Capture and Retention of Polystyrene Microspheres with Biomarkers in Optical and Optothermal Traps

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Abstract. The capabilities of optical and optothermal tweezers for manipulating 2-μm polystyrene microspheres with kidney and thyroid antigens are experimentally demonstrated. The ability of arranging such objects in various configurations with subsequent fixation has been demonstrated. It has been shown that the detachment rates of modified polystyrene microspheres depend on the presence and type of antigen. Experiments on optical capture of polystyrene microspheres in a mixed solution of sodium tetraborate with the addition of sodium dodecyl sulfate (SDS) of different molar concentrations are carried out. It is shown that an increase in the molar concentration of SDS from 5 to 30 mM leads to a decrease in the detachment rate by 55%.

Keywords: optical tweezers; optothermal trap; laser manipulation; polymer microspheres; antigens; mixed solutions.

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1 Introduction

An optical trap (optical tweezers) is a method based on the use of the light power of a tightly focused laser beam to capture, hold and manipulate micro- and nanoscale objects [1–3]. In optothermal traps, a focused light field is used not only to generate optical power, but also to create a temperature gradient in the medium by absorbing laser radiation and, accordingly, convection currents that carry particles to the region of maximum radiation intensity [4–7]. Structured light fields significantly expand the functionality of both optical [2, 8–14] and optothermal [7, 15–19] traps. The methods are widely used in scientific research, as they allow precise manipulation of objects without physical contact, which minimizes the risk of damage. Micro- and nanoparticles of various natures, sizes, and morphologies are used as objects of manipulation: polymer, silicon, metal, microdroplets of organic solutions [20], as well as erythrocytes, leukocytes, DNA molecules, proteins, single cells, bacteria, etc. [3, 21–23]. Since the efficiency of capture depends significantly on the size of the objects, their shape, optical and physical-chemical properties, the parameters of the trap (power and wavelength of laser radiation, distributions of intensity and/or phase of the light field, topological charge of the trap) are selected for each specific case. For example, the possibility of capturing is determined by the ratio between the refractive indices of the micro-object and the surrounding medium; to capture absorbing objects, it is necessary to create light traps with a special shape, etc. Optothermal traps are less sensitive to the characteristics of the object, but even in this case, the dynamic properties of the micro-object in the trap (and, accordingly, the efficiency of capture) are determined by its characteristics and require separate investigation.

Polymer microspheres modified with biomarkers (antigens, proteins, etc.) are of great practical interest as a basis for test systems for the rapid and effective diagnosis of various diseases, detection of infections and viruses, and they are also used in various analysis methods and as biosensors [24–27]. Considering the wide range of applications for diagnostic and biotechnological tasks, both fundamental and applied, it is interesting to explore the capabilities of laser manipulation of the microobjects under consideration. Microspheres labeled and unlabeled with biomarkers are of interest from the point of view of optical manipulation as objects of the same size, practically indistinguishable externally, but with different physicochemical properties, as objects for sorting.

The aim of this article was to study the capabilities of an optical and optothermal trap for manipulating polystyrene microspheres with kidney and thyroid antigens. It was important to demonstrate the operability of schemes of an optical trap and our previously proposed optothermal traps [7, 16, 17] for these types of objects, as well as to test the sensitivity of optical tweezers to the objects under consideration i.e. to test the ability to distinguish between unmarked and marked with different antigens microspheres using the optical tweezers technique. The sensitivity of the optical tweezers was assessed by measuring the rate of detachment of the microsphere from the trap – the maximum rate of retention of the captured object in the trap. The parameter allows one to estimate the rigidity of a particular trap for capturing a specific object under given condition, and it is quite easy to measure. For the case of known characteristics of the surrounding solution (distilled water), the optical trapping forces were calculated. The detachment rates of polystyrene microspheres in a mixed solution of sodium tetraborate with the addition of sodium dodecyl sulfate (SDS) of different molar concentrations were also measured.

