Optical levitation and manipulation of stuck particles with pulsed optical tweezers

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We report on optical levitation and manipulation of microscopic particles that are stuck on a glass surface with pulsed optical tweezers. An infrared pulse laser at 1.06 μm was used to generate a large gradient force (up to 10^{-9} N) within a short duration ($\sim$45 μs) that overcomes the adhesive interaction between the particles and the glass surface. Then a low-power continuous-wave diode laser at 785 nm was used to capture and manipulate the levitated particle. We have demonstrated that both stuck dielectric and biological micrometer-sized particles, including polystyrene beads, yeast cells, and *Bacillus cereus* bacteria, can be levitated and manipulated with this technique. We measured the single-pulse levitation efficiency for 2.0 μm polystyrene beads as a function of the pulse energy and of the axial displacement from the stuck particle to the pulsed laser focus, which was as high as 88%. © 2005 Optical Society of America

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The optical tweezer technique has become a powerful tool for manipulation of micrometer-sized particles in three spatial dimensions, typically by use of continuous-wave (cw) lasers.[1,2] It has been routinely applied to manipulate living cells, bacteria, viruses, chromosomes, and other organelles,[3] and was recently applied to the study of molecular motors,[4] colloid physics,[5] and polymers[6,7] and to the control of optically trapped structures.[8] The combination of Raman spectroscopy with optical tweezers allows optically trapped living cells and other particles to be characterized.[9,10] The trapping force generated by the cw tweezers (with an average power below hundreds of milliwatts to prevent photodamage[11]) is typically of the order of 10^{-12} N.[3] This weak force is sufficient to confine the suspended particles in liquids but not to levitate and manipulate the particles that are stuck on the glass surface owing to the strong binding force. In this Letter we describe a pulsed optical tweezers technique that uses a pulsed laser for levitation of the stuck particles and a low-power cw laser for successive trapping and manipulation. The large peak gradient force (of the order of 10^{-9} N) produced by the pulsed laser allows the binding interaction between the stuck particle and the glass surface to be broken such that the levitated particle can be captured and manipulated by the cw laser.

Pulsed laser beams have been used in quasi-cw femtosecond tweezers,[12] laser microdissection and laser-pressure catapulting (LPC),[13,14] and laser microsurgery.[15] In laser microdissection, pulsed UV or IR laser microbeams are used to precisely cut around and collect the regions of interest of specimens that are due to laser-based ablation to the specimens. LPC is a method of pushing the microdissected samples into the microcentrifuge cap by means of a strong UV laser, in which a gas pressure force under the specimen is caused by laser ablation as a result of the extremely high photon density within the focal laser spot.[13] Berns *et al.* combined cw IR optical tweezers with either pulsed UV or Nd:YAG laser microbeams, which function as optical scissors.[15]

The mechanism of the pulsed optical tweezers used in our system is different from that of laser microdissection and LPC.[13] First, the force used to levitate the stuck particles is the gradient force rather than the laser ablation or the gas pressure in LPC. Second, in our scheme the focus of the pulse beam was aligned to coincide with the focus of the cw trapping beam and to be a few micrometers away from the target particle, at which the gradient force acting on the stuck particle is the maximum. The nonzero distance z from the particle to the laser focus is essential for generating the effective gradient force because the trapping force can be expressed as $F=kz$ for small z, where k is the force constant.[3] However, if the laser focus is too far from the particle (for large z), F will decrease to zero. The optimum distance depends on the size, shape, and relative index of refraction of the target particle.[2,3] In addition, since the IR pulsed laser was not directly focused on the target particles and the pulse energy was relatively low, laser ablation of the specimens was not produced. Therefore the laser-induced damage to the stuck biological particles by the IR pulsed tweezers was minimized.

The experimental scheme is shown in Fig. 1(a). The pulsed optical tweezers were composed of two parts: a cw trapping system and a pulsed levitation

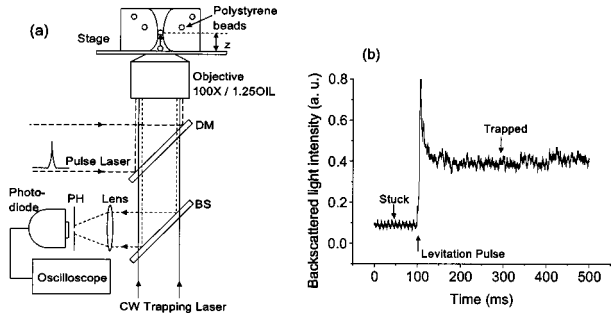

Fig. 1. (a) Experimental setup of pulsed optical tweezers: DM, dichroic mirror; PH, pinhole; BS, beam splitter. (b) Backscattered light intensity recorded as a struck polystyrene bead is levitated with an IR pulse and jumps into the cw trap.

