REGARDING THE OPTICAL TRAPPING FORCES ON MICROPARTICLES

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Cu un fascicul laser bine focalizat se pot capta şi manipula particule (din material biologic sau dielectric) având dimensiuni de ordinul nanometrilor sau micrometrilor. In această lucrare se discută cazul captării particulelor din dioxid de siliciu (silice) cu ajutorul unui microscop inversat şi a unui fascicul laser gaussian. In partea teoretică se prezintă procedurile pentru măsurarea forțelor care acționează asupra particulei captate. Randamentele optice în experimente în care se folosesc "pensetele optice" au fost de 2-12%, cu valorile superioare pentru măririle 20x sau 40x ale microscopului. S-a calculat o creştere cu 24-50% a puterii laserului necesară pentru captarea particulelor de silice atunci când câmpul optic (placa microscopului) se deplasează cu 20-60 μm. Se discută, de asemenea, diferența între forțele de captare dacă se consideră viteza locală a fluidului sau viteza deplasării câmpului optic, precum şi modificarea valorilor acestor forțe la creşterea temperaturii.

Working with a strongly focused laser beam it is possible to trap and manipulate particles (of biological or dielectric material) in a size range from nm to μm. In this paper the case of the trap of silica particles by means of an inverted microscope and a Gaussian laser beam is discussed. In the theoretical part the procedures for measurement of forces acting on a trapped particle are presented. In experiments using optical tweezers the optical efficiencies were in the range of 2-12%, with upper values corresponding to 20x or 40x magnification of microscope. An increase of 24-50% in the laser power was calculated to trap silica particles if the displacement of stage is 20-60 μm. The difference between trapping forces considering either local speed of the fluid or the boundary speed as well a change of these forces due to the temperature increase are also discussed.

Keywords: optical tweezers, laser beam, single-beam trap, viscous drag force, trapping forces

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Introduction

The optical trapping forces are involved in so called "optical tweezers", which consist in a strongly focused beam passing through a high numerical aperture microscope objective. Even if they have been invented over 20 years ago, the optical tweezers remain an important tool for many applications in physics, chemistry and biology because of various materials of trapped particles (silica, polystyrene, biological cell etc.). Since 1986, when Ashkin *et al* [1] has reported first experimental results regarding optical trapping of dielectric microparticles by a single-beam gradient force, a wide variety of optical traps were investigated involving scattering and gradient forces acting on a trapped microparticle. The scattering force is proportional to the optical intensity and distance in the direction of the incident light while the gradient force is proportional to the gradient of intensity; obviously, the gradient force draws the bead in the direction where the optical intensity is larger (that is, towards the axis of laser beam). Both forces balance at the focus where the particle is stable trapped. Any small displacement from this position results in a restoring force which pushes the particle back to the equilibrium position. To obtain a stable 3D trapping the gradient force must dominate the scattering force.

Nowadays it is more and more interesting to measure the optical forces induced by the single-beam laser acting on the microparticle immersed in a fluid with known viscosity. In the literature two regimes were analysed regarding the size (d) of the trapped particle compared with the wavelength of the laser (λ): *Mie regime* (for $d \gg \lambda$) in which the optical technique is applicable, and *Rayleigh regime* (for $d \ll \lambda$) where a much more rigorous model of the dipole is supposed.

In this paper we shall discuss the trapping and manipulating of microparticles especially force measurements by using optical tweezers. We present the case when a silica microparticle is first trapped and then we move the microscope stage with *nm* accuracy till the particle escapes from the trap. In this state we may calculate the linear velocity and viscous drag force applying the Stokes' law. Also, according to Ashkin's papers [2] the optical force can be calculated knowing the laser power and the trapping efficiency. In *Experimental part* this procedure will be detailed. When the transversal optical force overpasses the drag force the particle falls out from the trap.