2 Materials and Methods

The micromanipulation samples were polystyrene microspheres (Alpha Nanotech Inc.) of 2 µm size, pure and with thyroid antigens or kidney antigens. The finished samples – microspheres with kidney antigens or thyroid antigens mixed with distilled water – were provided by the Analytical Research and Development Laboratory of Reaviz Medical University in sealed Eppendorf-type test tubes. To conduct experiments on optical manipulation, the sample tube was shaken to evenly distribute the microspheres in the liquid volume; 1 µl of the sample suspension, taken from the middle of the tube using a Thermo Fisher Scientific single-channel laboratory pipette, was mixed with 1 ml of distilled water and thoroughly mixed. Physiological saline (0.9% NaCl), physiological saline with sodium dodecyl sulfate, sodium tetraborate buffer solution ($Na_2B_4O_7$) and SDS solutions in sodium tetraborate with different molar concentrations of 5÷30 mM were also used as solutions.

The experiments were carried out using optical and optothermal trap techniques. A typical scheme of a structured optothermal trap is shown in Fig. 1. In the experiments, a diode-pumped solid-state laser (DPSS) was used. The radiation wavelength was 0.53 µm. The output power varied from 10 to 60 mW. The structured trap (a trap with a complex intensity distribution − in the form of a ring or a square contour) was formed by using a liquid crystal spatial light modulator (LC SLM) HOLOEYE PLUTO-2-NIR-011 (1080×1920 px, pixel size 8 µm, 256 grayscale, modulation depth 2π), operating in reflection mode. Phase masks supplied to the LC SLM were calculated using spiral beam optics [28]. An expanded collimated beam illuminated the LC SLM, and the formed structured beam was focused by a 90× microscope objective. Microparticles suspended in solutions cylindrical cuvette (diameter 30 mm, thickness 40 mm, liquid layer thickness – 1–1.5 mm) with an absorbing bottom: an orange OC 13 filter was used as the bottom. Due to the absorption of radiation with a wavelength of 0.53 µm, a temperature gradient and, accordingly, a convection flow arose.

The same circuit elements were used to form a point optical trap. The SLM could be switched off and used as a mirror. In our experiments, the phase distribution of the lens was set to the modulator and focusing was done through it. For experiments with optical tweezers, we used a cell with a transparent bottom (K8 glass).

In experiments on capturing and aligning up microspheres with antigens into a complex contour we have added albumin to the cell and increased the laser power to fix the formed structures. We have proposed and described a technique for fixing microobjects along the boundaries of a structured trap in Ref. [17].

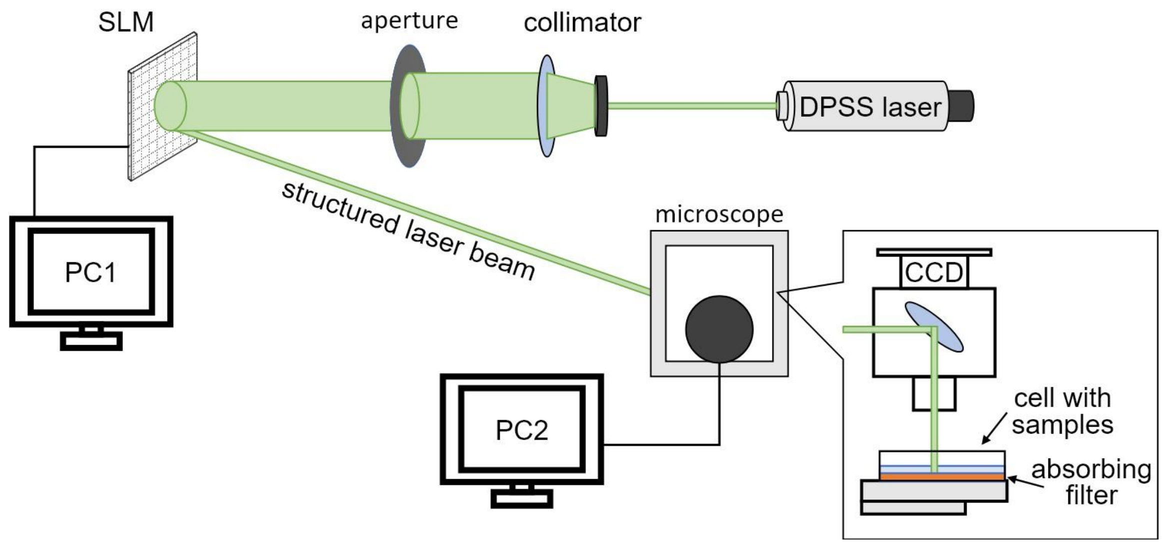
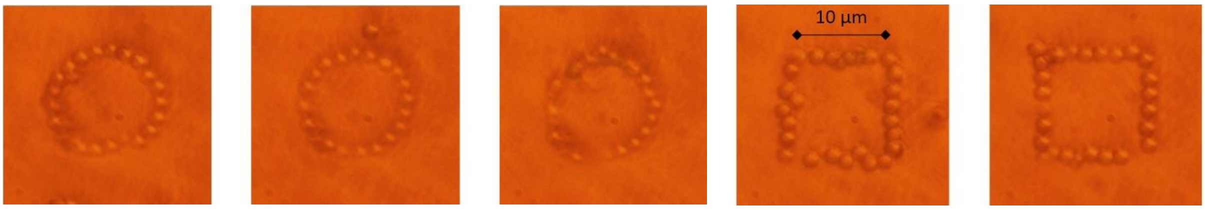


