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Pulse Laser Assisted Optical Tweezers for Biomedical Applications*

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Abstract— **Optical tweezers which enables to trap micron to nanometer sized objects by radiation pressure force is utilized for manipulation of particles under a microscope and for measurement of forces between biomolecules. Weak force of optical tweezers causes some limitations such as particle adhesion or steric barrier like lipid membrane in a cell prevent further movement of objects. For biomedical applications we need to overcome these difficulties. We have developed a technique to exert strong instantaneous force by use of a pulse laser beam and to assist conventional optical tweezers. A pulse laser beam has huge instantaneous laser power of more than 1000 times as strong as a conventional continuous-wave laser beam so that the instantaneous force is strong enough to break chemical bonding and molecular force between objects and obstacles. We derive suitable pulse duration for pulse assist of optical tweezers and demonstrate particle manipulation in difficult situations through an experiment of particle removal from sticky surface of glass substrate.**

I. Introduction

Radiation pressure force exerted by a focused laser beam can hold a single particle sized from 100 nm to several tens micron in diameter [1]. This phenomenon is called as laser trapping [2] and is utilized for micromanipulation of objects under an optical microscope and for investigation of force related phenomena such as adhesion of biomolecules [3], mechanical property of DNA [4] and other biomolecules [5], and cellar functions against to external force [6][7]. Under optical tweezers condition radiation pressure has an order of several pN to several hundred pN, which are same order of molecular forces between biomolecules. That is quite suitable for measurement of small force, although there are some problems such as unable to remove adhered particles and steric barrier in a cell condition. To apply medical applications optical tweezers should be improved further more. We have developed a technique to exert strong instantaneous force by use of a pulse laser beam instead of a continuous wave laser beam in conventional optical tweezers and to use for assistance of conventional optical tweezers [8]. In this manuscript a suitable pulse duration for pulse laser assisted optical tweezers is derived from motion equation and an experimental demonstration of particle manipulation in difficult situations is presented through an experiment of particle removal from sticky surface of glass substrate.

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II. Principle of Pulse Laser Assisted Optical Tweezers

A. *Radiation force exertion by a pulse laser beam*

In conventional optical tweezers a continuous wave (CW) laser has been used as a trapping laser, because stable laser light gives stable trapping and manipulation of objects. When objects are adhered on substrate surface or blocked by steric barrier like lipid membrane inside a cell, however, radiation force by a CW laser beam is not strong enough to break adhesion or steric structure. In such cases strong instantaneous force will help to overcome these difficulties. We thought up to use a pulse laser beam to generate strong instantaneous force on objects.

Since radiation pressure is proportional to instantaneous power of light, we may expect strong instantaneous power of pulsed laser beam gives strong instantaneous force. A pulse laser beam is focused and irradiated to an object to be manipulated as same to optical tweezers with a CW laser beam. Radiation force by a pulse beam exerts strong force instantly as energy compressed in short duration of pulse beam. For stable manipulation we also introduce a CW laser beam co-axially to pulse laser beam. According to instantaneous laser power of pulse beam we expect that radiation force of more than 1000 times can be applied to the object. By use of this strong instantaneous force, we can break strong bonding formed between surfaces of object and substrate and/or destroy steric barriers such as lipid membrane inside a cell.

B. *Suitable pulse duration for assist*

In pulse laser assisted optical tweezers pulse duration is one of key factors. As decrease of pulse duration, instantaneous power and force exerted by the same pulse energy become strong, although a certain period of time is required to move objects. We expect that a suitable duration exists for pulse laser assisted optical tweezers. Therefore we tried to derive the suitable duration.

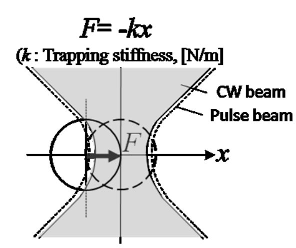

Figure 1. Schematic of radiation force generation on a particle. A CW beam and a pulse beam are incident onto a particle. A broken line indicates equilibrium position of the particle.

