Published in final edited form as:

Proc SPIE Int Soc Opt Eng. 2010 August 27; 7762: . doi:10.1117/12.862364.

Towards Stable Trapping of Single Macromolecules in Solution

Arijit Kumar De*, Debjit Roy, and Debabrata Goswami

Department of Chemistry, Indian Institute of Technology Kanpur, UP – 208016, India

Abstract

The implementation of high instantaneous peak power of a femtosecond laser pulse at moderate time-averaged power (~ 10 mW) to trap latex nanoparticles, which is otherwise impossible with continuous wave illumination at similar power level, has recently been shown [De, A. K., Roy, D., Dutta, A. and Goswami, D. "Stable optical trapping of latex nanoparticles with ultrashort pulsed illumination", *Appl. Opt.*, 48, G33 (2009)]. However, direct measurement of the instantaneous trapping force/stiffness due to a single pulse has been unsuccessful due to the fleeting existence (~ 100 fs) of the laser pulse compared with the much slower time scale associated with the available trapping force/stiffness calibration techniques, as discussed in this proceeding article. We also demonstrate trapping of quantum dots having dimension similar to macromolecules.

Keywords

Optical tweezer; femtosecond pulses; Rayleigh particles

1. INTRODUCTION

Trapping and manipulating single molecules has been made possible through the implementation of light-induced pressure using optical tweezers or single-beam gradient optical traps [1]. In such experiments, usually a macro-molecule, having few tens of nanometer in dimension, is tethered to a micron-sized bead which is trapped and the process can be observed in far-field microscopy [2]. Thus the forces thus measured are never the true forces acting on the single molecules which demands trapping without tethering. Although direct trapping of such objects (dimensionally similar to macro-molecules) have been demonstrated using continuous-wave (CW) laser beam, it demands the use of quite high average power. In addition, video microscopy is difficult due to their sub-diffraction dimension (although recently there has been major advances in sub-wavelength scale far-field microscopy called 'super-resolution microscopy' [3]). In this regard, we have shown that ultrafast pulsed excitation can do the same job at quite low average power and a combination with non-linear fluorescence microscopy leads to the detection of trapping Rayleigh particles (*i.e.* dimension of the particle is much smaller than the wavelength of light used) [4, 5]. In this article, we present a comparative discussion on using ultrafast

pulsed and CW illumination schemes to trap Rayleigh particles. We also show trapping of quantum dots having macromolecular dimension.

2. METHODOLOGIES

We used a completely home-made table-top tweezer [6] the detail of which is discussed elsewhere [4, 5]. Briefly, the output from a titanium sapphire oscillator (Mira 900-F pumped by Verdy5, Coherent Inc.), producing ~ 120 fs pulses (centered at 780 nm) at 76 MHz repetition rate, was sent to the designed inverted microscope (equipped with white light Köhler illumination) through a telescope and a pair of steering mirrors after expanding the beam. A dichroic beam-splitter sent the beam to a 100X oil-immersion objective (UPlanSApo 1.40 NA, Olympus Inc.) which traps the particles and collects the back-scattered two-photon fluorescence. The epi-fluorescence (or the white light) is transmitted through the dichroic beam-splitter and collected by a photo-multiplier tube (or imaged by a CCD camera with 350 k pixel, e-Marc Inc.). The photo-multiplier tube signals were fed into an automotive oscilloscope or 'picoscope' (Pico Technology Ltd) triggered by a rotating-disk optical chopper (having 30 slot wheel) which is run at 800 Hz by a tunable frequency driver (MC1000A, Thorlabs Inc.). Fluorescent polystyrene microsphere with 4.1 μm diameter and quantum dots with ~ 16 nm average diameter (F8858 & Q21031MP, Molecular probes Inc.) were used for trapping. Dilute and slightly alkaline solution of the samples were well sonicated and immediately used for trapping. The sample stage was attached to a mechanical stage (UE1724SR driven by ESP300, Newport) which was connected to a tunable velocity controller (ESP 300, Newport Inc.).