Fig. 1 The scheme of structured optothermal trap.



(a) (b) (c) (d) (e)

Fig. 2 Results of experiments on optothermal fixation of 2 µm latex microspheres with kidney cells immobilized on the surface in the form of a ring (a−c) and a square (d−e) due to albumin coagulation at a radiation power of 60 mW. In all cases (a−e), a uniform distribution with small differences can be observed.

3 Experiments and Discussion of Results

3.1 Experiments with an Optothermal Trap: Capture, Alignment along the Trap Boundary, and Subsequent Fixation

In experiments with an optothermal trap, the transfer of microparticles with kidney antigens by convection flows and their alignment along the trap boundary was recorded. The radiation power for capturing and moving microparticles was 20−30 mW. A 2.5% albumin solution was used for fixation. The particles actively moved towards the trap from all distances within the microscope's field of view and aligned fairly evenly along the ring or square's border. Fixation of microobjects on the substrate was achieved by a short-term increase in laser power to ~60 mW, which led to coagulation of albumin. Fig. 2 shows examples of frames from a video of microsphere fixation. It is evident that in all cases it is possible to evenly arrange and fix the microspheres along the trap boundary.

Microparticles with thyroid antigens could not be aligned along the trap boundary. This was due to the very slow movement of microparticles at laser powers of 10−30 mW. Increasing the power to 60 mW did not result in active particle movement. Further increasing the power under the given experimental conditions was not possible due to albumin coagulation.

In Refs. [16, 17], we have demonstrated the capture and alignment of latex microspheres of various sizes (2 and 4 µm) along a ring with a total laser radiation power in the working zone 27÷100 mW and a 40× magnification of the microobjective. In Refs. [7, 16], the radial and vertical components of the convection flow in point and ring optothermal traps were modeled depending on the laser radiation power. The results of the simulation, confirmed by the results of the experiments, showed that when working with an optothermal trap, it is crucial to adjust the power level of the laser light in a manner that ensures the particles are captured by the trap, but not carried upwards by the rising convective currents of the fluid. Although the ring-shaped optothermal trap is characterized by a less sharp increase in the vertical component of the flow velocity as it approaches the illuminated region compared to the point-shaped trap, this dependency is quite significant for latex particles. Therefore, the virtual absence of influence of laser radiation power on the behavior of microparticles with thyroid antigens most likely indicates a change in their adhesive properties.

3.2 Experiments with a Point Optical Trap: Determination of the Detachment Rates of Microspheres

Experiments were conducted to study the capture and motion of polystyrene microspheres and polystyrene particles with antigens using an optical trap. We determined the maximum velocities of movement (detachment rates) of these particles. The breakaway force method was used to estimate the maximum speed of motion. A cell with a drop of each suspension was mixed with 1 ml of distilled water and placed on the microscope stage. A separate microsphere was captured by a point optical trap (a focused beam of laser radiation) and moved in a straight line. The speed of movement was increased until the microparticle escaped out of the beam. Experiments were repeated at least 5 times with each microparticle, using at least 10 microspheres in each series.

The release of microparticles occurs at the moment when the capture force, which is dependent on the power of the laser radiation, light beam parameters and the size and relative refractive index of the microobject, equals the viscous friction force determined by the Stokes Eq. (1):

$$F_{drag} = 6\pi\eta rV, \quad (1)$$

where r is the radius of the microsphere, η is the viscosity coefficient, V is the velocity. Thus, the optical trapping force was calculated using the Eq. (1), where the viscosity coefficient η for distilled water was equaled to 0.001 Pa×s (at a temperature of 20 °C). The calculation results are presented in Table 1 and, for clarity, in Fig. 3.

