OPTICAL TWEEZERS BASED ON NEAR INFRARED DIODE LASER

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Abstract

Emission from a single-mode 100 mW diode laser at 840 nm is used to create optical tweezers: the trapping laser beam is introduced into a microscope and focused by the objective. The microscope also allows monitoring of the motion of the trapped particles. The optical tweezers were monitored with objectives having different numerical apertures between 0.65 and 1.3. The optical trapping of polystyrene spheres with a radius between 0.11 and 7.45 μm and of biological objects, the flagellated alga Tetraselmis, with typical dimensions of 8×8×13 μm³ were studied. The efficiency of the optical tweezers has been characterized through a parameter Q and compared with theoretical models. © 1997 Society of Photo-Optical Instrumentation Engineers. [1083-3668(97)01103-9]

Keywords optical tweezers; near infrared diode laser; microscope; single-beam laser trap.

1 INTRODUCTION

Optical trapping allows noninvasive manipulation of microscopic objects by means of a focused single laser beam. The intense electric field of the laser beam induces an electric dipole moment in the particle to be trapped: if the laser frequency is lower than the absorption frequency of the particle, the induced electric dipole moment is in phase with the applied field and, owing to the so-called dipole force, the particle reaches a stable balance at the position where the electric field has its maximum value. Thus dielectric particles are trapped near the focus of the laser beam and can be manipulated by displacing the optical trap, in a configuration denoted as “optical tweezers.” 1 The laser wavelength chosen should be far from visible and UV regions, where biological cells can be damaged by radiation absorption, and far from the maximum water absorption peak centered at 1.4 μm.

In the qualitative picture of a single-beam optical trap given by Ashkin and coworkers2-5 for dielectric spheres with dimensions larger than the optical wavelength, the forces produced by the focused beam can be derived from an analysis of the ray optics through the sphere. The amplitude of the trapping force increases with the aperture angle of the focused light beams. Thus efficient optical tweezers are created when the laser light is tightly focused, for instance by a high numerical aperture (NA) microscope objective. The optical trapping force depends on the diameter of the particles and decreases for small particles because of the reduced contribution of large-angle refraction.

If initially the applications of optical tweezers were based mainly on Nd:YAg lasers, more recently interest has largely been concentrated on the use of diode lasers. Furthermore, attention has been concentrated on the spectral region around 750 to 850 nm, where a minimum in water absorption takes place. The availability of high-power diodes with excellent coherence provides economical and easily handled laser sources. Afzal and Treacy4 demonstrated the first operation of optical tweezers based on a laser operating at 840 nm. The output beams of diode lasers are astigmatic and with a flat, non-Gaussian intensity profile. As a consequence of this poor quality of the laser beam, optical trapping by laser diodes could not be as efficient as with He:Ne or Nd:YAG lasers. This question has been examined by Bakker Schut et al.5 by measuring the trapping forces originated by laser diodes and finding them comparable to those created by Gaussian beams having the same intensity. A standard compensation of the elliptic shape of laser diodes can be obtained through the use of anamorphic prisms, and Escandon et al.6 have examined the trapping efficiency with that compensation for the ellipticity. We have demonstrated previously that radiation around 850 nm is suitable for the creation of optical tweezers.7-9 The present work describes our experimental progress in the use of optical tweezers using a laser diode. In addition to the trapping of Dunalie11a salina and Halobacterium halobium already reported,7-9 we present here the optical trapping of the flagellata alga Tetraselmis.
In our study based on the simple setup of an optical microscope, because the particles are monitored through the same optical port by which they are trapped, a whole plane of the sample, transverse to the laser propagation direction, is in the focus. This configuration has allowed us to measure the transverse and axial components of the optical force acting on the trapped species. The efficiency of the optical tweezers is described by a parameter \( Q \) introduced in Ref. 3 and the \( Q \) values can be derived from the measurements of the transverse or axial forces acting in the optical tweezers. It should be noted that even if optical tweezers represent a well-established instrument with wide applications, and considerable work was concentrated in the determination of the optical forces acting on different objects, the agreement between theory and measurement of the forces is still unsatisfactory. As stated by Svoboda and Block,\(^{10}\) it is still unclear whether the quality of the measurements or the incompleteness of the models is responsible for such a disagreement.

