Single-beam trapping in front of reflective surfaces

Alexandr Jonáš and Pavel Zemánek

Institute of Scientific Instruments, Academy of Sciences of the Czech Republic, Královopolská 147, 612 64 Brno, Czech Republic

Ernst-Ludwig Florin

Cell Biology and Biophysics Programme, European Molecular Biology Laboratory, Meyerhofstrasse 1, D-69117 Heidelberg, Germany

Received May 7, 2001

We show that the optical trapping of dielectric particles by a single focused beam in front of a weakly reflective surface is considerably affected by interference of the incident and reflected beams, which creates a standing-wave component of the total field. We use the two-photon-excited fluorescence from a trapped dyed probe to detect changes in the distance between the trapped beam focus as the focus approaches the reflective surface. This procedure enables us to determine the relative strengths of the single-beam and the standing-wave trapping forces. We demonstrate that, even for reflection from a glass–water interface, standing-wave trapping dominates, as far as 5 μm from the surface. © 2001 Optical Society of America

OCIS codes: 140.7010, 170.4520, 190.4180.

The single-beam optical trap (SBT) has become a routinely used experimental tool in the fields of molecular and cell biology, colloidal chemistry, and surface analysis within the past 15 years.[1–3] In the classic trapping scheme, a trapping probe (a dielectric particle) is confined in a tightly focused laser beam,[4] and its equilibrium position is, apart from external disturbances, essentially fixed relative to the trapping beam's focus. Recently an alternative to optical trap generation was suggested that exploits the interference of counterpropagating incident and reflected laser beams in front of a highly reflective slide.[5,6] In this case, the periodically modulated Gaussian standing wave (GSW) created by superposition of the two beams generally contains multiple trapping positions—the standing-wave traps (SWTs)—that are spatially fixed with respect to the reflective slide. Generation of the oscillating GSW component of the total field, however, is not limited to surfaces whose reflectivity approaches 100%.[5] Thus, in a wide class of experiments in which the SBT is used as a force transducer[7] or as a tool for fabrication of mesoscopic structures[8] and in which one operates in close proximity to a liquid–solid interface, the reflected beam is present and the probe is subject to the combined forces of a SBT and SWTs. Consequently, the response of the probe to the displacements of the SBT caused by movement of the beam focus within the sample can change significantly. Some volume elements can even become inaccessible to SBT manipulation.

In this Letter we investigate the extent to which the reflection from a dielectric interface modifies optical trapping in the SBT. The experimental setup shown in Fig. 1(a) is a standard one that is used for optical trapping. The whole system was built around an inverted optical microscope (Carl Zeiss Axiovert 35). The trapping beam emitted by a Nd:YVO_4 laser (λ = 1064 nm; Spectra-Physics T20-B10-106Q) was focused to a diffraction-limited spot by an objective lens (Carl Zeiss Plan Neofluar 100×; oil immersion; N.A. of 1.3) to create the SBT.[4] The objective lens was mounted upon a piezoactuator (Physik Instrumente PiFoc P 721), which served for the precise positioning of the SBT along the optical axis. During the axial movement of the trap through the sample, the axial position of a fluorescent trapped probe with respect to the beam focus could be monitored with nanometer resolution by two-photon excitation by an infrared trapping beam.[9] The fluorescent light was collected by the objective and measured by a photomultiplier (Hamamatsu R2949), and the resultant signal was digitized by a data acquisition board (Jäger Electronics, Germany, ADWin F5). The sample chamber [Fig. 1(b)] consisted of a coverslip at the bottom (objective side) and a slide reflecting the incident beam at the top (condenser side). The distance between the slide and the coverslip was set by spacer latex beads with a nominal diameter of 21.4 μm. Orange fluorescent latex beads (Molecular Probes; diameter, 216 ± 8 nm) suspended in deionized water were used as the trapping probes. These beads are smaller then the spacing of the GSW antinodes (400 nm for the trapping wavelength in water) and therefore can be viewed as local probes in an analysis of the trapping force profile.