As can be seen from the results, application of antigens leads to a change in detachment rate. At that, detachment rates and forces of optical trap for polystyrene with thyroid antigens are significantly lower than those for polystyrene microspheres or for polystyrene with kidney antigens. This is consistent with the results of optothermal trap experiments and is apparently associated with change in adhesive properties of microparticles upon application of different types of antigens.

Table 1 Experimental values of the detachment rate and strength of the optical trap.

Microobjects under study	Solution	Detachment rate, µm/s	Optical trapping force, fN
2µm polystyrene microspheres	distilled water	39.4 ± 6.3	742.2 ± 133.6
2µm polystyrene microspheres with thyroid biomarkers	distilled water	20.3 ± 5.5	382.0 ± 101.4
2µm polystyrene microspheres with kidney antigens	distilled water	44.9 ± 9.9	845.8 ± 192.3
2µm polystyrene microspheres	saline solution (0,9% NaCl)	31.0 ± 4.2	–
2µm polystyrene microspheres	saline solution +5 мM SDS	21.0 ± 3.5	–

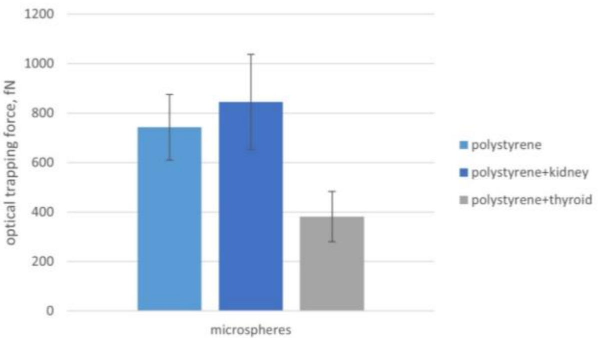


Fig. 3 Dependences of optical trapping forces for different types of 2-µm microspheres in distilled water at a laser radiation power of ~20 mW.

Table 1 also shows the values of the detachment rate of polystyrene microparticles suspended in saline and saline with the addition of SDS. A decrease in the detachment rate is observed. This is explained by a slight increase in the refractive index of saline compared to the refractive index of water, i.e. a decrease in the relative refractive index of polystyrene in saline, as well as an increase in the viscosity of saline. Thus, the optical trapping force, which is directly proportional to the relative refractive index of the particle in the medium, decreases, and the viscous friction force increases. The addition of sodium dodecyl sulfate further increases the viscosity of the solution, which leads to a decrease in the detachment rate. Since the exact values of the viscosity coefficient for the solutions used are unknown, the optical trapping force was not calculated.

From the point of view of biotechnological applications of optical tweezers, manipulation in various buffer solutions, including those with the addition of organic compounds, is of particular interest. Experiments were carried out to measure the detachment rate of 2-µm polystyrene particles (without biomarkers) in SDS solutions in $Na_2B_4O_7$ of different molar concentrations. In this series of experiments, we increased the power to 60 mW, as this allows for increased speed and accuracy of micro-object capture without the risk of damaging the biomarker. The results are presented in Table 2 and in Fig. 4.

Table 2 Experimental detachment rates of 2-µm polystyrene microspheres in $Na_2B_4O_7$ solutions with the addition of SDS of different concentrations.

Solutions under study	Detachment rate, µm/s
$Na_2B_4O_7$	194.5 ± 31.5
$Na_2B_4O_7$ + 5 мM SDS	164.7 ± 27.5
$Na_2B_4O_7$ + 10 мM SDS	151.9 ± 27.1
$Na_2B_4O_7$ + 20 мM SDS	130.4 ± 26.0
$Na_2B_4O_7$ + 30 мM SDS	106.9 ± 14.8

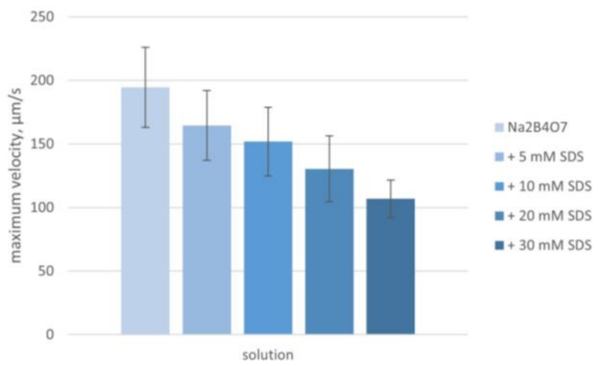


Fig. 4 The detachment rate of 2-µm polystyrene microspheres depending on the molar shell of SDS in $Na_2B_4O_7$. Laser radiation power was ~60 mW.

