

## Trap Position Control in the Vicinity of Reflecting Surfaces in Optical Tweezers<sup>†</sup>

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Shift of the trap position from the laser beam waist of optical tweezers is studied experimentally in the presence of a reflecting surface in the vicinity of the focal plane. A standing wave is formed owing to the interference of waves forming the waist and reflected from the surface. The standing wave is shown to affect significantly the resulting trap position. The distance between the surface and the stable optical trap as a function of the trapped particle size is studied numerically. A new method to stabilize the position of the microparticle relative to the surface is proposed. The localization accuracy is determined by the Brownian fluctuations in optical tweezers and is about 10 nm for effective trap stiffness of  $4 \times 10^{-5}$  N/m.

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Methods of optical manipulation of micro- and nanoparticles have been actively developed in recent decades. The application of optical tweezers is a widely used method based on the formation of a potential well for dielectric microobjects located near the waist of a tightly focused laser beam [1]. Changing the focus position allows trapping and manipulation of microobjects in the center of the potential well. The trapped particle displacements in the optical trap are determined by forces acting on the particle; hence, the optical trap can be used as a dynamometer on the microscale. Force measurements performed using optical tweezers are called photonic-force microscopy [2]. The optical tweezers technique has widespread applications in biophysics and biomechanics in study of characteristics of macromolecules and single cells, for instance, DNA [3, 4] and RBC [5, 6], and in measurement of interaction forces between living cells [7, 8]. Besides the biological problems, measurements of forces in optical traps can be performed during the research of surface-enhanced optical effects [9] and analyses of the magnetic interaction of microparticles [10].

Optical manipulation methods involving evanescent fields allow exceeding the diffraction limit in optical localization of microobjects [11] and open new ways in optical sorting [12]. Microparticles can be localized close to the surface where total internal reflection [13, 14] or surface-plasmon excitation [15, 16] is achieved. Forces acting on polystyrene beads located in a surface-plasmon evanescent field near a thin gold film were experimentally shown to be increased by factors of ten in surface-plasmon reso-

nance compared to nonresonant conditions [17]. Precise force measurements in such schemes require fixed distance between the microparticle and the surface; however, it can change uncontrollably owing to local variations of temperature, humidity and other factors. Nevertheless, to date, no technique has been proposed to control the distance between the trapped particle and the surface during long experiments.

In the case of a reflecting surface, for instance, metal, the standing wave arises from the interference of waves that form the waist with waves that are reflected from the metal surface. This wave together with the trapping beam waist has a significant influence on the microparticle localization [18]. The trap stiffness in the beam propagation direction was shown to be noticeably increased owing to the high gradient of the electromagnetic field intensity in the standing wave. Thus, the formation of the standing wave is expected to result in a shift of the spatial position of potential energy minimum from the optical tweezers focal waist. However, the trapped microparticle behavior in case of the focal waist displacement relative to a reflecting surface has not been studied to date.

In this letter, the influence of the standing wave arising from the interference of waves forming the waist and reflected from the surface on the position of the optical tweezers trap is experimentally studied. A technique to determine the distance between the surface and the trapped particle in the presence of a standing wave is proposed.

The optical trap is formed by the radiation of a single-mode diode laser with the wavelength of 975 nm (Fig. 1). The laser beam is focused in the sample chamber by an oil-immersion plan semiapochromat objective with NA of 1.3. The objective lens is supplied

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with micrometer and piezoelectric feeds moving the beam waist along the optical axis to control the focal waist position relative to the metal surface. The chamber consists of two cover glasses 100  $\mu\text{m}$  thick with a gap of about 20  $\mu\text{m}$ . A semitransparent silver film 40 nm thick is deposited on the surface of the upper glass using thermal vacuum evaporation. The gap is filled with a water suspension of polystyrene particles with a diameter of 1  $\mu\text{m}$  and concentration of  $5 \times 10^6 \text{ mL}^{-1}$ . The trapped particle position is determined by the quadrant photodiode detecting trapping beam radiation scattered on the particle. The imaging of the trapped particle is realized using LED radiation directed to the sample by a lens system and the objective. The image of the objective lens field of view is registered with a CCD camera.