In the present work we report additional data for the dependence of the \( Q \) value on some parameters of the optical tweezers, by trapping polystyrene spheres. While the dependence on the dimensions of the spherical particles has been already studied, we have used different microscope objectives and examined the \( Q \) dependence on the microscope's numerical aperture. In particular we have investigated the operation of optical tweezers with a microscope objective having a low numerical aperture, an experimental condition that was defined as the lower limit for laser trapping by Ashkin.\(^3\) We have found that even if the dependence on the particle radius and on the microscope numerical aperture is that expected by the theoretical models, our \( Q \) values are different from the theoretical ones.

As is standard in an optical tweezers operation, we characterized our system through measurements of the critical velocity, defined as the largest velocity at which the viscous medium surrounding the particle may be moved, the particle remaining trapped. This quantity determines the force required to release a particle after it is trapped. This critical velocity implies that during the release of the particles the potential energy of the optical trap is provided by a viscous force acting on the trapped particle moving against the surrounding fluid.

This characterization of the optical trap is not complete, and we have introduced and measured another quantity—the capture range velocity. We consider this quantity an additional test of the optical trap’s efficiency. The capture range velocity is determined as the maximum velocity of moving particles at which the optical tweezers forces are able to slow down and trap the particles. In order to trap particles by slowing down their motion, large radiation forces are required for fast-moving particles. In the slowing stage, the initial kinetic energy of particles moving with a velocity smaller than the capture velocity is reduced so that those particles are trapped by the potential barrier of the optical trap.

For the measurements of the capture range velocity, we started from a sample of particles having a thermal velocity distribution and measured the velocity of those particles which were trapped. In order to obtain a reliable value of the capture velocity, we performed statistical averages on several successive operations of the optical tweezers. These measurements are more time-consuming and are also affected by large uncertainties associated with the statistical averages. However, they are very appropriate for characterizing the trapping operation of moving or swimming particles, for instance, in applications of cell sorting and classification.

The forces acting on a trapped particle are briefly discussed in Sec. 2. The experimental setup based on a laser diode propagating through a microscope is described in Sec. 3. The determination of the capture range velocity is reported in Sec. 4. Results relative to the transverse and axial forces and the relative efficiency of the optical tweezers operating at 840 nm are reported in Secs. 5 and 6. The conclusions in Sec. 7 include a discussion on the possible sources of the disagreement between our measurements and other results reported in the literature.

### 2 Forces of a Single-Beam Laser Trap

The forces exerted by radiation on a particle are classified as scattering force, or radiation pressure, and gradient force, or dipole force.\(^1\) The scattering force is proportional to the optical intensity and points in the direction of propagation for the incident laser beam. The gradient force is proportional to the gradient of the light intensity and points in the direction of the intensity gradient.

The forces acting on a trapped particle are schematically drawn in Figure 1. The system has a cylindrical symmetry around the \( z \) axis, which represents the microscope axis and also the propagation direction of the laser beam. Assigning a positive sign to the \( z \)-axis forces pointing upward, the laser beam propagates along in the negative direction. The transverse force \( F_t \) is produced mainly by the radial component of the gradient force because the contribution of the scattering force is quite small. Along the \( z \) axis, the particle experiences the axial force \( F_a \) as a result of the axial gradient force component pointing toward the beam focus, \( F_{z,\text{grad}} \), and of the scattering force \( F_{sc} \) pushing the particle out of the focus. If the laser beam propagates in the vertical direction, as in our optical tweezers, the motion of the particle along the \( z \) axis is determined also by the force of gravity \( F_g \) and the buoyancy force \( F_b \) created by the liquid, these forces having opposite directions. The axial equilibrium of the
particle with $F_a=0$ is obtained with the particle placed below the objective focus, where $F_{z\text{ grad}}$ balances both the $F_{sc}$ and the $F_g-F_b$ forces. When the trapped species are placed in a liquid drop on a microscope slide, their motion is perturbed by the thermal convection within the liquid and by the adhesion of the liquid drop either to the objective or to the microscope slide.

