How the size of a particle approaching dielectric interface influences its behavior

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ABSTRACT
The influence of size of the trapped object on its position near the dielectric interface is studied experimentally. The trapping beam is reflected on a surface and creates weak standing wave component in resulting field distribution. This component causes unwanted jumps of the trapped particle, when the beam waist moves axially in the surface vicinity. Particles of different sizes are more and less influenced by the standing wave, respectively. The position of the trapped particle is measured with quadrant photodiode and photomultiplier tube at the same time.

Keywords: optical tweezers, two-photon fluorescence, particle position detection, standing wave

1. INTRODUCTION
The optical tweezers has become commonly used technique in micro- and nano-world, in the areas of colloidal chemistry, cell surgery and surface analysis.1–3 The original configuration uses laser beam, which is strongly focused by objective with high numerical aperture – single beam trap (SBT). The equilibrium position of the trapped particle is slightly beyond the laser beam waist, where the scattering and gradient forces are equal. Recently, usage of Gaussian standing wave (GSW) for the optical trapping was suggested.5, 6 In this setup, the laser beam is reflected from the mirror layer on the bottom slide of the sample space, so that the incident and reflected beams interfere and create spatially oscillating maxima and minima in axial direction. The standing wave traps (SWT) are thus multiple axially-separated optical traps, whose properties are defined by the position of the laser beam waist above the reflective surface. The axial positions of SWTs are fixed with respect to the reflective slide. However, the generation of GSW is not limited to near $R = 100\%$ reflective mirrors.5 Even when common glass bottom slide is used ($R = 0.4\%$), the GSW component of the resulting field can be observed near the interface and influences the equilibrium position of the trapped particle up to several $\mu m$ from the bottom slide.7, 8 So the GSW affects all experiments, where particle is held by SBT in the surface vicinity, such as fabrication of mesoscopic structures,9 photonic force microscopy3 or using optical tweezers as force transducer.10

As the laser beam waist (and thus the SBT position) moves towards the bottom slide, the particle approaches subsequent weak SWTs. The combination of the SBT and weak SWT forces cause that the equilibrium position of the particle (global minimum of the trapping potential) tends to stay near the weak SWTs and the particle does not move synchronously with the focus. Since the influence of the GSW grows stronger near the interface, the particle trapped in SBT is more and more influenced by the position of the SWTs and exhibits jumping behaviour (see Fig. 2, left image). Particles of different sizes are influenced by the GSW variously and it was shown theoretically, that this unwanted effect can be suppressed by choosing appropriate microsphere radius8, 11 (see Fig. 2, right image). The resulting axial force of SWT on spheres of such size is decreased, because it is attracted by both nearby intensity maxima in the opposite directions by the gradient forces. The aim of this work is to present experimental results for several particle sizes, which are most, least and medially affected by the GSW, respectively, when approaching the dielectric interface (common glass) on the bottom of sample chamber.

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2. RESEARCH METHOD

2.1. Apparatus

The experimental set-up was built around conventional light microscope Olympus BX-50 (see Fig. 1). The classical arrangement of optical tweezers was used. Infrared solid state laser (Nd:YLF, Spectra Physics, 20-W-105C, \(\lambda = 1053\) nm, output diameter 2 mm, maximal output power 4 W) served as a laser light source. The light from pumping laser diodes was blocked with band-pass filter on 820 nm. The laser beam was transformed with two telescopes (2.75 mm – 8 mm and 60 mm – 175 mm lens with anti-reflective coating), so that the diameter of the beam in the entrance to microscope fluorescent port was about 20 mm. This layout allowed fine adjustment of the laser beam waist in the sample chamber, both in lateral and axial planes. The laser beam was reflected by dichroic mirror inside the microscope, was directed towards the microscope objective (Olympus Ph3 100x, oil immersion, N. A. 1.25, entrance aperture 6 mm) and entered sample chamber. The overfilling of the entrance aperture of the objective assures good trapping of microparticles in 3D.

![Experimental setup diagram](image)

The sample chamber was formed between a microscope slide and cover slip. Their distance was set with spacers (polystyrene spheres of 9.14 µm in diameter, Polysciences, Polybead). The sample itself consisted of inspected microparticles soluted in water. As probes, 5 types of red-fluorescent polymer beads of 490 nm to 930 nm in diameter were employed (Duke Scientific, R500 – R900, density 1050 kg/m³, refractive index 1.59 at \(\lambda = 589\) nm). The fluorescent dye absorbs radiation at 542 nm and emits light of \(\lambda = 612\) nm, so in the vicinity of laser beam waist, the non-linear effect of two-photon fluorescence (TPF) is visible. The whole sample chamber was sealed with gum adhesive to prevent evaporation of the buffer.