In Fig. 2, theoretical simulations of the axial trapping force and trapping potential are shown for the case of trapping in a beam modulated by the reflection from a glass–water interface. The force is calculated by the extended Lorenz–Mie theory,[10]

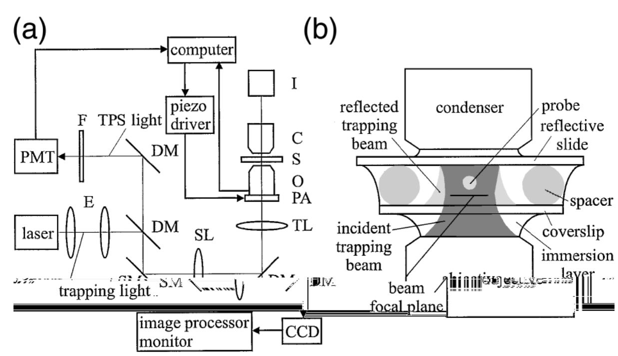

Fig. 1. (a) Experimental setup: PMT, photomultiplier tube; *F*, filter; DMs, dichroic mirrors; E, expander; SM, scanning mirror; SL, scan lens; TL, tube lens; PA, piezoactuator; O, objective; S, sample chamber; C, condenser; I, illumination. (b) Detail of the sample chamber.

0146-9592/01/191466-03$15.00/0 © 2001 Optical Society of America

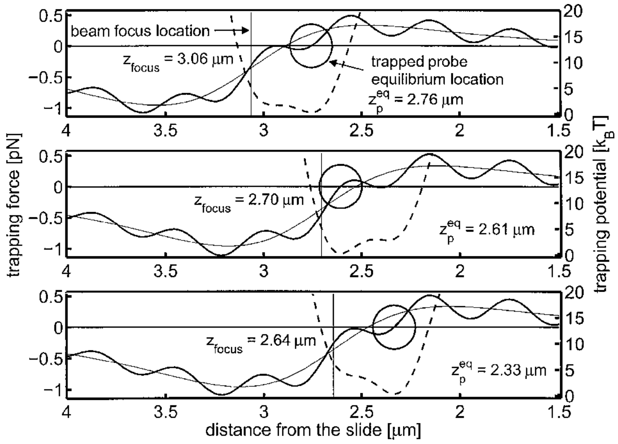

Fig. 2. Theoretical profiles of the trapping force (thicker solid curves) and the trapping potential (dashed curves) of the SBT modulated by the reflection from a glass–water interface at three distances z_{focus} of the beam focus from the reflective surface. The trapped probe is located at $z_p{}^{\text{eq}}$ (places of zero force and potential minima). The diameter of the circles corresponds to the actual probe size (216 nm) used in simulations and experiments. The other parameters are surface reflectivity, 0.4% (calculated from the refractive indices of glass, 1.51, and water, 1.33); focal spot size, 0.475 μm (estimated from a Gaussian fit to the two-photon fluorescence intensity profile obtained by scanning of a stationary probe fixed to a coverslip across the incident beam); and trapping power, 80 mW. Under these conditions the average contribution of the GSW component to the total intensity is less than 3%. For comparison, thinner solid curves show the pure SBT force profile (i.e., without reflection) for the same parameters.

and the potential is obtained as the integral of the force with respect to the axial coordinate. Without considering any aberrations or the reflection of the light scattered by the probe, we describe the total field incident upon the probe as the superposition of the original incident and reflected fifth-order-corrected Gaussian beams.[11] Figure 2 demonstrates that the distance of the trapped probe from the focus of the GSW-modulated beam changes when the focus is moved toward the slide (top to bottom in the figure). This is the result of the combination of the SBT and SWT force profiles, which leads to the existence of multiple positions of the zero trapping force. During the movement of the trapping beam's focus toward the slide, a stable equilibrium location of the probe (global minimum of the trapping potential) is created at successive zero-force positions, which are virtually fixed with respect to the slide (they lie in the vicinity of the GSW antinodes[5]). Thus the probe is confined near these discrete spatial locations and does not move synchronously with the focus, unlike for trapping with an unmodulated beam, in which only a single position of zero force exists, and the probe therefore follows the motion of the beam focus (thin solid curves in Fig. 2).

