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ISBN
9780819498601

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Publication Date
2014-03-04

DOI
10.1117/12.2040014

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Nima Khatibzadeh, Yesenia Rocha, Linda Z. Shi, Michael W. Berns, "Effects of media viscosity and particle size on optical trapping of microspheres," Proc. SPIE 8947, Imaging, Manipulation, and Analysis of Biomolecules, Cells, and Tissues XII, 89470G (4 March 2014); doi: 10.1117/12.2040014

SPIE.

Event: SPIE BiOS, 2014, San Francisco, California, United States

Effects of media viscosity and particle size on optical trapping of microspheres

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ABSTRACT

In this study, we investigated the effects of size and surrounding media viscosity on trapping of microspheres. A continuous wave ytterbium fiber laser with a 1064 nm wavelength was used to create an optical tweezers system for optical manipulation experiments. Briefly, the system consisted of an inverted microscope, and a 100X 1.4 NA oil immersion objective through which the laser beam converged to form the optical trap. The laser beam was collimated, steered, and coupled to the microscope through the epifluorescence microscope port. The laser power at the trap focal spot was determined by measuring the input power at the back aperture of the objective multiplied by the objective transmission factor at 1064 nm measured by a modified dual objective method. Polystyrene microspheres varying in diameter from 5 to 15 microns were suspended in liquid media in glass bottom petri dishes prior to trapping experiments. The microspheres were trapped at different trapping powers, and fluidic viscous drag forces where applied to the optically trapped microspheres by driving a computer controlled 2D motorized microscope stage at known velocities. The drag forces were calculated at the point that the microspheres fell out of the trap, based on the Stokes equation for flow around spheres. The data show a linear relationship between trapping force and trap power within the range of the microsphere diameters and media viscosity values used. The work includes calculation of the dimensionless trap efficiency coefficient (Q) at 1064 nm wavelength and the corresponding effects of media viscosity and microsphere size on (Q).

1. INTRODUCTION

Laser tweezers is a precise technique to manipulate nano and micron size objects and may act as an optical force transducer [1]. Given the noninvasiveness of the optical tweezers, it has been

Imaging, Manipulation, and Analysis of Biomolecules, Cells, and Tissues XII, edited by Daniel L. Farkas, Dan V. Nicolau, Robert C. Leif, Proc. of SPIE Vol. 8947, 89470G

extensively used for manipulation of biological specimens and for measurement of biological forces [2,3]. Determination of biological forces is essential for understanding biological phenomena such as sperm motility [4] and molecular motors [5,6], determination of the forces associated with organelle transport [7], compliance of the rotary motor located in bacteria flagellae [8], and in investigations on mechanical [9-11] and electromechanical properties of cellular plasma membranes [12].

Measurement of biological forces with optical tweezers may be performed by different methods, such as measuring displacement of a trapped microsphere from the trapping center by using a quadrant photodetector (QPD). In this method, the displacement of the microsphere in response to the forces exerted from the specimen can be calibrated to yield the force value. The trapping force *F* can also be estimated from the laser power at the focal volume by the following equation [13,14]:

$$F = \frac{QPn}{c} \quad (1)$$

where *F* is the trapping force (N), *P* is the incident laser power at the specimen (*W*), *c* is the speed of light (m/s) in vacuum, *n* is the refractive index of the surrounding medium, and parameter *Q* is the dimensionless trapping efficiency. In equation 1, the parameter *Q* is the fraction of the incident light momentum which is transferred to the trapped object, and varies from 0 to 1, and depends on the sample as well as the trapping beam properties. Given the correspondence between the trapping force and the in situ laser power, the knowledge of the force-power relationship enables optical determination of the forces and the use of optical tweezers as a force transducer.

2. MATERIALS AND METHODS

2.1. Optical tweezers setup

The laser beam from a continuous wave 1064 nm wavelength ytterbium fiber laser (PYL-20M, IPG Photonics) was collimated and steered through a series of mirrors and lenses, and coupled into the epifluorescence port of an inverted microscope (Zeiss Axiovert-135). A dual band laser dichroic mirror (Chroma Technology, z532/1064rpc) was used to reflect the infrared (IR) laser beam toward the microscope objective while simultaneously transmitting the light from the specimen to a camera. The laser beam was focused through a high numerical aperture (NA=1.4) oil immersion, Phase III, 100X objective (Zeiss Plan-Apochromat).