The axial particle position is detected in two ways. First, the TPF is employed. The intensity of the TPF radiation is directly proportional to the square of the intensity of the incident excitation light. Since the single beam trap stiffness was 3–4x higher in lateral direction than in axial one, it is possible to trace the axial position of the particle with respect to the laser beam waist with this method with high precision. In this set-up, the emitted red light was collected by microscope objective, passed dichroic mirror, tube lens of the microscope and was separated from the excitation light with IR filter. Then, it met the photomultiplier tube (Hamamatsu R1527) and was recorded in computer. Second, the quadrant photodiode (QPD) was used. The
laser light beam focused by the objective went through the sample chamber and was collimated again by the condenser. The output diameter of the beam was reduced by iris aperture of the condenser, so that its N.A. was 0.5. After reflecting on dichroic mirror it impacted the QPD (EG&G, UV-140BQ-4), which was placed in the back-focal-plane of the condenser lens. In this configuration, the signal of the photodiode was directly proportional to the axial position with respect to the laser beam waist, while it was possible to monitor also lateral deflections of the trapped particle.\textsuperscript{13, 14}

The sample chamber was attached to three-dimensional nano-positioning piezo stage equipped with capacitive sensors (Physik Instrumente, 517.3C, axial ranger 20 \( \mu \text{m} \)). The stage axial position was controlled with precise D/A converter (18-bit, \( \pm 5 \) V, home-made), so that closed-loop repeatability of the stage position was better than \( \pm 2 \) nm in axial direction. Signals from QPD and photomultiplier tube were recorded with fast data acquisition card (Computer Boards, PCI-DAS4020/12, 12-bit, 4 input channels).

2.2. Procedure

The trapping beam was set to 400 mW, so the power behind the microscope objective was about 50 mW. The particle of interest was trapped and pushed with the cover slip so far, that its TPF decreased to about 60\% of free-space TPF. Then the measurement began – whole height of the chamber was scanned with 20 nm steps. In each step, the particle was left 20 ms to settle in the new position and then 2048 samples from both QPD and photomultiplier with 60 kHz rate were acquired, processed and the mean value and standard deviation were recorded.

Using TPF for particle position detection has disadvantage of photobleaching – irreversible photochemical changes in the fluorescent dye, which cause exponential time decay of the TPF signal. Two following scans in the same direction are distanced the time interval \( \delta t \). Their corresponding signals in each step can be expressed as

\[
\frac{TPF(t + \delta t)}{TPF(t)} = \exp(-\delta t/\tau).
\]

So the three scans in each direction (six scans total) were obtained to get the photobleaching constant \( \tau \) and to eliminate the TPF signal decay. The whole procedure took about 5 minutes.

2.3. Processing of the data

After eliminating the TPF photobleaching, both TPF and QPD signals were smoothed with discrete Meyer wavelet approximation on level 3. The origin of the z-axis was set to the first maximum of the TPF signal and the TPF was normalized to value in this point. The QPD calibration was concerned with the range, where the trapped sphere was pushed by the microscope slide from the trapping position. In this place, the axial movement of the stage directly corresponds with the distance between the particle and the laser beam waist. Therefore, the slope of the edge was used to set the scale QPD signal. The signal of QPD slowly decreases as the beam waist approaches the sample space, mainly due to the spherical aberration caused by the refractive index mismatch between the coverslip and the water medium. We obtained approximation of the signal in the region of interest with low-pass filtering and by subtracting this shallow curve from the signal, we got unbent signal oscillating around zero. Next, the nearest maximum to the \( z_w = -4 \) \( \mu \text{m} \) was found and five subsequent maxima/ minima pairs were obtained from the QPD signal. Their average value was stored together with particle size. For the data processing, Matlab (Mathworks) with Wavelet toolbox was used.

3. EXPERIMENTAL RESULTS

3.1. Theoretical predictions

The distance between the incident laser beam waist and the bottom slide is \( z_w \). When the waist moves towards the slide, the distance between the trapped particle and the waist changes – we track the \( z_w - z_{sph} \) quantity in each \( z_w \) point with QPD and photomultiplier tube. In Fig. 2 (plots on left), there is a comparison between TPF emitted by the sphere and the deflection of the sphere from the beam waist position as a function of the waist-surface distance. The QPD signal is proportional to \( z_w - z_{sph} \), while the TPF signal is higher near the beam waist – the minima of TPF correspond to maxima of QPD (see the dotted line). These plots were
calculated\(^8\) for spheres with radius 0.345 \(\mu m\) and the particle size is outlined in bottom left corner of the QPD graph. The \(z_w = 0\) point was set in maximum of the TPF signal.