One can visualize the behavior described above by monitoring the intensity of the fluorescence, which is excited by the two-photon absorption of the trapping light in the dyed probe.[9] The procedure uses the fact that the intensity of the fluorescence [two-photon signal (TPS)] emitted from the trapped probe is proportional to the square of the local optical intensity and thus changes with axial displacement of the probe with respect to the beam focus. Experimentally obtained results for reflection from a glass slide placed in water are presented, together with a theoretical simulation, in Fig. 3. The top figure shows the normalized measured TPS plotted relative to the distance of the beam focus from the slide. The sawtoothlike structure of the signal is a consequence of shifts of the position of the trapped probe's equilibrium in the GSW-modulated optical field (see Fig. 2). While the beam focus approaches the slide, the GSW modulation depth increases with increasing ratio of the amplitudes of divergent reflected and incident beams. The influence of the GSW on the trapping force thus increases, and the smooth modulation of the probe's equilibrium position becomes abrupt jumps between successive equilibrium positions. This transition indicates the dominance of SWTs over the SBT and can be distinguished at a distance of approximately 5 μm from the slide. In the middle figure, a theoretical simulation of the TPS is shown. We determined theoretical values of the TPS by integrating the square of the local optical intensity at the equilibrium position over the probe volume, assuming a homogeneous distribution of the dye within the probe. Despite an idealized description of the incident wave (Gaussian beam), the coincidence of the measured and calculated TPSs is quite good. A comparison of the middle and the bottom figures clearly reveals the correlation between the TPS value and probe–focus distance. To exclude possible effects of the radiometric forces, we repeated the measurement with different power levels. The accent of the sawtoothlike shape of the TPS with increasing power has proved that the mechanism for modulation of probe position can indeed be reflection.

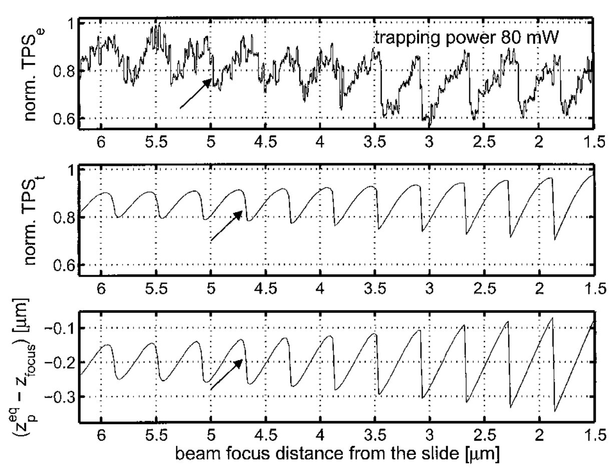

Fig. 3. Comparison of experimentally recorded TPS (top) with theoretical simulation (middle) for reflection from a glass–water interface. The simulation parameters are identical to those in Fig. 2 and correspond to experimental conditions. Bottom, calculated distance of the trapped probe from the beam focus. To show clearly the detailed features of signals, we display only a narrow interval of beam-focus-to-slide distances. Arrows indicate the transition between smooth modulation of the probe position and abrupt jumps.

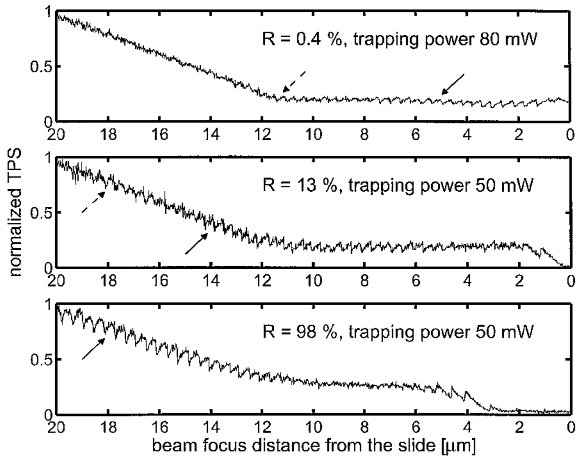

Fig. 4. Experimentally recorded TPS as a function of the distance of the beam focus from the reflective slide for three slide reflectivities. Initially the probe was brought into contact with the coverslip at the bottom of the sample chamber; then the beam focus was moved toward the reflective slide. Dashed arrows, first appearance of the TPS modulation; solid arrows, transition between smooth modulation and abrupt jumps. The overall slope of the TPS is caused by the spherical aberration that is due to refractive-index mismatch between glass and water.