The efficiency of an optical trap has been characterized by Ashkin through an adimensional efficiency parameter $Q$ defined as

$$Q = \frac{cF}{nP},$$

where $c/n$ is the light speed in the medium, $F$ is the trapping force, and $P$ is the incident laser power. The $Q$ parameter is defined to be independent of the laser power, because of the linear dependence of the force with laser power $P$ obtained within the RO model of Ref. 3. The definition of Eq. (1) applies to both the transverse force $F_t$ and the axial force $F_a$, so that the $Q_t$ and $Q_a$ values represent the transverse and axial efficiencies, respectively.

3 EXPERIMENTAL SETUP

Our optical trap was created by introducing into a compound microscope a linearly polarized transverse electromagnetic field beam from a diode laser with a typical non-Gaussian and more flat-top profile. The microscope allowed us to both observe and manipulate particles, according to the scheme originally proposed in Ref. 11. Our optical configuration used the radiation at 840 nm emitted from a 100-mW SDL-5311-G1 (Spectra Diode Labs) diode. The laser was collimated through a laser diode objective with focal length 6.5 mm. An anamorphic prism pair with a magnification factor of $3 \times$ corrected for the diode beam’s ellipticity. As in Ref. 6 the angle of rotation of each prism was adjusted to produce on the output the most circular laser beam shape. The resulting beam was focused through an $f=200$ mm lens mounted on an X-Y-Z translator system and injected into a modified commercial microscope (Swift M485B). A polarizing cube beamsplitter inserted between the eyepiece and the objective of the microscope, as shown in Figure 2, reflected the laser beam down into the microscope objective. Great care was taken for the laser beam diameter to properly fill the back pupil of the objective. Different immersion microscope objectives, listed later, were used for both focusing the beam and magnifying the sample image; all of them were rated for a 160-mm tube length. The image was further magnified by an eyepiece ($10 \times$) and observed through a CCD video camera on a monitor. Infra-red (IR) blocking filters between the cube and the eyepiece prevented most of the laser light diffused by particles from reaching the camera. The laser power transmitted through the microscope objective and reaching the sample was directly measured through a large-area power meter. Corrections for internal reflection due to the refractive index mismatch in the absence of the immersion fluid were applied. The parfocality condition for the trap and the specimen required a critical adjustment of the position for the $f=200$ mm lens.

The trapped polystyrene particles (density $1.06 \text{ g/cm}^3$) solved in deionized water and the trapped biological species, solved in a liquid, were deposited in a drop on the top of a microscope slide. This setup allowed us to trap and monitor on a TV screen, the polystyrene particles and the biological species freely moving within the sample. In a second part of the experiment, the microscope stage holding the sample could be moved by a step-
ping motor in a direction transverse to the laser propagation and parallel to the laser polarization, in order to modify the sample velocity in a controlled way. We have carefully verified that the movement of the sample was in a direction transverse to the laser beam and did not modify the axial trapping. All reported data on the trapped particles were obtained as the averaging of at least five independent measurements.

We have performed an initial set of measurements using a water immersion objective 40× by Nikon with a numerical aperture NA=0.65, Part Number 43475, corresponding to a maximum cone angle of θ_m=30°, and measured transmission coefficient at 850 nm of 46±0.2%. According to the analysis of Ref. 3, this θ_m angle is at the edge of the trapping capability. A more complete investigation of the optical tweezers operation was later performed using the following objectives with different magnifications:

- 55X/NA=0.9 oil immersion, by Fratelli Koristka, Part Number 103909, with θ_m≈37°, and transmission 36±0.1%;
- 100X/NA=1.0 water immersion, by Carl Zeiss Inc, Part Number 440087, with θ_m≈49°, and transmission 21±0.7%;
- 105X/NA=1.3 oil immersion, by Officine Galilei s.r.l. with θ_m≈60°, and transmission 16±0.5%.