1. Calculation and measurement methods of the optical trapping force

Theoretical analysis of the interaction between a particle and an electromagnetic wave consists in the description of the incident beam, the field due to the interaction of this incident beam and the particle and finally, the forces acting on the particle surrounded by the resulting field [3]. Single beam optical trapping requires a tightly focused laser beam with a spot diameter comparable with the trapping wavelength. To achieve this condition, high-quality oil immersion objectives of microscope are usually used. In this case the beam passes through a number of dielectric interfaces (optical elements inside the objective, immersion oil layer, coverslip and water layer) a fact which makes a correct description of the field in the focal region to be quite complex. The effects caused by diffraction of the beam on the objective back aperture and by spherical aberration due to the refractive index mismatch at the dielectric interfaces below the objective influence strongly the final distribution of optical intensity. In spite of this, the simple Gaussian beam is still the most frequently considered form of the incident beam even if it is only a paraxial solution of the wave equation and it does not treat properly the polarization components in the focused beam.

Description of the field produced as a result of the interaction of an incident beam and a spherical microparticle is a general problem studied by the generalized Lorenz–Mie theory. The force interaction between the total outer field and the particle can then be quantified on the basis of the momentum-conservation principle and on assumptions that a fluid surrounding the particle is isotropic, non-magnetic, linear in its response to the applied field, and also in hydrodynamic equilibrium. Theoretical procedures were developed for calculation the optical forces acting on a sphere placed in a single corrected Gaussian beam (CGB). It was assumed that an initial field distribution close to the dielectric interface is created as the interference of the incident CGB and CGB retroreflected from the surface. Because it is supposed that the retroreflected beam is always weak in intensity the final axial optical intensity distribution has an incident CGB envelope modulated by a weak standing-wave component with modulation depth proportional to $R^{1/2}$ (R is the surface reflectivity). Therefore, negligible low value of R (a water–glass interface has $R \approx 0.04$ %) can produce a standing-wave component. If a sphere is inserted into the interference field of the incident and retroreflected CGBs close to a reflective surface, a complex scattering–reflection event occurs.

However, to simplify and speed up the calculation we omitted multiple scattered fields between the reflective surface and the sphere. Therefore, the distribution of incident field that was scattered by the sphere is resulted only from the interference of the incident CGB and the counter-propagating reflected CGB.

This distribution is inserted into the above-mentioned generalized Lorenz–Mie theory. To speed up the calculation it is also assumed that the spherical object is located on an axis of coordinates system and it can thus employ the radial symmetry of the system.

There are several experimental procedures for the measurement of the forces (of the magnitude order of picometers) acting on a trapped particle immersed in a fluid. One of them is based on the brownian motion of the particle; another procedure is based on the comparison between transversal optical force and drag force in a fluid of known viscosity. Obviously, both scattering and optical forces are due to the interaction of light with the particle located in a place near the focus of the beam. For a good trapping the scattering force always acts along the direction of propagation whereas the gradient force pushes the particle in direction of increasing optical intensity. These two forces are balanced at the focus where the particle is stable trapped. However as Fig. 1 shows to obtain a stable 3D trapping the gradient force must dominate the scattering force. Any small displacement from the position where the particle is trapped results in a restoring force that pushes the particle back to the equilibrium position. If this displacement is due to collisions with another particle in the surrounding fluid, the particle has a random movement with averaged velocity equal to zero and constant power spectrum.

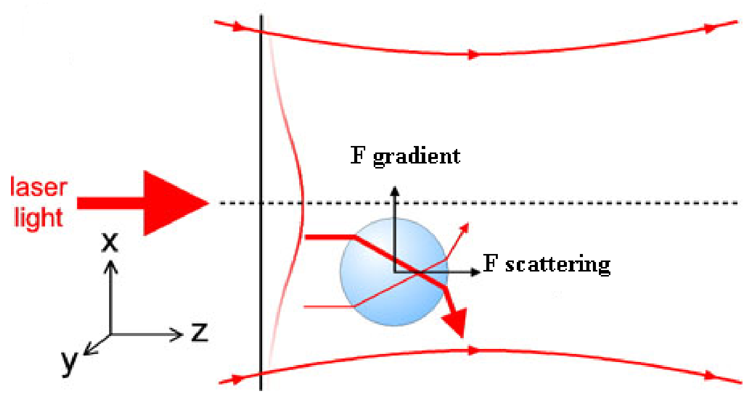

Fig. 1. The gradient and scattering forces acting on a microparticle subjected to exposure in a laser beam

It is well known that the radiation force F_p, exerted on a surface in a medium of refractive index n by a parallel light beam of power P, is given by $F_p = nP/c$, where c is the light speed in vacuum. As a correction, for a particle existing in an optical trap, where the light impinges from many directions, a dimensionless quantity Q is introduced to denote the efficiency of the trap. The value of Q takes into account the particle size and mass, the refractive index difference between the particle and surrounding medium, the density of the medium, the nature of laser beam as well as the laser power.

