power. At some power P of the beam, the corresponding end of the aggregate was pulled out of the trap. We drew a conclusion that at the given beam power, the optical trapping force F is equal to the interaction force between the red blood cells in the aggregate F_{int}.

The value of the force F can be determined using the calibration curve (Fig. 3). However, for a more accurate estimate, we should take into account that the physical characteristics of blood plasma are different from the physical characteristics of water. In this connection, we performed a separate calibration of the optical tweezers in the plasma. It was found that the rate of the plasma flow pulling the red blood cell out of the trap at the given laser beam power P is 96 ± 13 μm s^{-1}. The coefficient of the plasma viscosity was η = 0.001717 Pa s. Thus, by using Stokes' formula, we obtain F = 8.4 ± 1.1 pN. Therefore, the interaction force between red blood cells in the aggregate, deformed by means of the double-beam optical tweezers, is F_{int} = 8.4 ± 1.1 pN.

6. Conclusions

We have fabricated double-beam optical tweezers, suitable for measuring the force of interaction between red blood cells. The experiments have shown that the optical tweezers provide secure trapping of red blood cells and their aggregates. It has been also found that the trapping of a red blood cell in the optical trap for 15 min does not lead to any visible changes in it.

We have calibrated the optical tweezers by the method based on the use of viscous forces. We have obtained the experimental dependence of the optical trapping force on the laser beam power, which was close to linear. We have also demonstrated the possibility of measuring the interaction forces between red blood cells in the aggregate, deformed by the optical tweezers. The developed method can be used to assess the strength of linear aggregates of red blood cells under various conditions.

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References

1. Ashkin A., Dziedzic J.M., Bjorkholm J.E., Chu S. *Opt.Lett*., **11** (5) 288 (1986).
2. Neuman K.C., Block S.M. *Rev. Sci. Instr*., **75** (9), 2787 (2004).

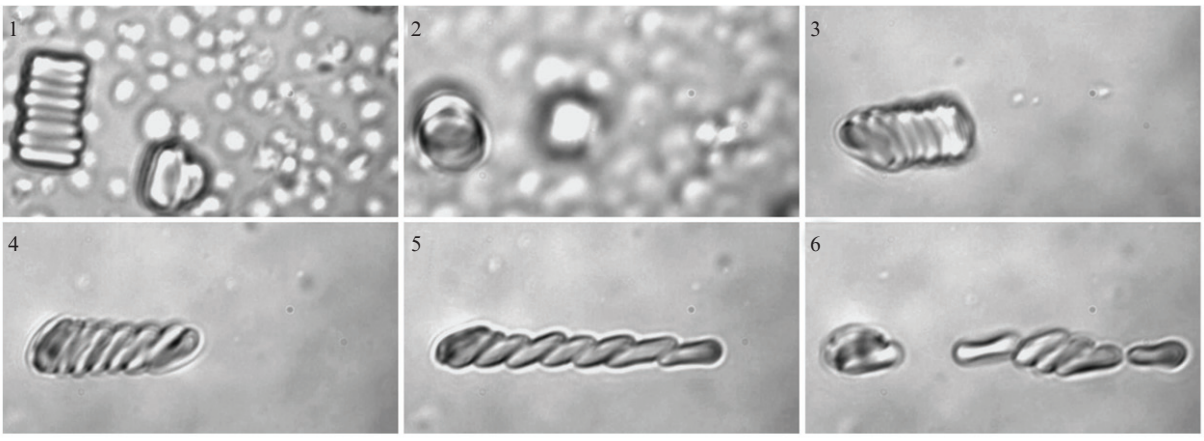

Figure 5. Stages of the process of measuring the interaction forces between red blood cells in an aggregate: 1 – the aggregate is captured with a single trap at the bottom of the cuvette; 2 – the aggregate is raised up from the bottom of the cuvette; 3 – the fluid flow rotates the aggregate in the horizontal plane; 4 – the aggregate is held by two traps by opposite ends; 5 – the laser beams are moved apart in opposite directions, the red blood cells begin to 'slide' from each other; 6 – the destruction of the aggregate (disaggregation).