To investigate how the extent of the region wherein the trapping is influenced by GSW depends on slide reflectivity R, we performed measurements with specially coated reflective slides. Figure 4 shows the normalized TPS plots for three values of R. The TPS profile for an ordinary glass slide ($R = 0.4\%$; top) is shown as the reference. Within ~ 8 μm from the start of the scan, no TPS modulation is seen. The particle is trapped in the pure SBT, and the TPS falls off smoothly, influenced only by spherical aberration.[9] Closer to the slide surface, the SWTs strengthen, and the TPS modulation becomes visible (details illustrated in Fig. 3). For the intermediate reflectivity slide ($R = 13\%$; middle), the smooth TPS modulation first appears 18 μm from the reflective slide surface, and the particle starts to jump at 14 μm. When a high-reflectivity slide ($R = 98\%$; bottom) is used, trapping is already affected at the bottom coverslip. After the first 2 μm of smooth variation, discontinuities in the TPS appear. Near the slide surface (closer than 4 μm), the strength of the SWTs in the GSW-modulated force profile is too big for us to overcome by moving the SBT. The particle is confined in the GSW and no longer follows the beam focus movement, which leads to a slow decay of the TPS to zero.

In conclusion, we have studied how single-beam trapping in its typical experimental configuration is influenced by reflection from the surface of the trapping cell. Interference of the incident and the reflected beams creates a GSW component of the field, which modulates the SBT force profile by introducing SWTs. For a given surface reflectivity, the depth of this modulation, which determines the relative strength of the SBT and the SWTs, depends on the distance of the trapping beam's focus from the surface. We used the TPS excited in the trapped fluorescent probe to monitor the distance of the probe from the beam's focus while it is moved toward the reflective surface. Thus we were able to detect the location at which the dominance of the SWTs over the SBT induces abrupt jumps in the probe position. We have demonstrated that, even for an ordinary glass–water interface with reflectivity as low as 0.4%, the SWTs dominate the trapping as far from the surface as 5 μm and affect the trapping to distances several times longer. To verify the interpretation of the experimental data, we tested surfaces of different reflectivities. We found that the extent of the SWTs' dominance grows with increasing R and can reach tens of micrometers for $R = 98\%$. As a result, the presence of the reflected beam causes some positions in the sample chamber to be inaccessible by manipulation of the SBT. This might be a critical factor in the application of the SBT, e.g., for the precise assembly of micrometer- and submicrometer-scaled structures.[8]

This research was supported by the European Molecular Biology Organization (grant ASTF 9499), the Grant Agency of the Czech Republic (grants 101/98/P106, 202/99/0959, and 101/00/0974), and the Grant Agency of the Academy of Sciences of the Czech Republic (grant C1065004/00). We thank Pavel Pokorný (Institute of Scientific Instruments) for preparation of the reflective slides and Alexander Rohrbach (European Molecular Biology Laboratory) for helpful discussions. A. Jonáš's e-mail address is sasa@isibrno.cz.

References

1. K. Svoboda, C. F. Schmidt, B. J. Schnapp, and S. M. Block, Nature **365,** 721 (1993).
2. J. Y. Walz and D. C. Prieve, Langmuir **8,** 3073–3082 (1992).
3. E.-L. Florin, A. Pralle, J. K. H. Hörber, and E. H. K. Stelzer, J. Struct. Biol. **119,** 202 (1997).
4. A. Ashkin, J. M. Dziedzic, J. E. Bjorkholm, and S. Chu, Opt. Lett. **11,** 288 (1986).
5. P. Zemánek, A. Jonáš, L. Šrámek, and M. Liška, Opt. Commun. **151,** 273 (1998).
6. P. Zemánek, A. Jonáš, L. Šrámek, and M. Liška, Opt. Lett. **24,** 1448 (1999).
7. K. Sasaki, M. Tsukima, and H. Masuhara, Appl. Phys. Lett. **71,** 37 (1997).
8. E. R. Dufresne and D. G. Grier, Rev. Sci. Instrum. **69,** 1974 (1998).
9. E.-L. Florin, J. K. H. Hörber, and E. H. K. Stelzer, Appl. Phys. Lett. **69,** 446 (1996).
10. J. P. Barton, D. R. Alexander, and S. A. Schaub, J. Appl. Phys. **66,** 4594 (1989).
11. J. P. Barton and D. R. Alexander, J. Appl. Phys. **66,** 2800 (1989).