Thus, the trapping force F will be given by:

$$F = \frac{n P Q}{c} \tag{1}$$

The above equation has been experimentally verified proving the transversal trapping force is proportional to the laser power involved in the experiment.

There are two main approaches for measuring the trapping force of an optical trap. One method is based on estimation of the trap stiffness (k) and then calculating the restoring force for the trapped particle, as the product of trap stiffness and the optical separation distance (x) between the center of the trap and the particle: $F_{opt} = k \times x$. The trap stiffness can be calculated by measuring the extent of the particle movement by diffusion. For such experimental technique the establishing of a power spectrum for the particle motion is required, and further a detector with a large bandwidth; due to such experimental difficulties this method is not commonly used.

The second method for measuring the trapping force consists in moving the fluid medium in which the trapped particle is suspended (it remains in a stationary state). This fact induces a viscous drag force F_d on the trapped particle that is proportional to the speed of fluid flow. Whether the velocity of the movement is increased or the laser power is reduced the particle may escape from the trap; at this stage the trapping force is considered to be equal to the viscous drag force.

The fundamental equations describing fluid dynamics (Navier–Stokes equations) lead to an expression for the viscous drag force on a sphere surrounded by a fluid flow; this force is referred to as the Stokes drag force, F_d. The expression for Stokesian flow is applicable only if the value of Reynolds criterion for the fluid flow (Re), given by (2) is much less than 1, allowing the inertia terms to be omitted from the Navier–Stokes equations.

$$\mathrm{Re} = \frac{\mathrm{v}\, \mathrm{d}\, \rho}{\eta} = \frac{\mathrm{v}\, \mathrm{d}}{\nu} \tag{2}$$

In the Reynolds' expression (2) v and d are the velocity of fluid and diameter of particle, respectively, and η and ν are the dynamic and kinematic viscosities. For a trapped particle of 6 μm diameter, suspended in distilled water moving at a velocity of 750 μm/s, the value of Re criterion is approximately 10^{-4} and therefore the Stokes drag law (equation (3)) is valid:

$$F_d = 3\, \pi\, d\, \eta\, v \tag{3}$$

where v is the velocity of the fluid flowing behind the particle.

Since the Stokes law is valid for a particle far from any boundary walls, a correction is often made to take into account the possible shear effects caused by the close proximity of a boundary wall. The correction includes a factor governed by the Faxen law which involves shear effects due to particle rotation. Thus, the corrected Stokes drag force for a sphere moving next to a single parallel wall is given by (4), where *a* is the particle radius and *l* is the distance from the boundary wall.

$$F = \frac{3\pi\eta d v}{1 - \frac{9}{16}\frac{a}{l} + \frac{1}{8}\left(\frac{a}{l}\right)^3 - \frac{45}{256}\left(\frac{a}{l}\right)^4 - \frac{1}{6}\left(\frac{a}{l}\right)^5} \qquad (4)$$

Using equations (1) and (4) the trapping forces and trapping efficiencies in many different circumstances have been evaluated. We assessed the reliability of such calculations where the fluid was moved with different velocity functions.

2. Experimental Part

The optical set-up used for trapping, manipulating and optical forces measurements was described in a previous work [5]. It consists in a Nikon TE-2000 inverted microscope with a *piezoelectric* stage MELLES GRIOT, programmed for *nm* displacements, a Hamamatsu spatial light modulator, placed in front of a dichroic mirror and a laser (yterbium) beam with continuous wave at 1064 nm.

All discussions presented in this paper are referred to spherical silica microparticles (2.34 μm diameter) immersed in water (1.33 refractive index) which are trapped with optical tweezers. The depth of the trapping was approximately 48,8 μm below the microscope coverglass.