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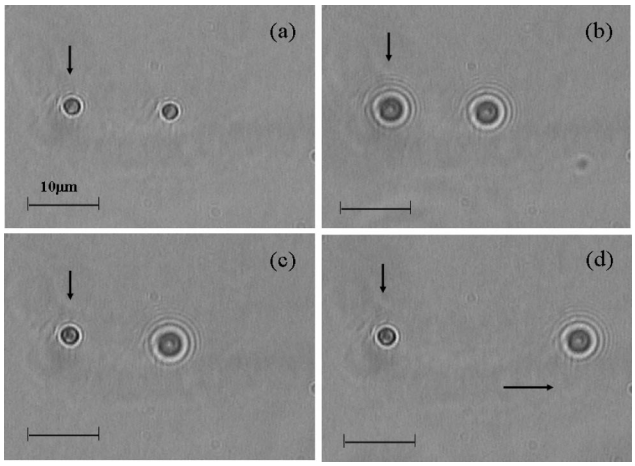

Fig. 2. Levitation and manipulation of a stuck polystyrene bead. (a) Two beads were initially stuck on the coverslip. (b) The beads were defocused with $z=8$ μm. (c) The marked bead was levitated with an IR pulse and jumped into the cw trap. (d) The levitated bead was trapped while the stage was moved.

system. The cw beam from a diode laser at 785 nm was made circular by a pair of anamorphic prisms and was introduced into an inverted microscope equipped with an objective (100×, 1.25 N.A.) to form a cw trap. A pulsed Nd:YAG laser (Quantel YG580), operated without Q switching at a repetition rate of 13 Hz, was used for levitation. A set of neutral-density filters and a half-wave plate ($\lambda/2$) in combination with a polarizer were used to change the pulse energy. The pulse energy was typically reduced to 300–500 μJ/pulse (with a peak power up to 10 W), and the power of the cw trapping laser was typically 18 mW at the beam focus. A computer-controlled shutter was used to select a single pulse that actually exposed the particle to be detached. A pair of lenses was used to precisely align the focus of the pulsed beam to meet the focus of the cw trapping beam on the microscope specimen plane. The focus of both the pulsed and cw beams was initially adjusted to be above the top of the stuck particle (with $z \sim 8$ μm). As a laser pulse was fired, the stuck particle was kicked off by the pulsed gradient force and then jumped to the trap position.

To detect the position jump of the stuck particle after the pulse levitation, we measured the backscattered light at 785 nm from the particle with a fast photodiode (through an $f=15$ cm lens and a 400 μm pinhole). A low-pass filter in the front of the photodiode was used to block the backscattered light at 1.06 μm such that the detected signal reflected the relative position of the particle inside the cw trapping beam. Figure 1(b) shows a typical plot of the backscattered light intensity as the function of time, recorded with a digital oscilloscope. Two distant intensity levels were observed; the lower is for the case when the particle is stuck on the coverslip, and the higher is for the particle in the trap after the pulse was applied. The transition time was typically less than 15 ms.

Figure 2 shows the levitation and manipulation by the pulsed optical tweezers of a stuck 2.0 μm polystyrene bead. The polystyrene spheres (Model P0020320PN, Bang Laboratories) were diluted in deionized water, and a drop of the fresh sample was transferred to a sample well in which some beads were found to adhere to the glass coverslip (Model NC9115219, Fish Scientific) after a few minutes. The beads were initially stuck on the coverslip [with $z=0$ in Fig. 2(a)]. Then we adjusted the objective to move the focus of the cw trapping beam above the surface ($z=8$ μm) such that the microscope was defocused on the beads, as shown in Fig. 2(b). The defocusing image of the marked bead indicates that the bead was not trapped by the cw laser beam. Then one IR pulse was fired to the marked stuck bead, and the bead was kicked off. Figure 2(c) shows the image of the beads after the levitation, which indicates that the marked bead had jumped to the cw laser trap (showing a sharp image) and the other bead was still stuck on the coverslip (showing a defocusing image). Now the bead has remained in the cw trap and can be manipulated by moving the microscope stage, as shown in Fig. 2(d).

The success in levitation relies on the magnitude of the pulsed gradient force and the binding condition between the stuck particles and the surface. For the given particles and aqueous solution, the force depends on the pulse energy and on axial displacement z; the binding condition may change from one particle to another. For the 2.0 μm polystyrene beads stuck on the glass surface in water we measured the single-pulse levitation efficiency as the function of the pulse