Since the oil-immersion objective is used in the experiments and the oil refractive index of 1.52 differs from that of water, the objective displacement  $\Delta d_0$  is not equal to the waist displacement  $\Delta d$ . The cone of rays incident at angle  $\theta$  in water (inset in Fig. 1) is considered in order to find the relation between  $\Delta d$  and  $\Delta d_0$ . The cover glass that separates the water and immersion oil has a refractive index close to that of the oil and is not taken into account. The solution of the geometric problem is defined by the following relation:

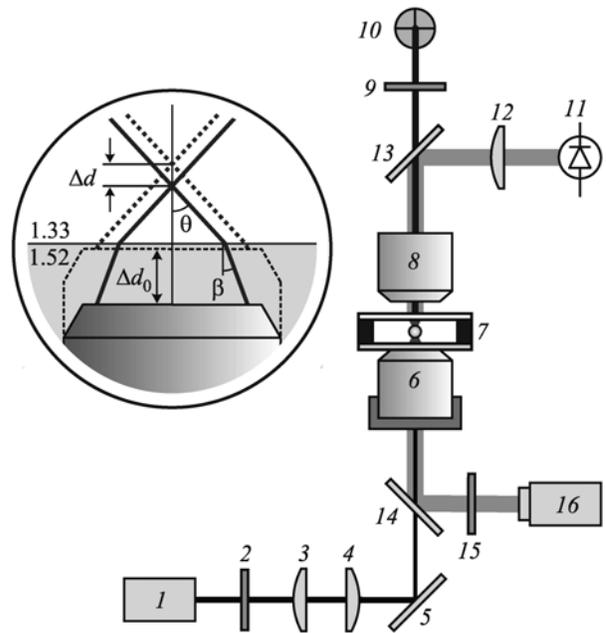
$$\Delta d/\Delta d_0 = \tan\beta/\tan\theta, \quad (1)$$

where angle  $\beta$  corresponding to propagation of rays in immersion oil is evaluated by Snell's law. In the case of small angles,

$$\Delta d/\Delta d_0 \approx \sin\beta/\sin\theta = 1.33/1.52. \quad (2)$$

Since the objective with a numerical aperture of 1.3 is used, angle  $\theta$  takes all values in the range from  $0^\circ$  to  $77^\circ$ . In practice, rays with different  $\theta$  meet at different distance from the surface and thus decrease the trap stiffness  $k_z$  along the optical axis. The particle equilibrium position is determined by an effective value of  $\theta$ . The numerical calculations for a Gaussian-shaped trapping beam show that the objective displacement of 1  $\mu\text{m}$  corresponds to the optical trap shift of 0.75  $\mu\text{m}$ .

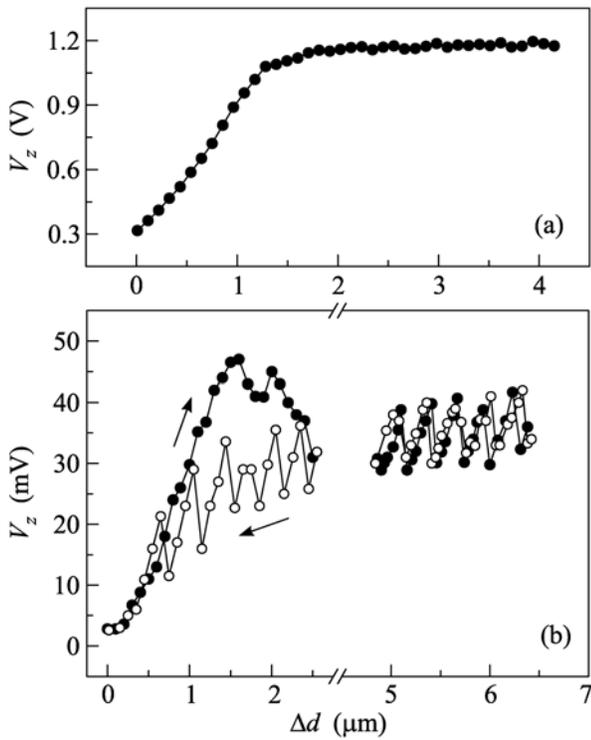
In the standard technique of detection of bead displacement in an optical tweezers trap [19], the radiation scattered on the particle is registered using a detector based on a quadrant photodiode. The output voltage  $V_z^{(\text{ins})}$  is proportional to the total current from all photodiode sections, which is linearly related to the particle shift from the trap center along the optical axis. On the other hand, the measurement of the mean value  $V_z = \langle V_z^{(\text{ins})} \rangle$  can be used for determination of the distance between the surface and the equilibrium position of the particle. In the case of the chamber sides made of transparent materials with low reflectance, the trap position is determined by the focal waist position, and the dependence of the  $V_z$  value on the objec-