In each experiment the microscope slide, coverslip and sample stage are all moved at the same velocity. Nevertheless, the fluid existing near the surface of the coverslip also moves with the same velocity but increasing distance from the surface decreases the velocity of the fluid. This implies that the velocity of the fluid surrounding the trapped particle has different values depending on how far the particle is from the moving surface, that is on the trap depth. The time span for which the stage is moved or the distance span over which it is moved could also change the local velocity of the fluid flowing behind the particle.

3. Results and Discussion

Fig. 2 presents the results of measured efficiency (Q) of the setup as a function of laser power for different objectives of Nikon microscope. It is noticed a constant value for efficiency when the laser power is changing from 0.2 W to 0.7 W; however, this limiting efficiency decreased for higher magnification in a range of variation of 2-12 %.

In fluid dynamics several procedures were described to obtain exact solutions of Navier–Stokes equation and, consequently, a velocity profile. For example, the Navier-Stokes equation was solved for a particular case when a triangular function is imposed for the velocity of the sample stage. According to the results reported by Wright *et al.* [4] the local velocity of fluid surrounding the trapped particle (actually, behind the particle) remains approximately constant during the travel of the stage at distances from 20 μm to 70 μm, for various sample stage speeds.

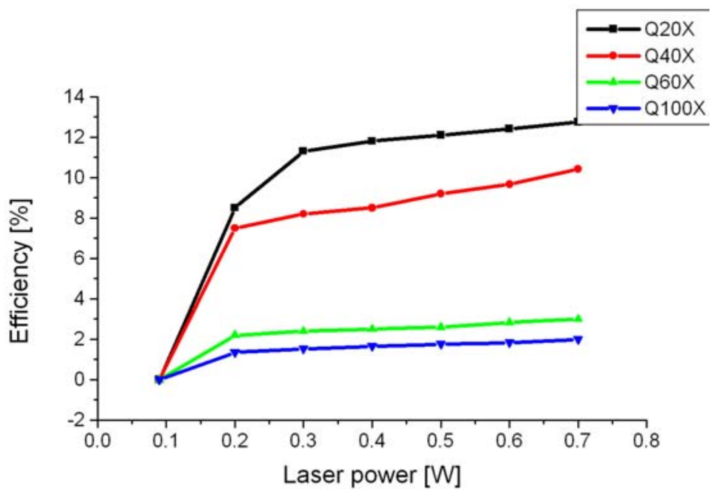

Fig. 2. The efficiency as a function of laser power for several magnifications of microscope

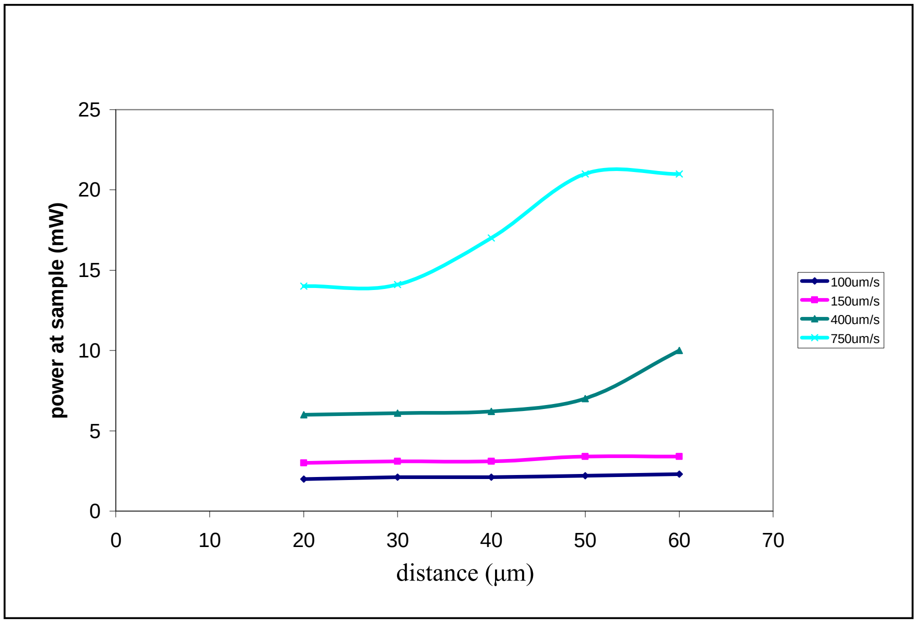

Fig. 3. Comparison of the trapping power at different distances in the displacement of sample stage for four stage speeds