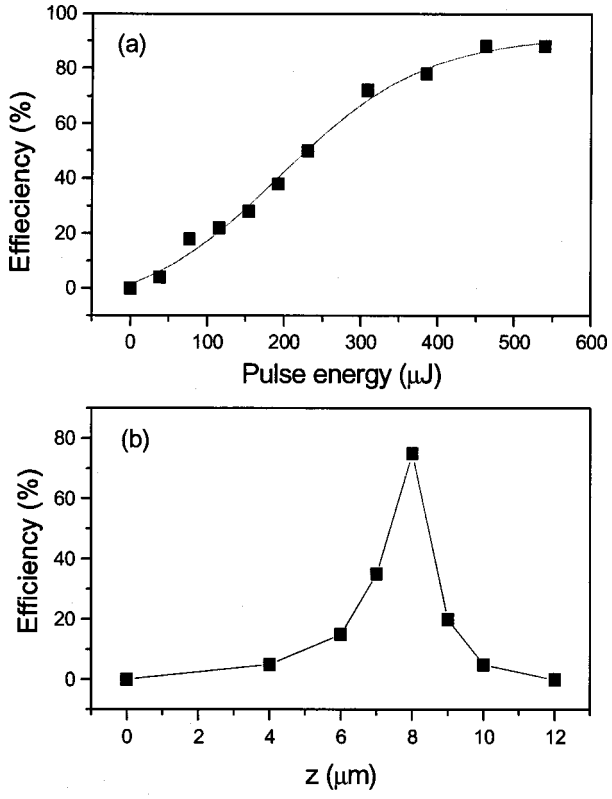

Fig. 3. Single-pulse levitation efficiency versus (a) pulse energy (in microjoules) at $z=8$ μm; (b) initial distance z (with a constant pulse energy of 385 μJ).

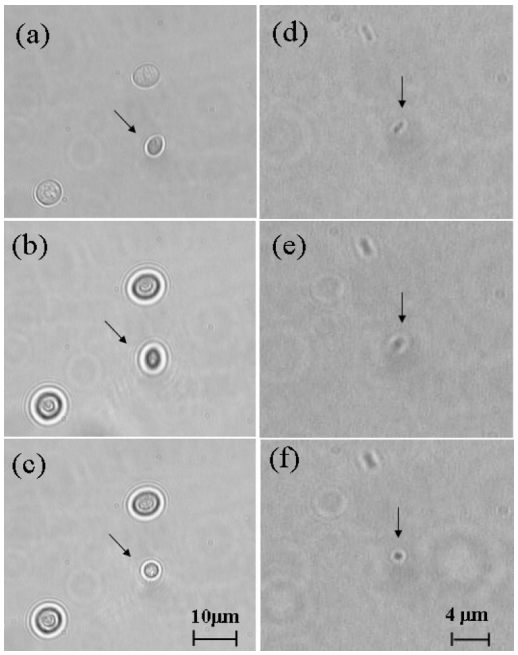

Fig. 4. Levitation and manipulation of (a)–(c) a stuck yeast cell and (d)–(f) a *Bacillus cereus* bacterium.

energy and initial axial displacement z. For this purpose we randomly selected 50 stuck particles for levitation, fired only one pulse for each particle, and then counted the number of particles that actually levitated to determine the levitation efficiency. Figure 3(a) shows the levitation efficiency versus the pulse energy (with a fixed z of 8 μm). The solid curve is the Boltzmann fit of the experimental data. One can see that the levitation efficiency was increased with the increase in pulse energy and can reach up to 88% at $\sim$450 μJ. Figure 3(b) shows the dependence of the levitation efficiency on displacement z with a constant pulse energy. For each displacement z, we tried to levitate 20 stuck particles. The optimum efficiency was observed at $z \sim 8$ μm for the 2.0 μm beads, and the leviation efficiency decreased drastically with a slight change in z. The optimum levitation efficiency corresponds to the maximum gradient force generated by the pulse beam.

Figure 4 illustrates that biological cells that are stuck on the glass surface can also be levitated with the pulsed optical tweezers. Figures 4(a)–4(c) show stuck yeast cells that were suspended in 0.1% NaCl solution, and Figs. 4(d)–4(f) show stuck *Bacillus cereus* bacteria suspended in a LB medium (with 0.01% NaCl). In these experiments we first focused the microscope to see the clear image of the stuck cells, as shown in Figs. 4(a) and 4(d), and positioned the target cell into the laser spot. Then the target cell was defocused with $z \sim 8$ μm [Figs. 4(b) and 4(e)], and then the IR laser pulses were fired such that the target stuck cells were levitated and moved to the cw trap [Figs. 4(c) and 4(f)]. The successful levitation of the stuck cells was verified by the clear cell images as well by their manipulation in either the x or the y direction by the cw beam. The binding interaction of the biological cells with the surface is usually stronger than that of the polystyrene beads, and in many cases a few individual pulses were required for levitation of a stuck cell.

In summary, we have developed pulsed optical tweezers for optical levitation and manipulation of the stuck micrometer-sized particles, including biological cells. We have measured the single-pulse levitation efficiency as a function of pulse energy and of the initial axial distance from the particle to the laser focus. We observed that the levitation efficiency can be as high as 88% for 2.0 μm polystyrene spheres. Pulsed optical tweezers open the possibility of manipulating stuck microparticles in aqueous environments and may find broad applications in cell biology and molecular biology.

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