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Pulsed optical tweezers for levitation and manipulation of stuck biological particles

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Abstract: We report on optical levitation and manipulation of microscopic particles that are stuck on a glass surface with a pulsed optical tweezers. Both the stuck dielectric beads and biological cells are demonstrated to be levitated.

OCIS codes: (170.4520) Optical confinement and manipulation; (140.7010) Trapping

Optical tweezers has become a powerful tool for capturing and manipulation of micron-sized particles, typically by using continuous-wave (cw) lasers [1, 2]. It has been routinely applied to manipulate living cells, bacteria, viruses, chromosomes and other organelles [3, 4], To reduce the photodamage to the trapped particles, the average power of the trapping lasers is usually limited to below hundreds of mW and near-infrared (NIR) or infrared lasers were used for trapping [3, 6]. The trapping force generated by the cw optical tweezers is typically in the order of 10^{-12} N [4, 5]. This weak force is efficient to confine micro-particles suspended in liquids, but not sufficient to levitate the particles that are stuck on the glass surface, where it has to overcome the binding force. Therefore, the stuck particles cannot be manipulated with the optical tweezers that only employs cw lasers.

In this paper, we describe a pulsed optical tweezers that employs 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 (in the order of 10^{-9} N) produced by the pulsed laser allows breaking the binding interaction between the stuck particle and the glass surface and then, the levitated particle is captured and manipulated by the cw laser. Pulsed laser beams have been used for femtosecond optical twezzers [7, 8], laser microdissection and laser-pressure catapulating (LPC) [9, 10], and laser microsurgery [11, 12]. The mechanism of the pulsed optical tweezers used in our system is different from that of laser microdissection and laser-pressure catapulating. The force used to levitate the stuck particles in this work is due to the gradient force, rather than laser-ablation or the gas pressure. The focus of the infrared pulsed beam was aligned to coincide with the focus of the cw trapping beam and to a few microns away from the target particle, at which the gradient force acting on the stuck particle is the maximum but leads to the minimum photodamage to the particle.

The experimental scheme is shown in Fig. 1. The pulsed optical tweezers is composed of two parts: the cw trapping beam and the pulsed levitation beam. The pulsed Nd:YAG laser (λ=1.06 μm) is used for levitation. The pulsed laser is operated without Q-switching. The pulse energy was reduced to 300~500 μJ per pulse at the sample.

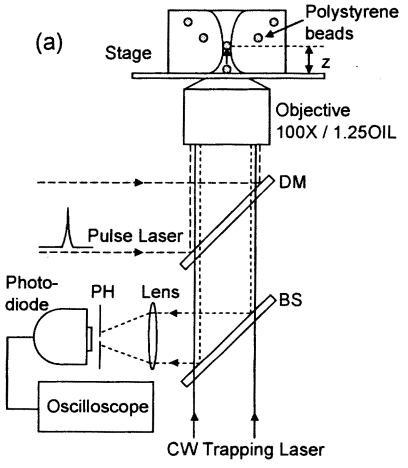

Fig.1. Experimental Setup of pulsed optical tweezers

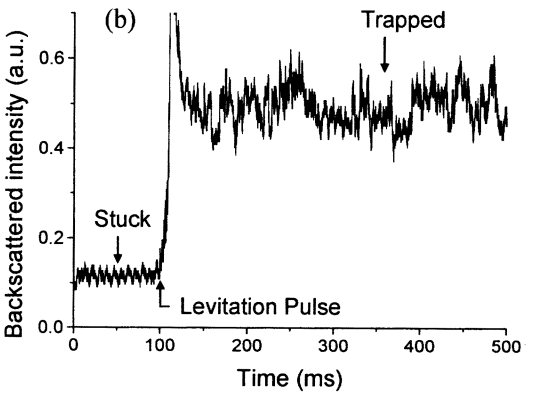

Fig. 2. Backscattered light intensity recorded at the detector as a stuck particle is levitated with an infrared pulse and jumps into the cw trap.

A diode laser (λ=785 nm) that is introduced into an inverted microscope equipped with an objective (100×, 1.25 NA) is used for cw trapping. The power of the cw trapping laser was typically 18 mW. The focus of both the pulsed and cw beams is initially adjusted to be above the top of the stuck particle (with an axial displacement z from the surface of the bottom glass plate). A fast photo-diode is used to record the back-scattered light from the stuck particle. As a laser pulse is fired, the stuck particle is kicked off by the pulsed gradient force and then jumps to the trap position. Fig.2 shows a typical plot of the backscattered light intensity as the function of time, recorded with a digital oscilloscope triggered with the levitation pulse. One can see a transition of the stuck particle from the stuck position to the trapped position within a typical transition time of less than 15 ms.

Fig. 3 shows the levitation and manipulation of a stuck polystyrene bead (2.0 μm diameter) suspended in water using the pulsed optical tweezers. Fig.3(a) shows the image of two beads stuck on the coverslip and we intended to levitate the one marked with an arrow which can not be manipulated using the cw laser trap. As a single infrared pulse is fired, the stuck bead is levitated and then manipulated with the cw beam.