In order to specify the characteristics of our setup, we determined the beam waist w_0 of the laser at the focus of the 40× objective through several independent measurements. w_0 was derived from the cone angle θ_m of the laser focused by the objective, making use of the far-field diffraction formula θ_m = λ / π w_0, valid for θ_m≤30°. Taking into account the correction due to the refractive index of water, the value w_0=1.5±0.1 μm was obtained. This beam waist value was in good agreement with an independent estimate (2±0.5 μm) based on the shadow of a wire with a diameter d between 10 and 30 μm. When the wire was placed in the focus of the beam, its shadow covered the entire laser spot in the case of w_0<d. Finally, in our previous work, an independent estimate of the beam waist was obtained by imaging on a CCD camera the light scattered from an emulsion of milk and water at 90° with respect to the focusing beam. For the 40× objective, a waist dimension of 3±1.5 μm was estimated in that measurement. Thus values derived are in good agreement and the three techniques provide an alternative to the knife-edge technique usually applied.10

In the trapping of small particles with a radius down to 0.115 μm, the magnification of the microscope objective and the eyepiece was not large enough to monitor the trapped particles. Thus we operated the microscope at a reduced working distance, so that the image of the trapped particles collected farther from the eyepiece was magnified, even at the expense of its sharpness. The operation of the microscope at a larger working distance could produce larger spherical aberrations in the laser beam focus, but no evidence appeared from our measurements.

4 Determination of the Capture Range Velocity

The 40× water immersion objective allowed us to trap polystyrene spheres (refractive index 1.2 with respect to water) of various sizes and also biological particles, for instance, the ellipsoidal-shaped flagellated alga Tetraselmis, with typical dimensions of 8×8×13 μm³. We were able to trap moving particles reaching the beam waist located 1 mm above the bottom of the microscope slide. When trapped, the particles were dragged for 60 μm by laterally displacing the waist of the f=200 mm lens. The light beam trapped only the particles that had a velocity within the capture range, i.e., velocities smaller in absolute value than the capture range velocity v_r. The velocity v_r was derived from a statistical analysis of the trapping operation for particles with different velocities: for a given laser power, v_r is defined as the velocity at which more than 50 percent of the particles passing through the focused volume around the laser waist (12×45×60 μm³) are captured by the trap. We measured the velocity v_r and observed a strong dependence of the capture velocity of the trapped particles on the laser power and the particle dimensions. Figure 3 shows our experimental results for the velocity v_r. The data for Tetraselmis were obtained only when their average speed was partially reduced from their typical 200 μm s⁻¹ value probably because of a reduced functional activity.
Latex spheres with radius of 1.0 and 3.2 μm were trapped in the best way, that is, with the largest capture velocity \( v_c \), at a given laser power. Less efficient results were obtained with those sized 0.5 μm and a dramatic worsening in trapping was obtained for those with \( r = 0.115 \) and 0.24 μm. It should be noted that the trapping of latex particles with such small radii was not reported previously for optical trapping at the 840-nm wavelength. The trapping of objects with transverse dimensions smaller than the beam waist is difficult because, according to the qualitative picture of Ref. 1, a small object experiences a nearly parallel set of flow lines without high-angle converging beams. For the large dimensions of *Tetraselmis* \((r \approx 10 \mu m)\), a less efficient performance was obtained. However, a direct comparison between the latex spherical particles and some nonspherical, optically inhomogeneous biological objects with unknown refractive indices is not straightforward.

5 CRITICAL VELOCITY, TRANSVERSE FORCE, AND EFFICIENCY

In order to quantify trapping performance, we measured the transverse force \( F_t \), as in Figure 1; i.e., the force exerted in a direction orthogonal to the beam axis. As previously performed by other authors (see Refs. 12 and 13), we used Stokes’ Law to determine the force needed to remove a spherical particle from a trap in a viscous medium

\[
F_t = 6 \pi \eta r v_c, \tag{2}
\]

where \( \eta \) is the viscosity of the medium (for water \( 1 \times 10^{-2} \) poise at room temperature), \( r \) is the radius of the sphere, and \( v_c \) is the critical velocity at which the particle is released from the optical trap. The determination of \( v_c \) and the use of Eq. (2) allow us to calculate \( F_t \).