Increasing the power compared to previous experiments resulted in an increase in the detachment rate, which decreased with increasing SDS concentration. The detachment rate decreases by 55% with increasing SDS molar concentration from 5 to 30 mM. It is well known that sodium dodecyl sulfate is an organic compound with the formula $CH_3(CH_2)_{11}OSO_3Na$ and is an anionic surfactant. Increasing the concentration of surfactants resulted in the microparticles not sticking to the surface, but their dynamic properties deteriorated.

As articles review shows, the dynamic behavior of microparticles in optical traps in organic and mixed solutions is highly complex. Thus, the authors of Ref. [29] note the independence of the rigidity of the optical trap from the refractive index of organic solvents when capturing polystyrene particles coated with a layer of fused quartz. The work shows that the rigidity of the trap, regardless of the refractive index, was about ~0.15 pN/nm for a large number of studied organic solvents (ethanol, isopropanol, acetone, DMF, and others), and the dynamics of microobjects in the trap was determined by the viscosity of the solution. In the work of T. Kužela et al. [30], a systematic study of the behavior of a micro-object in an optical trap depending on the concentration of a mixed solution is carried out for the first time. The authors investigate the dynamic behavior of polystyrene microparticles of 0.8 µm and 3 µm in mixed solvents, which include water with the addition of dimethyl sulfoxide, ethylene glycol and glycerol. It is shown that for all the solvents used, an increase in viscosity is observed with an increase in the molar concentration of organic compounds from 5 to 30 mM, which indirectly confirms the results of our measurements.

Experiments conducted with polystyrene microspheres showed that the type of solution and changes in the concentration of substances in the solution affect the interaction between the laser and the object, changing the characteristics of capture and retention. Studies of the dynamics of polymer microspheres under the influence of optical traps in various media are interesting from a fundamental point of view, because a change in concentration leads to a continuous change in the refractive index and viscosity of the solution. And they will be continued in the future.

4 Conclusion

Experiments were conducted with pure polystyrene microspheres and polystyrene microspheres with kidney and thyroid gland antigens using optical and optothermal trapping techniques. The ability of capturing and transferring polystyrene microspheres with kidney antigens from relatively large distances using an optothermal trap, aligning them into specific configurations formed by structured light traps, followed by fixation using an albumin solution and a short-term increase in power is demonstrated. In this work, traps in the form of a circle and a square boundary were used, but the light fields can be quite diverse.

It was shown that the speeds of detachment of microobjects from the optical trap are different for pure polystyrene microspheres and polystyrene with biomarkers, which in the future can be used to sort microobjects of the same size, but with different chemical and physical properties. However, a large number of experiments with subsequent image processing are required to collect statistics and calibration.

The detachment rates of polystyrene microspheres (without antigens) in various solutions were measured. It was shown that this value depends on the choice of solution and the concentration of dissolved substances. In particular, in a mixed solution of sodium tetraborate, the detachment rates of polystyrene microspheres decrease by 55% with an increase in the molar concentration of SDS from 5 to 30 mM, which may be associated with an increase in the viscosity of the solution in this range of molar concentrations. Thus, our experiments confirmed the possibility of controlling the capture rate by choosing a solution and changing the quantitative composition of the mixed solvent. The polystyrene microspheres used at this stage can be considered as model objects with known optical properties. The presence of a biomarker (antigen) significantly changes the physicochemical properties of the microspheres and, accordingly, their movement in the optical trap. On the other hand, the refractive index of the solution, its viscosity, solubility and other parameters can significantly affect the motion of an object in it. In the future, studies will be conducted on the movement of modified microspheres with various antigens in practically significant buffer solutions. One example is a buffer system based on Tween 80, which is used in diagnostic test systems that employ the indirect agglutination reaction. Studies of the movement of polystyrene microspheres in mixed solutions will also be continued.

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Disclosures

The authors declare that they have no conflict of interest.

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