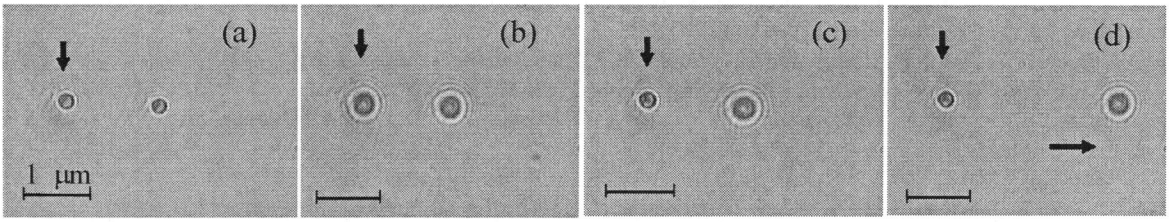

Fig. 3. Optical levitation and manipulation of a stuck polystyrene bead. (a) Two beads stuck on the coverslip (with z=0). (b) The beads were defocused before the application of the pulse (with z=8μm). (c) The marked bead was levitated after the application of the pulse and jumped into the cw trap. (d) The levitated bead is manipulated while stage was moved in +X direction.

We measured the single pulse levitation efficiency as the function of the pulse energy and the initial axial displacement z. The success in levitation relies on the magnitude of the pulsed gradient force and the binding condition between the stuck particles and the surface. Fig. 4(a) shows the dependence of the levitation efficiency on the displacement z with the pulse energy kept at 385 μJ for the 2.0-μm polystyrene beads stuck on the glass surface in water. Fig. 4(b) shows the levitation efficiency versus the pulse energy (for a fixed z of 8μm). The line curve is the Boltzman fit of the experimental data. One can see that the levitation efficiency is increased with the increase in pulse energy and can reach up to 88% at ~450 μJ.

Fig. 5 illustrates that biological cells that are stuck on the glass surface can also be levitated and manipulated with the pulsed optical tweezers. Fig. 5(a)-(c) are for stuck yeast cells that were suspended in 0.1% NaCl solution and Fig. 5(d)-(f) are for stuck *Bacillus cereus* bacterial cells suspended in a LB medium (with 0.01% NaCl). The same technique as described for polystyrene beads is used here. The binding interaction of the biological cells with the surface is usually stronger than that of the polystyrene beads and we found that in many cases a few individual pulses were required to levitate a stuck cell.

In summary, we have developed a pulsed optical tweezers for optical levitation and manipulation of the stuck particles. Both polystyrene beads and micron-sized biological cells (yeast and bacteria) are demonstrated. We have measured the single pulse levitation efficiency as a function of the pulse energy and the initial axial displacement. The single pulse levitation efficiency can be as high as 88% for 2.0-μm polystyrene spheres. Pulsed optical tweezers opens the possibility of manipulating stuck micro-particles in an aqueous environment and may find broad applications in cell biology and molecular biology.

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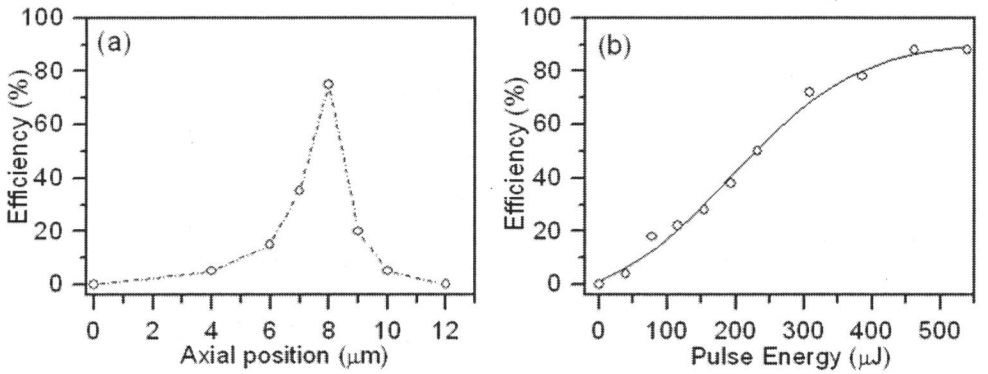

Fig. 4. (a) Levitation efficiency versus the initial distance (z) in μm between the particle position laser focus (with a constant pulse energy of 385 μJ). (b) Single pulse levitation efficiency versus pulse energy in μJ at z=8 μm.

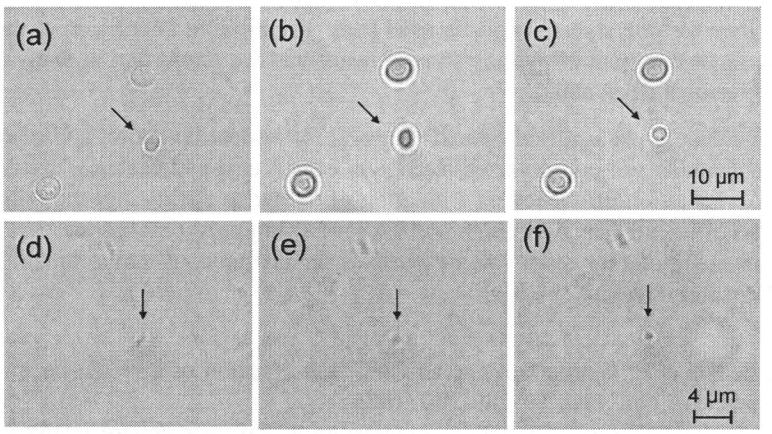

Fig. 5. Levitation and manipulation of a yeast cells and a Bacillus cereus bacterium that are stuck on the coverslip. (a) Yeast cells stuck to the coverslip. (b) The yeast cells were defocused before the application of the pulse. (c) The marked yeast cell was levitated with infrared pulses and jumped into the cw trap. (d) Two Bacillus cereus bacteria stuck on the coverslip. (e) The stuck bacterial cells were defocused. (f) The marked bacterium was levitated and jumped into the cw trap.