To measure \( v_c \), we trapped the latex spheres near the bottom of the water drop, where they did not experience a convective motion, and moved the microscope stage by a micrometric screw and a stepping motor, dragging the liquid containing the trapped particles. To compare results for different objectives, we used 1-μm radius latex spheres clearly visible by all the objectives and easily trapped, according to the characterization presented in Sec. 4. The trapping of the latex particles near the bottom of the slide, while the slide was displaced for distances up to 110 μm, was very sensitive to the condition of the spheres (for instance, after a few hours of exposure to air, they will stick to the glass) and to the viscosity of the medium (rigorously clean deionized water being constantly used). Moreover, by using a microscope slide cleaned by immersion in an ultrasonic wave cleaner for several minutes, we measured a value of \( v_c \) twice as large as that measured in the same conditions using a microscope slide cleaned only by immersion in ethanol or acetone. Apart from these precautions, the \( v_c \) measurements were straightforward because the particles near the bottom of the slide were not perturbed by convective motion. In the \( v_c \) measurements, the velocity was controlled and precisely measured from the calibration of the stepping motor so that the relative uncertainty was better around 5 percent. The negative aspect of our measurement with particles at the bottom of the water drop was that the near presence of the microscope slide, between 1.5 and 2 μm, required a correction to the force of Eq. (2), as discussed in Ref. 14.

In order to perform measurements with the oil immersion objectives, we deposited on top of the microscopic slide a drop of oil optically matching the objective and the sample. For the 55× objective having a 1.35 working distance, we used a microscope slide with a groove for the sample and a 0.17-mm coverslide. For the 105× objective having a working distance around 0.2 mm, this setup did not allow us to focus on the bottom of the drop, so we created a very thin cell, inserting the solution between a flat slide and the coverslide separated by 35-μm spacers.

We measured \( v_c \) for the \( r = 1-\mu m \) latex particles as a function of the incident laser power. Figure 4(a) gives the measurements of the critical velocities as function of the laser power for the four microscope objectives listed above. From these measurements, we calculated the transverse force, shown in Figure 4(b), according to Stokes’ formula of Eq. (2), including the adhesion corrections for the nearby microscope slide. At a given laser power, the data reported in the figure show a large increase of the
trapping force with the numerical aperture of the focusing objective. Because a greater NA objective also presented larger transmission losses, changing the NA and different ranges of incident laser power were explored. For all the objectives, the trapping force was between $0.7 \times 10^{-7}$ and $3.5 \times 10^{-7}$ dyne.

Our measurements have shown an unexpected nonlinear dependence of the trapping force $F_t$ on the laser power. Such a nonlinear dependence was not discussed before by other authors operating optical tweezers. The nonlinear behavior may be explained in terms of crosstalk between transverse and axial responses of the trap. The axial equilibrium position of the particle in the trap originates from the balance between the forces listed in Sec. 2. Increasing the laser power increased both $F_{a,\text{grad}}$ and $F_{\text{sc}}$, while $F_t$ and $F_b$ remain constant. As a consequence, the equilibrium position of the trapped particle is shifted toward the laser beam waist, where the trapping forces are reduced. The transverse force $F_t$ that is measured by our setup depends on the $z$ position of the particle and decreases toward the beam waist. Thus at increased laser power, the particles move to an equilibrium position where the transverse force, and hence the $Q_t$ value, is reduced. As a consequence of this nonlinear dependence, the $Q_t$ value determined by Eq. (2) was not independent of the laser power and its maximum value occurred at the applied laser power just above the minimum one required for trapping.

The maximum value for $Q_t$ for the different objectives is shown in Figure 5(a). The uncertainty was around $\pm 20\%$ because of the contributions from $\nu_t$ and from the incident laser power. Owing to the strong dependence of the trap efficiency on the microscopic converging angle $\theta_m$, the use of a larger converging angle provided an increase by a factor of 3 in the performance of the trap. There was no relevant difference between the 100× immersion objective, with $\theta_m=49^\circ$, and the 105× oil immersion one, with $\theta_m=60^\circ$, even for an increase of $\theta_m$ by 11°. Probably for that oil immersion objective the laser beam experienced quite large optical distortions. It should be noted that the second oil immersion objective, the 55× with $\theta_m=37^\circ$, produced results in agreement with the remaining ones. Figure 5(b) contains our data for the dependence of the $Q_t$ value on the latex particle radius by using the 40× objective. Because we have measured nearly the same value of $\nu_t$ for particles with radii of $r=0.5$, 1, and 2.1 $\mu$m, it is determined from Eqs. (1) and (2) that the measured $Q_t$ values increased linearly with the radius. For the remaining radii, the $Q_t$ value was nearly constant.