**Fig. 1.** Optical tweezers setup. (1) laser, (2) neutral optical filter, (3, 4) beam expander, (5) metal mirror, (6) objective, (7) chamber with a sample, (8) condenser, (9) color optical filters, (10) quadrant photodiode, (11) light diode, (12) collimator lenses, (13–15) dichroic filters, (16) CCD camera. Inset: illustration of the interrelation of the objective lens displacement  $\Delta d_0$  and the waist shift  $\Delta d$ .

tive displacement is monotonic. An example of the experimental dependence  $V_z(\Delta d)$  for the upper side of the chamber made of cover glass with a refractive index of 1.52 is shown in Fig. 2a. When  $\Delta d = 0$ , the waist forms above the glass surface, so the particle is pressed to the surface, and its displacement from the waist is large. As  $\Delta d$  increases, the waist position approaches to the surface and  $V_z$  grows. Finally, when the waist shifts within the sample, the particle is localized in the waist, but a weak increase in  $V_z$  is observed, which can be used to obtain the value of distance between the particle and the surface.

When surfaces with high reflectance are used in the experiment, the standing wave is formed near the reflecting surface. The standing wave significantly influences the optical trap potential; thus, the trapped particle position ceases to be determined unambiguously by the travel of the objective lens. The dependence of the photodiode voltage  $V_z$  on the waist displacement  $\Delta d$  for the case of the upper side of the chamber made of glass covered with a semitransparent silver film 40 nm thick is shown in Fig. 2b. The film reflectance value is 0.9 at the wavelength of 975 nm. Although the angular aperture of the objective lens exceeds  $70^\circ$  and the reflected light intensity decreases rapidly with the increase in distance from the surface, the distortion of the trap potential is observed at distances more than 10  $\mu\text{m}$  from the surface. The depen-

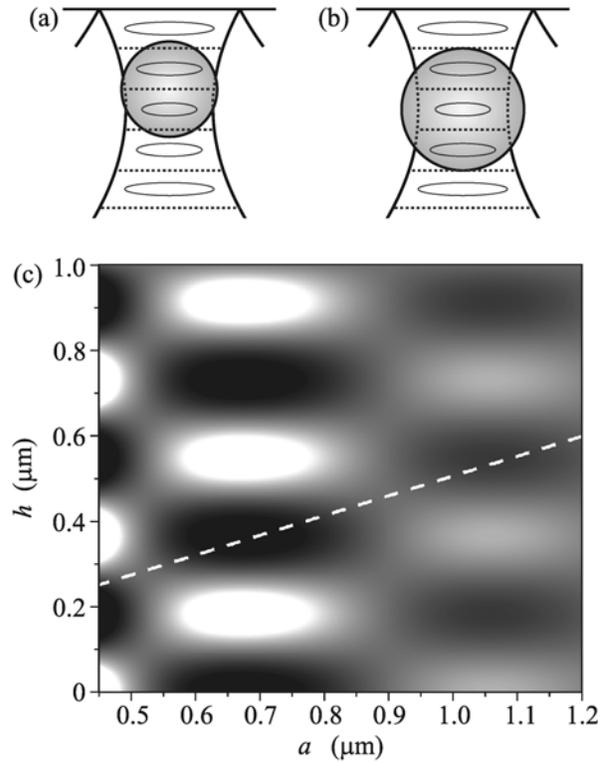


**Fig. 2.** Photodetector voltage signal  $V_z$  versus the waist displacement  $\Delta d$ . (a) The upper side of the chamber is a cover glass. (b) The surface of the upper side of the chamber is coated with a silver film. The closed and open circles correspond to an increase and a decrease in the distance between the surface and the particle, respectively.

dence  $V_z(d)$  is a nonmonotonic sawtooth function. In the vicinity of the reflecting surface, the function shape differs for the trapped particle traveling to the surface or from it.