3. Akhmanov S.A., Nikitin S.Yu. *Physical Optics* (Oxford: Clarendon, 1997; Moscow: Izd-vo MSU, 1998).
4. Grier D.G. *Nature*, **424**, 810 (2003).
5. Fedosov I.V. *Kogerentno-opticheskie metody v izmeritel'noi tekhnike i biofotonike* (Coherent Optical Methods in Measuring Techniques and Biophotonics) Ed. by V.P. Ryabukho and V.V. Tuchin (Saratov: Satellit, 2009) p. 59.
6. Nikitin S.Yu., Priezzhev A.V., Lugovtsov A.E., Maklygin A.Yu. *Journal 'Physics' of the Publishing House 'First of September'*, No. 2 (939), 53 (2012).
7. Khokhlova M.D., Lyubin E.V., Zhdanov A.G., Rykova S.Yu., Krasnova T.N., Sokolova I.A., Fedyanin A.A. *Proc. SPIE Int. Soc. Opt. Eng*., **7715**, 77150M (2010).
8. Berg M.J., Hill S.C., Videen G., Gurton K.P. *Opt. Express*, **18** (9), 9486 (2010).
9. Kinnunen M., Kauppila A., Karmenyan A., Myllyla R. *Biomed. Opt. Express*, **2** (7), 1803 (2011).
10. Collins M., Kauppila A., Karmenyan A., Gajewski L., Szewezyk K., Kinnunen M., Myllyla R. *Proc. SPIE Int. Soc. Opt. Eng*., **7376**, 7376 (2010).
11. Ramser K., Hanstorp D. *J. Biophotonics*, **3** (4), 187 (2010).
12. Bruns T., Becsi T., Talkenberg M., Wagner M., Weber P., Mescheder U., Schneckenburger H. *Proc. SPIE Int. Soc. Opt. Eng*., **7376**, 7376OM (2010).
13. Kumar R., Sarasvati S., Shakher C., Mehta D.S. *Proc. SPIE Int. Soc. Opt. Eng*., **7376**, 7376OG (2010).
14. Bor-Wen Yang, Zhe Li. *Biomed. Opt. Express*, **1** (4), 1217 (2010).
15. Guck J., Ananthakrishnan R., Mahmood H., Moon T.J., Cunningham C.C., Kas J. *Biophys. J*., **81**, 767 (2001).
16. He'non S., Lenormand G., Richert A., Gallet F. *Biophys. J*., **76**, 1145 (1999).
17. Lim C.T., Dao M., Suresh S., Sow C.H., Chew K.T. *Acta Materialia*, **52**, 1837 (2004).
18. Ghosh A., Supurna Sinha, Dharmadhikari J.A., Roy S., Dharmadhikari A.K., Samuel J., Sharma S., Mathur D. *Phys. Biol*., **3**, 67 (2006).
19. Huruta R.R., Barjas-Castro M.L., Saad S.T.O., Costa F.F. *Blood*, **92** (8), 2975 (1998).
20. Fontes A., Fernandes H.P., Thomaz A.A., Barbosa L.C., Barjas-Castro M.L., Cesar C.L. *J. Biomed. Opt*., **13** (1), 014001 (2008).
21. Guck J., Ananthakrishnan R., Cunningham C.C., Kas J. *J. Phys.: Condens. Matter*, **14** (19), 4843 (2002).
22. Dharmadhikari J.A., Mathur D. *Current Science*, **86** (10), 1432 (2004).
23. Khan M., Mohanty S.K., Sood A.K. *Pramana – J. Phys*., **65** (5), 777 (2005).
24. Bronkhorst P.J.H., Grimbergen J., Brakenhoff G.J., Heethaar R.M., Sixma J.J. *British J. Haematol*., **96**, 256 (1997).
25. Priezzhev A.V., Ryaboshapka O.M., Firsov N.N., Sirko I.V. *J. Biomed. Opt*., **4**, 76 (1999).