![Figure 5](https://www.spiedigitallibrary.org/journals/Journal-of-Biomedical-Optics/d JULY 1997 d Vol. 2 N o. 3/337)

We have compared our data with published experimental results obtained at different wavelengths and large NA objectives, and also with the theoretical models of several authors, even if it might be inappropriate to directly compare the data taken with Gaussian laser beams and those with diode lasers. For the $r=2.5-\mu$m radius, the theory of Refs. 16 and 17 has predicted a value of $3.4 \times 10^{-7}$ dyne for the optical force of linearly polarized laser light, focused in a waist $w_0=2\lambda$ and directed upward with a laser power of 2.3 mW, which is large enough to levitate the particles. In our setup, the condition $w_0=2\lambda$ is satisfied for the 40× objective and the lowest force we measured for the $r=3.2-\mu$m radius was $4.2 \times 10^{-7}$ dyne for the laser power of 5.7 mW.

Taking into account the refractive index of the latex particles, the theoretical values of Refs. 16 and 17 correspond to $Q_t=0.33$ for our conditions; this value is not in agreement with the measured 0.16 value. In Refs. 2, 3, and 18, the RO model for large particles, with $r \gg 5 \mu$m, predicts for $\theta_m=60^\circ \pm 70^\circ$ an optical efficiency $Q_t$ of 0.35, which is more than two times larger than our best value. On the other hand, for the same $\theta_m=60^\circ$, the more accurate calculations based on the EM model performed by Wright, Sonék, and Berne' predict a value of $Q_t=0.16$ for $r=0.5 \mu$m silica particle, a value well confirmed experimentally by the same authors. For our $r=1-\mu$m latex particles, this value is expected to increase, owing to the larger refractive index and to the larger radius. On the contrary, our value is only $Q_t=0.12$. Moreover for latex particles of the same radius, d’Helon et al. in trapping with an

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*Simmons et al.* used an Nd:YAG laser up to 140 mW for trapping measurements of 1-μm-diameter beads and reported in their Fig. 7 a deviation from the linearity between force and laser power.
He:Ne laser reported $Q_t = 0.035$, while for $r = 1.5\,\mu m$ latex particles, Sato et al.,$^{19}$ using a 1.3-μm diode laser, reported a $Q_t = 0.13$ value. The disagreement does not change, considering our measurements with other radius particles. The trapping force created by a laser diode at 840 nm has been measured by Afzal and Treacy$^4$ for latex particles with radius of $1\,\mu m$, using an inverted microscope with levitation of the trapped particles. The efficiency $Q_t = 0.13$ of those authors with a $100\times / 1.3$ objective is in good agreement with our value of $Q_t = 0.12$ using an equivalent objective. Later Baker Schut et al.$^5$ obtained a value of 0.09 for the axial $Q$ using a 780 nm diode laser without compensation for the laser astigmatism. Finally Escandon et al.$^6$ using a 790-nm diode laser, measured $Q_t$ values between 0.30 and 0.37 with anamorphic prism magnification 2× and 3×, respectively.

The continuous lines in Figure 5 represent a fit of our data using the functional relation determined by previous authors. In Figure 5(a) for the $Q_t$ dependence of the transverse efficiency $Q_t$ on NA, we used the values calculated in Ref. 13 with the RO model. That model predicts a linear dependence of the $Q_t$ value on the cone angle, even if, quite surprisingly, such an increase was not observed experimentally by Wright, Sonek, and Berns.$^{13}$ A predicted linear dependence of $Q_t$ on $\theta_m$ closely reproduces our experimental observations when a scaling factor is applied to the theoretical predictions. For the dependence of $Q_t$ on the particle radius, the most accurate calculations of $Q_t$, presented in Ref. 20, predict in the Rayleigh regime at radius $r < 0.5 \mu m$, an $r^3$ dependence. At larger radii, the increase with radius is less strong, and at radii larger than 5 μm, a nearly constant $Q_t$ value is obtained. For the interval of radius explored in our experiment, we derived from a fit of the numerical results of Ref. 20 that $Q_t$ increases toward a limiting value with an $\exp(-r)$ dependence. That dependence is also supported by our data, as shown by the continuous line in Figure 5(b).

