

Femtosecond optical tweezers for *in-situ* control of two-photon fluorescence

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Abstract: We perform a comparison of optical tweezing using continuous wave (cw) and femtosecond lasers. Measurement of the relative Q-values in the femtosecond and cw regimes shows that femtosecond optical tweezers are just as effective as cw optical tweezers. We also demonstrate simultaneous optical tweezing and *in-situ* control of two-photon fluorescence (at 400nm) from dye-doped polymer microspheres. By switching the 800 nm tweezing laser source between femtosecond and cw regimes, we turned the fluorescent signal from the tweezed particle on and off while maintaining an equivalent tweezing action. Femtosecond lasers can thus be used for optical tweezing and simultaneously utilized to induce nonlinear multi-photon processes such as two-photon excitation or even photoporation.

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1. Introduction

Optical tweezers [1-3] have become a powerful tool in the modern biological laboratory. The relative simplicity of the apparatus lends itself readily to numerous applications. They offer a non-invasive method for manipulating objects from 25 nm to tens of microns in size by using the optical gradient force. In the biological arena, optical tweezers have advanced significantly our understanding of molecular motors such as actin/myosin and kinesin walking on microtubules [4]. Other notable advances have taken place in the last decade. These include the use of light patterns for rotation [5] and creation [6] of 3D structures and the use of dynamic holograms for manipulation of micron-sized particles [7-9], multi-harmonic generation from optically-trapped particles [10, 11], three-dimensional imaging [12], photonic-force microscopy [13] and new wavelengths for optical traps [14].

Until now, virtually all optical tweezers have typically utilized continuous-wave (cw) laser sources that typically need only a few mW of cw power to instigate tweezing in three dimensions. While cw tweezers have been tremendously successful, femtosecond lasers provide impressive pulse peak powers that allow access to nonlinear processes within trapped particles, such as multi-photon absorption and harmonic generation. Femtosecond lasers are also undergoing a revolution where their footprint sizes have recently been reducing dramatically [15]. A key feature of such sources is the ability to switch conveniently between the cw (non-modelocked) and femtosecond-pulse regimes. Malmqvist and co-workers [11] have demonstrated second harmonic generation from optically trapped nonlinear Rayleigh particles using a femtosecond titanium-sapphire laser. Additionally, Xing and co-workers [16] have presented numerical modeling and theoretical analysis which predicts that femtosecond tweezers are entirely feasible. However, to our knowledge no demonstration of femtosecond optical tweezers in the Lorentz-Mie or Mie regime (where particle size is comparable to or much larger than the wavelength) has yet been reported, nor has there been any experimental comparison between cw and femtosecond optical tweezers involving any particle size.

In this paper we investigate the use of femtosecond lasers for optical tweezing in the Lorentz-Mie regime. We use a titanium-sapphire laser operating at 800 nm and a single-beam gradient force optical trap (optical tweezer) to investigate the lateral trapping force (Q-value) [17] on 1.28 μm silica spheres. The trapping forces were measured with the titanium-sapphire laser operating in cw and modelocked (femtosecond) regimes, and we can report that in the femtosecond regime ($Q = 0.1319 \pm 0.025$) the tweezing action is equally effective as that in the cw regime ($Q = 0.1301 \pm 0.043$) at similar average powers. This verifies that it is the average power rather than the peak power that is the key parameter for optical trapping. This also indicates that trapped-particle diffusion between successive femtosecond pulses is negligible.

A distinctive advantage in using femtosecond tweezers rather than cw tweezers is that the characteristically high peak powers of femtosecond pulses can be used to exploit nonlinear optical processes. We demonstrate this by achieving simultaneous two-photon fluorescence excitation and femtosecond optical tweezing of fluorescent (dye-doped) microspheres.

Although a similar experiment has been reported using cw optical tweezers [18], the fluorescent two-photon signal achieved with a femtosecond tweezer is much greater as expected. For a very low average (tweezing) power level (~5 mW) we can maintain optical tweezing and enhance any two-photon fluorescence observed in contrast to using a cw laser. An appealing aspect of this work is that, by simply switching the titanium-sapphire laser between cw and modelocked regimes, we turn the fluorescent signal from the tweezed particle on and off *in situ* while easily maintaining tweezing action. This technique thus allows optional and controllable ‘self-marking’ of tweezed nonlinear particles, as well as increased sensitivity of fluorescence studies at average powers well below the thermal damage threshold of biological samples. The use of femtosecond lasers for optical trapping in combination with multiphoton processes means for the first time we open up the prospect of a simplified optical microscope where a single laser source may facilitate trapping and subsequent imaging of the trapped sample by two-photon microscopy. This offer significant cost advantages combined with scientific versatility into the microscope apparatus.