3. RESULTS AND DISCUSSIONS

Although the use of high instantaneous peak power of laser pulses has been shown to be able to trap Rayleigh particles theoretically [7] and demonstrated experimentally [4], the force calibration by viscous drag [8] or trap stiffness calibration by Brownian fluctuations [9] has been found to yield same results for both CW and pulsed regimes of operations; this has led to conclusions that ultrafast pulsed excitation has the same efficiency of CW excitation. The fallacy lies in the fact that both viscous drag as well as fluctuation based methods involve much slower time scales compared with the interaction time (or pulse-width) of a femtosecond light pulse and effect of individual pulses are completely washed out; thus such experiments furnish information only on cumulative force exerted by many pulses which may be computationally obtained by sampling over many pulses [9, 10] and experimentally only the average power appears to be the deciding factor [7, 9]. This is precisely the reason no effect of pulse chirping on trapping efficiency has been reported [9]. A true approach will be to calibrate the instantaneous force exerted by a single pulse which has been hitherto rendered un-achievable although its effect has been realized experimentally [4].

The escape velocities (along both parallel and perpendicular direction to laser polarization) have been found to be the same for the 4.1 micron sized bead under pulse and CW excitation schemes, as shown in figure 1. However, pulsed excitation leads to stable trap of tiny quantum dots of ~ 16 nanometer size which can be observed via background-free two-photon

fluorescence as shown in figure 2. The absence of distinct steps (one step corresponds to one particle) in the signal as before [4] is due to multiple trapping as well as the wide size distribution (~10 to 20 nanometers) coupled with very low non-linear fluorescence signal. This can be taken care of by using lock-in detection of fluorescence from quantum dots with sharp size distribution. Increasing the power does not help as it also increases the forward scattering force leading to unstable traps. Also the repetition rate needs to be higher so that the trapped particle does not leave the focal volume (~ 10 femto-liter) during the dead-time between the pulses.

4. CONCLUSIONS

Thus we have shown, although using the gigantic instantaneous peak power of femtosecond laser pulses we can trap tiny Rayleigh particles stably at quite low average power level, the instantaneous trapping force/stiffness due to a single pulse is difficult to measure and in every endeavor the cumulative effect of many pulses renders the measurement obscure. Further use of optimized pulsed excitation to efficiently trap single macromolecules is presently being investigated in the authors' lab.

ACKNOWLEDGEMENTS

AKD and DR thanks CSIR, India for graduate fellowship. We thank DST, India and Wellcome Trust Foundation, UK for funding.

REFERENCES

- [1]. Ashkin A, Dziedzic JM, Bjorkholm JE, Chu S. Observation of a single-beam gradient force optical trap for dielectric particles. *Opt. Lett.* 1986; 11:288. [PubMed: 19730608]
- [2]. Bustamante C, Macosko JC, Wuite GJL. Grabbing the cat by the tail: manipulating molecules one by one. *Nature Rev. Mol. Cell Biol.* 2000; 1:130. [PubMed: 11253365]
- [3]. Hell SW. Microscopy and its focal switch. *Nature Met.* 2009; 6:24.
- [4]. De AK, Roy D, Dutta A, Goswami D. Stable optical trapping of latex nanoparticles with ultrashort pulsed illumination. *Appl. Opt.* 2009; 48:G33.
- [5]. De AK, Roy D, Goswami D. Towards spatio-temporal control in optical trapping. *Proc. SPIE.* 2009; 7400:7400G-1.
- [6]. De AK, Roy D, Saha B, Goswami D. A simple method for constructing and calibrating an optical tweezer. *Current Science.* 2008; 95:723.
- [7]. Wang L, Zhao C. Dynamic radiation force of a pulsed Gaussian beam acting on a Rayleigh dielectric sphere. *Opt. Express.* 2007; 15:10615. [PubMed: 19547415]
- [8]. Agate B, Brown CTA, Sibbett W, Dholakia K. Femtosecond optical tweezer in-situ control of two-photon fluorescence. *Opt. Ex.* 2004; 12:3011.
- [9]. Shane JC, Mazilu M, Lee WM, Dholakia K. Effect of pulse temporal shape on optical trapping and impulse transfer using ultrashort pulsed lasers. *Opt. Ex.* 2010; 18:7554.
- [10]. Xing Q, Mao F, Chai L, Wang Q. Numerical modeling and theoretical analysis of femtosecond laser tweezers. *Opt. Laser Technol.* 2004; 36:635.

Figure 1. Variation of escape velocity with average laser power parallel to the laser polarization (left) and perpendicular to it (right) under pulsed (solid circles) and CW (void triangles) excitations with linear fits.

Figure 2.
Two-photon fluorescence signal indicative of trapping ~16 nm quantum dots.