As shown in Fig. 1 a particle shifted from optical axis is pulled back in a beam spot. The magnitude of acting force f_{trap} by radiation pressure can be assumed as $f_{trap} = -kx$, where x is displacement from an equilibrium point and k [N/m] is trapping stiffness of optical tweezers. Also we assume that the particle is in water or in other viscous medium and describe a motion equation of the particle as

$$-kx = m\frac{d^2x}{dt} + 3\pi\eta d\frac{dx}{dt} \quad (1)$$

where η is viscosity coefficient, d and m are diameter and mass of the particle, respectively. The second term of right side in eq. (1) represents viscous drag force f_{drag} on an assumption of Stokes' law ($f_{drag} = 3\pi\eta dv$) of viscous drag force on a spherical particle moving in velocity v.

From this equation movement of the particle becomes damping motion or oscillation motion. We can determine them from a discriminant of characteristic equation of eq. (1). In conventional optical tweezers maximum force exerting by radiation pressure is around 100 pN, therefore the motion is always damping motion. In case of pulse laser beam the maximum force becomes around 1000 times as large as conventional case. The motion possibly becomes an oscillation motion. According to this we use the fastest damping time in damping motion as a reference of suitable pulse duration for particle manipulation.

The fastest damping motion occurs in case when the discriminant becomes zero. From this relation we can derive a condition of trapping stiffness k in the fastest damping motion as

$$k = \frac{(3\pi\eta d)^2}{4m} = \frac{27\pi\eta^2}{2d\rho} \quad (2)$$

where ρ is density of the particle material. Also the fastest damping time γ is derived from above equations as

$$\gamma = \frac{d^2\rho}{9\eta} \quad (3)$$

In actual application in bioscience field optical tweezers is often used for manipulation of 1-μm diameter particle. If we assume 1-μm diameter particle in water (viscous coefficient of 1.02×10^{-3} [Pa•s]) for this calculation, the required trapping stiffness becomes 44 mN/m and damping time is calculated as 110 ns. From above discussion we conclude the suitable pulse duration is around 100 ns to 1000 ns in case of manipulation of particles sized in several micrometers under water condition. Since the damping time is proportional to the square of the particle diameter from eq. (3), suitable pulse duration also varies with particle diameter. Suitable pulse duration for a certain applications should be chosen from diameter of the particle to be manipulated.

III. EXPERIMENTAL DEMONSTRATIONS OF PARTICLE MANIPULATION

As we derived the suitable pulse duration for pulse laser assisted optical tweezers, we have performed optical tweezers experiment with a long duration pulse laser. The long duration pulse laser which we used is a Q-switched Nd: YAG laser (Lotis Inc., model LS-2130, 1064-nm wavelength), which was specially developed to achieve a pulse beam with suitable duration. Pulse duration of the pulse beam is 160 ns in full width of half maximum (FWHM) and maximum pulse energy is 50 mJ at a repetition rate of 10 Hz. We established a whole setup on an inverted microscope (Nikon Inc., TE2000) with a high numerical aperture (NA) objective (Nikon Inc. PlanApo x60 WI, NA=1.20) combined with a conventional optical tweezers, which consists of a continuous wave (CW) Nd: YVO_4 laser (Spectra Physics Inc., BL-106C, wavelength of 1064 nm) and other optics. A pulse beam from the pulse laser is combined with the CW laser beam at a polarizing beam splitter and introduced into the microscope. Spot positions of both laser beams are always located at the same position. Also axial positions of both laser spots are carefully adjusted by changing the position of focusing lens in pulse laser beam optics.

We performed to manipulate particles fixed on glass surface as an example of particle manipulation in difficult situations. First particles were adhered on cover glass surface by ionic interaction between particles and substrate. Each particle was extracted one-by-one by irradiation of the pulse laser beam. Once a particle was removed from substrate, the particles can be transferred to a new position by moving a CW laser beam spot, and fixed at the position. Fig. 2 shows sequential images of this manipulation. Spot positions of both laser beams always located at equivalent place and therefore particles A and D in (a) and (d), respectively, could be caught by the CW beam immediately after the particle were extracted by the pulse beam. During the particle transfer the trap position was lifted up around 3 μm by moving objective lens to avoid collision with other particles. According to this other particles B and C fixed on glass surface were out of focus during transferring the particle A. When particles were going to be fixed, we moved particles to glass surface 1 μm more after contact to the surface to ensure particle fixation. The objective was moved by piezo actuator (PI Polytech Inc.). In this demonstration we used 1% APS aqueous solution to disperse particles and to adsorb the particle on glass surface repeatedly.