2. The optical tweezers

As is already well known, optical tweezers use a strongly focused laser beam to confine and manipulate microscopic particles in three spatial dimensions. For particles larger than the wavelength of laser light, optical confinement (trapping) occurs when the light is refracted through a transparent particle, causing an exchange of momentum. The particle is drawn into the region of highest local intensity, and a well-trapped particle will remain close to the position of the beam focus when it is translated, both in horizontal and vertical directions. Typically only a few mW of laser power are required for three dimensional optical trapping.

For our investigation (Fig. 1), we made use of a titanium-sapphire laser operating at 800 nm. When modelocked, the femtosecond pulses were produced at a repetition-rate of 80 MHz (pulse spacing of 12 ns). The output from the laser was first passed through a neutral density (ND) filter to reduce the power to the few milliwatts required for tweezing. The beam was expanded with a simple telescope to fill the back of the ×100 objective lens. The beam focus was situated between a microscope slide and coverslip, containing the sample microspheres in a water solution. A CCD camera observed the trapped microspheres, illuminated by a white light source, as well as any induced fluorescence. Because our laser was Kerr-lens modelocked (KLM), its operation could be switched conveniently between cw and femtosecond regimes.

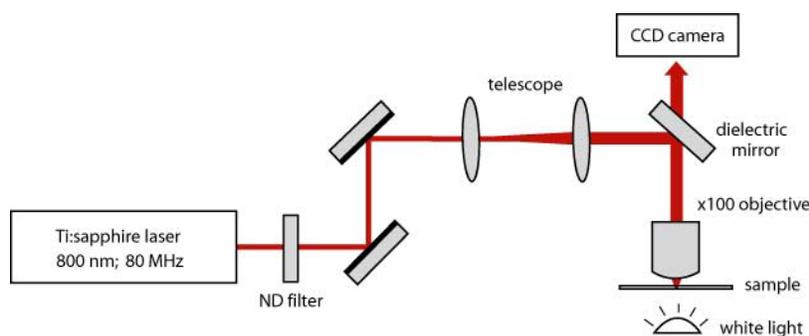


Fig. 1. Experimental set-up for the femtosecond and cw optical tweezers

The degree to which a microsphere is effectively trapped (the trapping efficiency) can be quantified by a dimensionless value, Q , defined in eq.(1)[17] as:

$$Q = \frac{3\pi\eta d v k c}{n P_{twz}} \quad (1)$$

η is the viscosity of the suspending medium, d is the particle diameter, v is the maximum lateral velocity at which the particle remains trapped, k is a correction factor ($k = 1$ for trapping of particles far from the chamber walls, as is the case here), c is the speed of light, n is the refractive index of the suspending medium and P_{twz} is the average tweezing power at the beam focus. A high trapping efficiency is also strongly dependent on a high mode quality of the Gaussian laser trapping beam and any aberrations present.

A well established technique to determine the ‘Q-value’ of the optical tweezer involves the lateral translation of the sample slide at known increasing velocities until the confined particle is no longer held in the trap. It is normal to perform this measurement on a number of particles and at various power levels to deduce an average. In the next section, we make a measurement of Q-values for the above tweezers under cw and femtosecond illumination conditions.

3. Femtosecond versus continuous wave optical tweezers

When a laser operates in the femtosecond regime, the output beam dimensions may be slightly different from when the laser is operating in a continuous wave. This is as a result of the intracavity oscillating field being focused more tightly within the laser crystal under femtosecond conditions due to the optical Kerr effect. Given the requirement on trapping efficiency for a high quality Gaussian beam shape and also the potential change in the Q-value from any beam deviation we might induce, we measured the horizontal and vertical beam profiles of the output beam from the titanium-sapphire laser in both cw and modelocked regimes. Figure 2(a) and Fig. 2(b) illustrate the horizontal and vertical beam profiles respectively, where the 1/e diameters, D , for each profile are indicated.

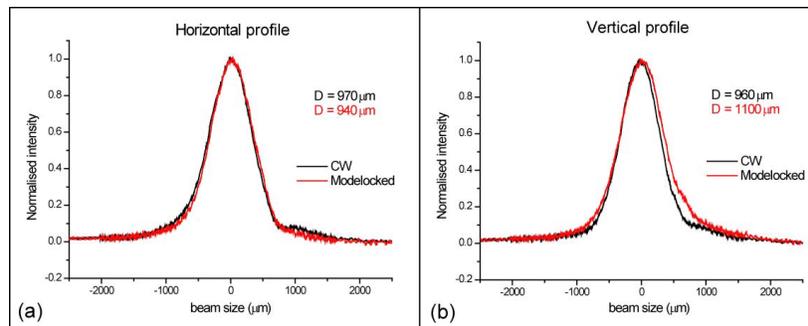


Fig. 2 (a) Horizontal and (b) vertical profile of the output beam from the titanium-sapphire laser in both cw (non-modelocked) and femtosecond (modelocked) operations.