6 AXIAL FORCE AND EFFICIENCY

While optical tweezers operation are easily characterized in the transverse direction, it is possible to obtain only limited information on the axial force produced by optical tweezers. As already indicated,$^{13,20}$ we determined the critical value $P_c$ of laser power where the axial radiation force balanced the resultant $F_g - F_b$ of the gravity and buoyancy forces. For values of laser power larger than $P_c$, the particles were trapped; for values of laser power smaller than $P_c$, the suspended particles fell. Such a determination of $P_c$ for a given radius sphere provides only a lower limit to the force $F_{z,\text{grad}} - F_{sc}$ relative to that power: the particles fall only when the force $F_{z,\text{grad}} - F_{sc}$ is larger than the optical trapping force. We measured the axial forces for latex particles with radii $r = 1.0, 3.2, \text{ and } 7.5 \,\mu m$. Our measurements are in agreement with the expected increase of the axial force with the third power of the radius, even if our values are systematically lower than those measured in Ref. 13. However the $Q_a$ values had a large uncertainty, around 50 percent, because of the difficulty of measuring the laser power $P_c$ where release of the trapped particles occurred. For the particles of $r = 3.2 \,\mu m$, our lower limit to the trapping force is $8 \times 10^{-9}$ dynes, with an axial $Q_a$ value of 0.0078 for the $40\times$ objective. An axial $Q_a$ value of 0.013 was obtained for the $100\times$ objective. From the measurements with $r = 7.45-\mu m$ particles and $40\times$ objective, an efficiency of $Q_a = 0.12$ was derived. It is interesting to compare the values of the axial and transverse efficiencies for particles with a given radius. For the $r = 3.2-\mu m$ particles, for both the efficiencies we used, the $Q_a$ values are one order of magnitude smaller than the corresponding $Q_t$ values, as also predicted and measured in Ref. 13.

7 CONCLUSION

The operation of optical tweezers based on an 840-nm diode laser has been described. We used in our optical tweezers different microscope objectives and examined the performance of the tweezers as a function of the objective numerical aperture. We have demonstrated that an NA = 0.65 low-aperture objective allows us to create optical tweezers with a good performance: this water immersion objective, having the advantages of a large transmission and a large field of view, is able to trap objects with dimensions between 0.1 and 7.45 μm radius. The measured $Q$ values increase linearly with the numerical apertures, even if our objective with the largest numerical aperture did not present the expected increase. We have measured the capture range velocity, which is the maximum velocity at which moving particles can be trapped. This velocity represents a useful indicator for the operation of an optical trap operating on moving biological species. We tested our trap by measuring the capture velocity of Tetraselmis. Note that a capture velocity measured by Liu et al.$^{21}$ on sperm cells had a value comparable to ours. We also measured the critical velocity that represents a standard approach for the characterization of optical tweezers. The two different velocities define the capture and release operations of the optical tweezers, respectively. From the measured transverse critical velocity for $r = 1-\mu m$ polystyrene spheres as a function of incident laser power, we verified that the transverse force and the transverse efficiency increased with the numerical aperture of the objective, even if the increase is not as large as expected from theoretical estimates. The
axial radiation force has been measured under conditions of minimum laser power and a lower limit to the axial efficiency $Q_a$ determined.

The measured values of transverse and axial efficiencies, with a maximum value of 0.13, are not in agreement with the values derived in the theoretical models of optical tweezers, nor are they in good agreement with measurements of other authors, who, however, used lasers at different wavelengths or different microscope configurations. Instead, a good agreement exists between our results at weak laser power and the previous measurement performed by Afzal and Treacy using 840-nm laser radiation. Regarding the comparison with the theoretical models, the measured $Q$ values can be underestimated because of several factors: (1) the absence of a precise control on the position of the trapped particle within the laser focus and the strong dependence of the $Q$ value on the position and (2) the presence of radiometric forces. The influence of spherical aberration cannot be excluded because our trap operates near the bottom of our container, with the laser beam propagating through the liquid. Previous authors have reported a large decrease in the trapping efficiency when particles were trapped far down into the liquid. Notwithstanding the deficiencies of the models, the optical traps are able to operate even a large range of experimental parameters, such as laser wavelength, power and beam waist size, and particle dimensions. This broad range of operation and efficiency makes optical traps a versatile tool for scientific research on biological species.

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