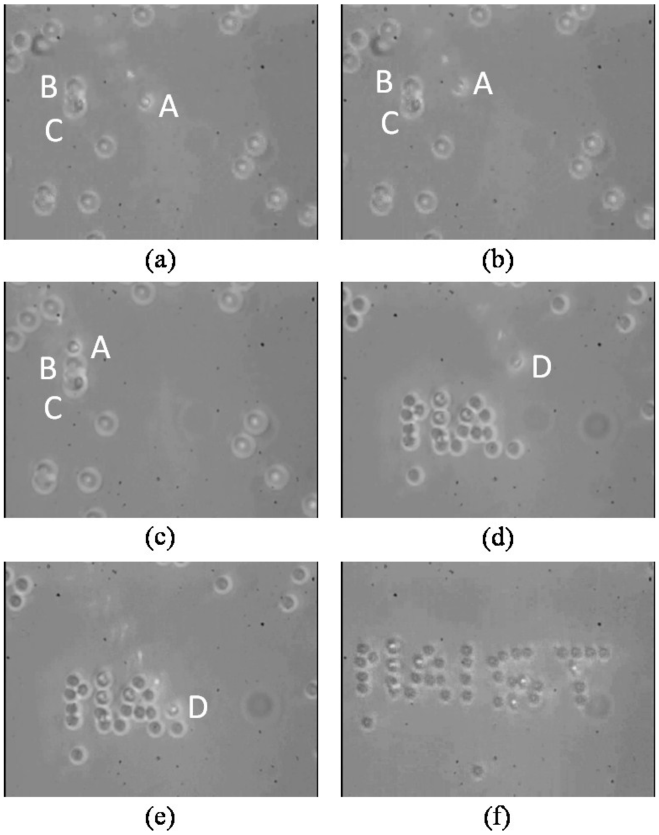

Figure 2. Sequential manipulation of adsorbed particle on cover glass surface. A particle A was irradiated by both pulse and CW laser beams (a), caught and transferred to new place and fixed again (b)-(c). A particle D was also manipulated to form a pattern (d)-(e). Finally a pattern was depicted with adhered particles (f). Image size of each picture is arround 80µm x 60µm.

IV. Effect of Pulse Assist in Different Pulse Durations

We have also performed pulse laser assisted optical tweezers with other durations of pulse lasers of 6 ns and 150µs. From a series of experiments we have investigated features of force exerted by each laser, manipulation capability of 1- to 5-micron diameter particles and eruption of laser induced dielectric breakdown (LIB), and performance of particle extraction from APS treated surface of glass by each laser. Table I summarizes each result of those investigations on pulse assist by different duration pulse laser beam. In terms of *in vivo* manipulation of cells 160-ns pulse duration is the most effective among them.

V. Conclusion

We have developed a pulse laser assisted optical tweezers with a suitable duration pulse laser beam. The suitable duration derived from particle motion in optical trap is 100 ns to 1000 ns in case of manipulation of a particle in water condition. We have successfully demonstrated effectiveness of a suitable duration pulse laser for pulse assist on optical tweezers and suppression of laser induced dielectric breakdown (LIB) phenomena. We have started to apply this scheme to cell manipulations. We hope this idea will be a ground breaking technology of optical tweezers.

TABLE I. Assist effects in PLAT with various pulse durations LIB is abbrebiation of Laser induced dielectric break down.

	6 ns (Pulse beam)	**160 ns (Pulse beam)**	**150 µs (Pulse beam)**
Feature of Force	Instantaneous strong force	Instantaneous Strong force	Instantaneous strong force
Suitable manipulation & effects	LIB is occurred. Not suitable for manipulation	Extraction, push in, hold and manipulation	Extraction, push in, hold and manipulation
Particle extraction from APS processed surface	Not Good Hardly performed, LIB is occurred	Good	Not Good Strong laser energy is needed (2.5 mJ more)

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