In making comparisons between the trapping efficiency under cw and femtosecond operation, we determined that the observed small difference in beam profiles illustrated in Fig. 2 had a negligible effect on the determination of Q-values which follow.

We first obtained optical tweezing of silica spheres ($d = 1.28 \mu\text{m}$; *Bangs Laboratories*) in water ($n = 1.33$; $\eta = 1 \times 10^{-3} \text{ Nsm}^{-2}$), held between a microscope slide and coverslip, and placed beneath the $\times 100$ objective in Fig. 1. The maximum lateral velocity at which the particle remains trapped, v , together with the measured average laser power at the $\times 100$ lens focus, P_{twz} , was then used to calculate the Q-value from eq. (1). This was repeated for ten different spheres, and at three laser power levels. The entire procedure was then repeated when the titanium-sapphire laser was operating in the femtosecond regime. Average laser tweezing powers of less than 5 mW is all that was required throughout the experiment, based on a measured 20 % transmission of the $\times 100$ objective lens.

Table 1 provides a summary of the overall Q-value for each regime, including an experimental error calculated as the sample standard deviation. It is clearly evident that, as both laser regimes imply a Q-value within experimental error of each other, femtosecond optical tweezers are just as effective as cw optical tweezers, and that it is the average power of

the laser source (rather than the peak power) that dominates the optical trapping force. As a further confirmation of this, we performed three further Q-value determinations at different laser powers, using both the femtosecond and cw tweezers on the exact same trapped silica sphere. The difference between the average Q-value in the two regimes was only 2 %.

Table 1. Comparison of Q-values between cw and femtosecond optical tweezers for 1.28 μm spheres

Tweezer regime	Q-value
Continuous wave	0.1301 ± 0.043
Femtosecond	0.1319 ± 0.025

4. *In-situ* control of two-photon fluorescence using femtosecond optical tweezers

The observation of two-photon fluorescence excitation in cw optical tweezers has been used as an *in-situ* probe to study the physiology of an optically confined sample [18]. In the study, a cw laser operating at 1064 nm was used to confine cell specimens while simultaneously exciting visible fluorescence from micron-sized cellular regions. A single focused Gaussian beam excited a human sperm cell tagged with a fluorescent dye emitting at a peak wavelength of 620 nm. A near-square-law dependence of the fluorescent intensity on incident trapping-beam laser power was measured, which is consistent with a two-photon absorption process.

There exist a number of other fluorescent dyes and proteins that can be induced to fluoresce at various excitation wavelengths within the visible spectrum. Green fluorescent protein (GFP), for example, absorbs light principally at 395 nm, while fluorescing at a peak of 509 nm. Cyan, yellow and blue fluorescent proteins also exist, and such dyes and proteins are used frequently in biology as visible markers.

Here we demonstrate how, by using femtosecond-laser-based optical tweezers, the fluorescent signal from biological markers such as GFP could be significantly enhanced. The peak power reached by femtosecond pulses is typically 10^5 times higher than the corresponding cw regime at the same average power. Not only does this increase in peak power directly amplify any fluorescence signal, but indirect multi-photon absorption processes can also be utilized in conjunction with the optical tweezing. Care must of course be taken to obviate any photobleaching in such operation. By switching the laser between cw and femtosecond regimes we can turn the multiphoton signal on and off using our laser system.

To demonstrate this, we used the femtosecond titanium-sapphire laser operating at a centre wavelength of 800 nm to achieve simultaneous two-photon fluorescence excitation and femtosecond optical tweezing of fluorescent polymer microspheres ($d = 1.0 \mu\text{m}$). The polymer microspheres (*B100, Duke Scientific*) are characterized by an excitation band around 400 nm. Fluorescence emission occurs at peaks of 445 nm and 473 nm (Fig. 3). With the titanium-sapphire laser operating at 800 nm, any observed fluorescence is therefore generated as a result of a two-photon absorption process.