These theoretical profiles were further analyzed and overall effect of GSW on particles of 0.1 – 0.5 \(\mu m\) radii was quantified.\(^8\) The quantity \(z_w - z_{sph}\) was introduced as average of five subsequent jump lengths starting from the first jump which occurs for \(z_w < 4 \mu m\) (see Fig. 2, right plot). The resulting plot shows clearly the tendencies of the effect together with chosen particle sizes for experimental work – these were commercially available sizes close to sensitive region, insensitive region and medial region of the curve, respectively.

![Figure 2](image1.png)

Figure 2. Theoretical calculations of particle behaviour in weak GSW.\(^8\) Left plot: Dependence of deflection of particle with radius \(a = 0.345 \mu m\) from the beam waist as a function of waist-surface distance \(z_w\) (top) and TPF signal of the particle in the same position (bottom). Right plot: Influence of the particle size on the length of jumps in the resulting field (see section 3.1). In both cases: wavelength \(\lambda = 1.053 \mu m\), refractive indices \(n_{sph} = 1.585\) and \(n_{water} = 1.332\), and water-glass reflectivity \(R = 0.4\%\).

3.2. Measurement results

The measurement series are divided into three groups. Particles highly sensitive to the GSW are represented by spheres of radius 0.345 \(\mu m\). The example of the resulting measurement can be found on Fig. 3. The plot consists of experimentally obtained TPF and QPD \((z_w - z_{sph})\) signals, respectively, in the region of interest (located between \(-4 \mu m\) and the glass bottom slide at 0 \(\mu m\)). Both signals were calibrated accordingly to description in 2.3. The inset shows processed and unbent signal of \(z_w - z_{sph}\) near \(-4 \mu m\). It is shown, that the saw-tooth profile of QPD signal is well-defined and each maximum of \(z_w - z_{sph}\) corresponds to minimum of TPF profile. The second group, medially sensitive particles, is represented with two sizes of the spheres – radii 0.3 \(\mu m\) and 0.465 \(\mu m\), respectively (see Fig. 4). The low sensitive particles were spheres of radii 0.245 \(\mu m\) and 0.41 \(\mu m\) (see Fig. 5). All insets have the same Y-axis scale, so the signal magnitude can be compared qualitatively with naked eye.

![Figure 3](image2.png)

Figure 3. Measurement of spheres in high-sensitive region (radius 0.345 \(\mu m\)).
Figure 4. Measurement of spheres in medium sensitive region – radii 0.3 μm (left image) and 0.465 μm (right image).

Figure 5. Measurement of spheres in low sensitive region – radii 0.245 μm (left image) and 0.41 μm (right image).
To compare the measurement with theoretical dependence, we acquired large number of $z_{w} - z_{sph}$ values for each of the particle sizes. Their average values and standard deviations are put in the Fig. 6 together with the theoretical calculation. It can be seen, that the behaviour of the particles of radii 0.465 µm, 0.245 µm, 0.41 µm and 0.3 µm are in coincidence with the theoretical prediction. As the laser beam waist enlarges going through the medium due to spherical aberration, the differences between experimental and theoretical results for particles of 0.345 µm in radius occur. Another source of deviations is in variation of radii of the spherical particles (coefficient of variation is upto 3% from the mean diameter).

![Figure 6. Comparison of experimental results with theoretical prediction (see text).](image-url)

4. CONCLUSIONS

In this article we demonstrated experimental research on behaviour of optically trapped particles approaching the dielectric interface. It was shown that even common glass bottom slide with reflectivity $R=0.4\%$ creates GSW strong enough to deflect particle from its SBT equilibrium position. We measured this behaviour on particles of radii 0.245 µm, 0.3 µm, 0.345 µm, 0.41 µm and 0.465 µm.

The experimentally obtained data were compared to theoretical prediction of the effect. We have proved that for particle sizes 0.245 µm and 0.41 µm in diameter, the unwanted jumps in weak GSW are minimized, whereas when using particle of 0.345 µm radius as SBT probes, the movement exhibits larger jumps when approaching the surface.

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