2.2. Laser exposure and dosimetry

A triple objective method which is a modified dual objective method [15] was used to determine the objective transmission coefficient at 1064 nm. Using this method, it was determined that the transmission through the objective was 25% at the trapping wavelength. In these experiments,

laser power in the focal spot was varied from ≈20 to 55 mW as described for each individual experiment.

2.3. Microsphere trapping

Single polystyrene microspheres with different diameters were trapped using the 1064 nm wavelength optical tweezers described above. The fluidic viscous drag forces were applied to the trapped microsphere by driving the motorized microscope stage at known velocities. The viscous drag force values were calculated based on the Stokes' equation for fluid flow around spheres with low Reynolds numbers (Re<<1):

$$F_{drag} = 6\pi\mu r v\beta \qquad (2)$$

where F_{drag} is the viscous drag force (N), μ is the dynamic viscosity (N.s/m^2) of the surrounding media, r is the radius of the microsphere (m), parameter v is the velocity of the fluid flow (m/s) passing the microsphere. β is a correction factor for the viscous drag force arising from proximity of the trapped microsphere to the bottom surface and can be calculated from equation 3 [14]:

$$\beta = \left[1 - \frac{9}{16}(r/h) + \frac{1}{8}(r/h)^3 - \frac{45}{256}(r/h)^4 - \frac{1}{16}(r/h)^5\right]^{-1} \qquad (3)$$

where h is the distance above the surface of the center of the trapped microspheres. The velocity of the fluid flow was increased until the trapped microsphere dropped out of the trap. The polystyrene microspheres with diameters of 4.5 µm (Polysciences Inc., Warrington, PA, USA), 10 µm (Polysciences Inc., Warrington, PA, USA), and 15 µm (Molecular probes, Eugene, OR, USA) were used. The surrounding media viscosity values were varied between 1-7 cP for each microsphere size.

2.4. Stage movement and control

Controlled transverse motions in the x and y directions were provided using a microstepper-motor driven stage for inverted microscopes (Ludl Electronic Products, BioPrecision2, NY, USA). The stage was driven by the LabView (LabView 8.5.1, National Instruments, TX, USA) based RoboLase III system software through which the stage could be controlled and driven in either x or y directions at given velocities over desired distances with a minimum movement resolution of 200 nm.

2.5. Viscous media preparation

Methyl cellulose (Sigma Aldrich, M7140, St. Louis, MO, USA) was used to increase the viscosity of the microsphere suspension solutions according to a previously described method [16].

3. RESULTS AND DISCUSSION

The trapping force-power relationships of the microspheres were obtained over a range of ≈20-55 mW in the focal volume. The trapping efficiency was calculated based on the force-power data and using equation 1. In our previous report [16], we have analyzed the effects of adding methyl cellulose on the refractive index of the media and no significant changes in the refractive indices were observed upon addition of up to 2% (w/w %): the refractive indices remained unchanged at ≈1.33. Therefore, this value for the parameter *n* was used in the studies reported here.

A typical trapping force-power relationship is shown in Figure 1 for polystyrene microspheres 15 µm in diameter in media with 3 cP viscosity. The trapping force increased as a function of power at the focal volume. A linear regression was used to model the force-power relationship of the microspheres (solid red line on the graph).