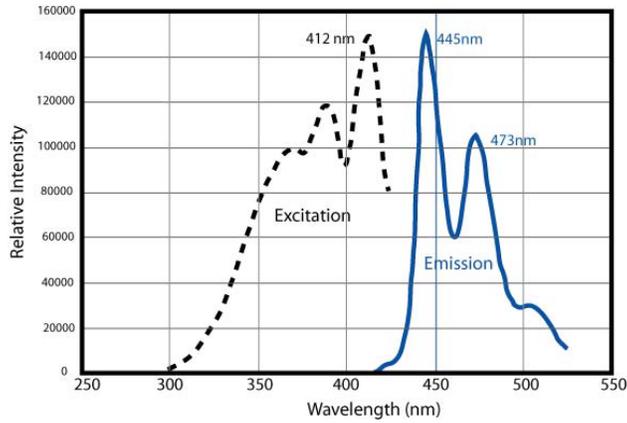


Fig. 3 Excitation and emission characteristics of the blue fluorescent polymer microspheres [www.dukescientific.com]

Placing a sample of the blue fluorescent microspheres in water, and repeating the above procedure, we achieved successful tweezing in both the cw and femtosecond regimes. When using the femtosecond optical tweezers, the tweezed particle exhibited a strong fluorescence intensity, discerning it easily from the surrounding medium and un-tweezed spheres. This strong fluorescence, which was absent when using the optical tweezers in the cw regime, was measured as a function of tweezing power in order to confirm the quadratic nature of the two-photon absorption process. Figure 4 shows our measured data (incorporating fractional errors in fluorescence intensity (10%) and tweezing power (5%)) plotted as logarithms. (The straight line indicates a quadratic reference).

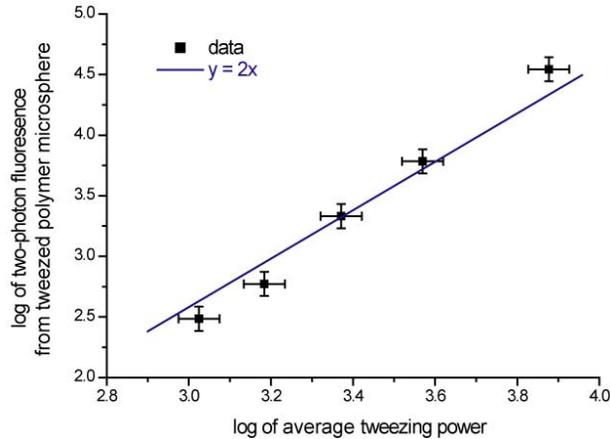


Fig. 4 Logarithm of average tweezing power versus logarithm of fluorescence intensity from tweezed polymer microspheres. The straight line $y = 2x$ describes the quadratic nature of the two-photon absorption process.

In Fig. 5 we present a movie sequence which demonstrates optical tweezing of these blue fluorescent microspheres. The movie begins with the titanium-sapphire laser operating in the femtosecond regime, and a microsphere being tweezed while exhibiting a strong two-photon fluorescence. Approximately two thirds of the way through the movie, the laser is switched to the cw regime. At this point, the fluorescent signal disappears but tweezing of the same sphere is easily maintained at the same strength. This procedure is entirely reversible and allows optional and controllable 'self-marking' of tweezed nonlinear particles. There is also

the possibility of increased sensitivity in fluorescence studies at average powers well below the thermal damage threshold of biological samples.



Fig. 5 (2.6MB) Movie (monochrome) of simultaneous two-photon excitation fluorescence and femtosecond optical tweezing of dye-doped 1.28 μm polymer microspheres. While tweezing of the same microsphere is maintained, the fluorescent signal is turned off by simply switching the titanium-sapphire source laser from the femtosecond regime to the cw regime. This procedure is entirely reversible ([3.5MB version](#)).

5. Conclusions and summary

We have performed a comparison of optical tweezing using continuous wave and femtosecond laser sources. We find that it is the average power that dominates the tweezing action and obtain near identical Q -values for tweezing microspheres in both the femtosecond and cw regimes. As demonstrated here, the use of a femtosecond laser in optical tweezing offers some distinct advantages in that multi-photon processes can be exploited in conjunction with effective tweezing action. We have demonstrated this for two-photon excitation with optical tweezers, and switched the two-photon signal from the trapped object on and off as desired. This may have applications when tracking and observing biological fluorophores. Given that a tweezing power of just a few mW is adequate to achieve femtosecond and cw optical tweezing, the recent development of compact femtosecond laser sources for biological and medical applications are of particular interest [19].

We conclude by mentioning a potential avenue for further investigation. In the biological arena, we are investigating a combination of tweezing and guiding [20] using femtosecond laser pulses for the DNA transfection of cells [21], because femtosecond laser pulses can be used to transfect cells *in vivo*. Our work forms a step forward in developing a simplified multi-functional optical microscope that can perform both multiphoton imaging and optical trapping simultaneously. The very same laser can then be used for moving and imaging cells, and even photoporating or cutting into the cell membrane for DNA transfection.

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