The findings of these authors support our experimental data shown in Fig. 3 and can be used to explain, in part, the increased laser power required for stage moving at larger distances and the different magnitudes of this power with increasing stage speed. However, their theory of change in localized speed with distance leads to an estimated increase in the laser power required to trap the particle of, at most, 3 %, a percentage which is too small to explain the 50 % increase of power in our measurements, as can be seen in Fig. 3. Also, the use of the stage speed rather than the local fluid speed to calculate the transversal trapping force would produce a 3pN larger force at a stage speed of 750 μm/s and 20 μm amplitude of distance.

Moreover, it is possible that the sphere will be rotated if there is a significant velocity gradient across it. If the trapped sphere is rotating, it will loose energy from the trapping system and thus could decrease the efficiency of the trap. Since the sphere has a finite size (of 6 μm, in our experiments) the top and bottom of the sphere ly at different trap depths and therefore are subjected to different local fluid speeds. A velocity difference across the sphere at a trap depth of 20 μm may be calculated for different distances to the sample stage.

To see if the rotation of the particle influences the efficiency of the trap, we will assume the relevant energies involved. The maximum velocity difference across the sphere is estimated as being approximately 11 μm/s causing the rotation of the surface of the sphere with a maximum velocity of 5.5 μm/s. The corresponding rotational kinetic energy of a sphere of 6 μm diameter (rotating at 5.5 μm/s) is of the order of 10^{-25} J. However, the energy of laser required to move a 6 μm sphere at a distance ten times greater than its diameter under a 42 pN force is of the order of 10^{-15} J. Therefore, the energy associated with moving the fluid past the particle is much larger than the rotational energy of the sphere, indicating that the efficiency of the trap is unlikely to be affected by particle rotation.

When the sample stage was moved with a velocity of sinusoidal waveform, with the same maximum speed produced by the triangular velocity waveform, the laser power required to hold the particle was shown to be less. In this case, an increase in power required to trap a particle was about of 24 % when the particle was subjected to fluid flow, caused by a constant stage speed (of 150 μm/s), as opposed to a sinusoidal stage velocity, for a distance moved of 20 μm. Both these effects are partly due to the local speed of the fluid flow past the particle, although the percentage increase in power required to trap the silica particle is one order of magnitude greater than the percentage increase in the calculated local speed, for different amplitudes of distance. It is to be mentioned that no complete explanation for this effect has been given.

Since the method of equating the forces associated with an optical trap with the Stokes drag force is so commonly used, these results could have far-reaching implications. Besides a difference in trapping force of approximately 3 pN when considering the calculated local speed of the fluid rather that the boundary speed, another effect that should be considered is the change in the viscosity of the medium with temperature, potentially caused by the sample illumination or the trapping laser beam. We estimated that a change in sample temperature between 25 and 40^{0}C may involve a change in the viscosity of the fluid and therefore a change in the trapping force of 10.1 pN, when the stage is moved at a speed of 750 μm/s. Thus, the experiments using optical trapping to measure piconewton forces should be aware for showing the influence of various parameters on force measurements.

Conclusions

In experiments using optical tweezers for trapping or manipulating silica microparticles the optical efficiencies are in the range of 2-12 %, with upper values at lower magnifications of microscope.

Unexpectedly, it has been found that the minimum power required to held a particle in an optical trap against a fluid which flows with a constant speed is dependent on the amplitude of distance travelled by the sample stage (or the time of the application of the fluid flow). An increase of 24-50 % in the power required to trap the particle with the amplitude of stage displacement was also observed when the sample stage was driven with a sinusoidal velocity function.

A larger trapping force is necessary when considering the calculated local speed of the fluid rather that the boundary speed as well as the change in the viscosity of the medium with temperature.

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