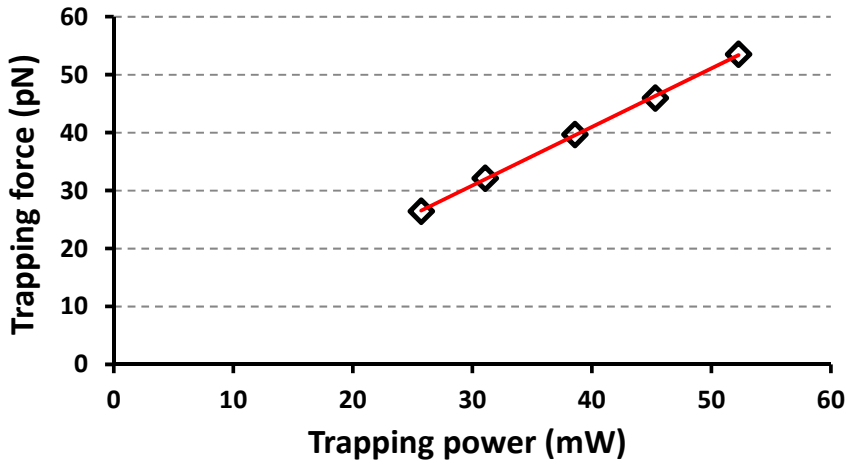

Figure 1- The trapping force-power relationship of 15 µm polystyrene microspheres trapped at 1064 nm wavelength in 3 cP media.

The trapping efficiency of the microspheres was calculated over a range of powers based on equation 1 (Figure 2). The trapping efficiency was unchanged over the power range examined.

Similar experiments were conducted on 15 µm microspheres in media with 1 and 7 cP viscosities. The trapping force-power data is similar to the results at 3 cP, and the trapping efficiency (*Q*) was independent of the surrounding media viscosity.

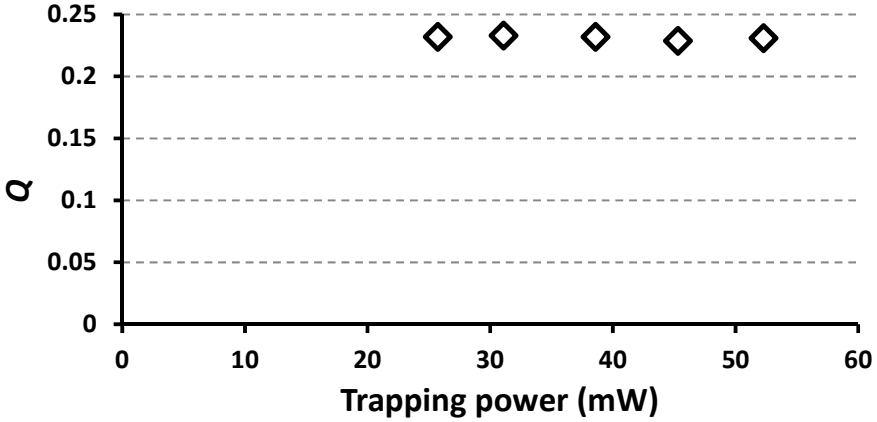

Figure 2- Calculated trapping efficiency (*Q*) versus trapping power for 15 µm polystyrene microspheres trapped at 1064 nm wavelength in 3 cP media.

Force-power experiments were extended to microspheres with diameters of 10 and 4.5 µm. Similar to experiments at 15 µm, there was an increase in force values at higher powers at the laser focal point. For each size microsphere, *Q* was independent of viscosity. With respect to the microsphere size, *Q* increased with increasing microsphere diameter. The mean±S.D. values of the *Q* for 4.5 µm microspheres was ≈0.1436±0.003, for 10 µm microspheres was ≈0.1923±2e-3, and for 15 µm microspheres ≈0.23±3e-3.

4. CONCLUSIONS

These results are important as a first-order approximation to the forces that can be applied either directly to biological objects or by means of microsphere handles attached to the biological specimen. Of particular interest is the determination that *Q* did not change over the force-power ranges investigated, and was independent of viscosity. The general trends illustrated by the microsphere measurements are certainly applicable to biological objects although their *Q* values may require more investigation due to the structural difference between the biological specimens and the polystyrene spheres used in this study.

ACKNOWLEDGEMENTS

We would like to thank Dr. Steve George's lab at University of California, Irvine for help with the Anton Paar rheometer device. This research was partially funded by a grant from the Beckman Laser Institute Foundation, and a grant from the Air Force Office of Scientific Research, AFOSR Grant # FA9550-08-1-0384.

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