The nonmonotonic shape of the dependence is explained considering that the equilibrium positions of the particle are determined by the standing wave as a first impact factor, while the focal waist position makes a secondary contribution. Depending on the particle diameter, the center of the bead can be stably located either at nodes (Fig. 3a) or at antinodes of the standing wave (Fig. 3b). When the waist starts to shift down, the distance between the waist and the surface increases. If the particle remains motionless, the increase in its displacement from the waist and, accordingly, in photodiode voltage  $V_z$  is observed. At a certain waist position, the bead starts to sense the neighboring potential well and can be localized in it, which causes the abrupt decrease in  $V_z$ . The period of the leaps corresponds to the standing wave period.

The curve shape for small values of the distance from the surface depends on the direction of movement of the objective lens. If the objective focal waist approaches the surface, the particle behavior remains the same—it changes its location stepwise. If the distance between the waist and the surface increases, the



**Fig. 3.** Standing waves in the vicinity of reflecting surface. (a) Equilibrium position lies at a node. (b) Equilibrium position lies at an antinode. (c) Potential energy distribution obtained in the Rayleigh approximation ( $a$  is particle diameter,  $h$  is distance between the surface and the center of the particle). The energy is normalized to the particle volume; light areas correspond to high values. Dashed line: the particle touches the surface; the states under this line are not realized.

dependence corresponds to the case of a stuck particle that does not move [20]. This is because the antinode nearest to the surface is the deepest potential well for the trapped particle compared to the other antinodes of the interference pattern.

The configuration of the stable positions of the particle in the standing wave is obtained under the assumption that the microparticle does not disturb the optical trap. In this case, the potential energy is represented as an integral over the particle volume [21, 22]:

$$W = -\int \alpha \mathbf{E}^2(\mathbf{r}) d\mathbf{r}, \quad (3)$$

where  $\alpha$  is the specific particle polarizability. In the plane wave approximation, integral (3) is proportional to the following:

$$W \propto - \int_{h-a/2}^{h+a/2} \sin^2\left(\frac{\pi z}{\Lambda}\right) \left[\left(\frac{a}{2}\right)^2 - (z-h)^2\right] dz, \quad (4)$$

where  $\Lambda = \lambda/(2n)$  is the standing wave period,  $a$  is the particle diameter,  $n$  is the refractive index of liquid in the chamber, and  $h$  is the distance between the surface and the particle center.

The plot of the dependence  $W(h, a)$  is shown in Fig. 3c. The calculation parameters are  $\lambda = 975$  nm and  $n = 1.33$ . The standing wave period in the water is 365 nm. Dark areas on the plot correspond to potential energy minima and, therefore, to stable equilibrium positions. The diameter of particles used in the experiment is  $a = 1$   $\mu\text{m}$ , which is shown from the calculation to correspond to the case shown in Fig. 3b. If the particle is localized at the three nearest antinodes, the gap between the surface and the particle is equal to 50 nm. During measurements, uncontrollable changes in the distance between the waist and the surface are possible, for example, because of local temperature variations. However, the particle position is mostly determined by the standing wave field and follows the surface. Since the voltage  $V_z$  strongly depends on the waist displacement  $\Delta d$  (Fig. 2b), even small changes in the distance between the particle and the surface lead to significant changes in  $V_z$ . For experiments requiring precise particle localization relative to the surface, these changes can be compensated by the feedback that moves the objective if  $V_z$  changes. In this case, the distance between the particle and the surface can be fixed with accuracy corresponding to the Brownian fluctuations in optical tweezers—about 10 nm for effective trap stiffness of  $k_z = 40$  pN/ $\mu\text{m}$ .

In conclusion, the effect of trap displacement relative to the focal waist in the vicinity of reflecting surfaces in optical tweezers is studied experimentally. The trapped particle equilibrium position shifts owing to the standing wave arising from the interference of waves that form the waist and waves that are reflected from the surface. The calculations of the distance between the surface and the stable optical trap as a function of the trapped particle size are carried out. The application of the standing wave for optical tweezers measurements requiring precise particle localization relative to the surface makes it possible to stabilize the particle position at fixed distances from the surface and to improve the measurement accuracy.

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