# Optical Tweezers 3D Photonic Force Spectroscopy 

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#### Abstract

: Since optical tweezers trapped microspheres can be used as an ultrasensitive force measurements technique, the knowledge of its theoretical description is of utmost importance. However, even the description of the incident electromagnetic fields under very tight focusing, typical of the optical trap, is not yet a closed problem. Therefore it is important to experimentally obtain whole accurate curves of the force as a function of wavelength, polarization and incident beam 3D position with respect to the center of the microsphere. Theoretical models for optical forces such as the Generalized Lorenz-Mie theory, can then be applied to the precisely evaluated experimental results. Using a dual trap in an upright standard optical microscope, one to keep the particle at the equilibrium position and the other to disturb it we have been able to obtain these force curves as a function of $\mathrm{x}, \mathrm{y}$ and z position, incident beam polarization and also wavelength. Further investigation of optical forces was conducted for wavelengths in and out Mie resonances of the dielectric microspherical cavities for both TM and TE modes.


KEYWORDS: Optical Tweezers, Mie Resonances, Spectroscopy, Generalized Lorenz-Mie Theory, Microspheres, Polarization.

Optical tweezers have become an important tool for biological manipulations and cell mechanical properties measurements ${ }^{1-4}$. These measurements use the displacement from equilibrium position of a microsphere as the force transducer. Therefore, the calibration procedure requires the use of good models for the optical force in microspheres. Geometrical optics has been used when the particle dimensions are much greater than the light wavelength, and Rayleigh scattering theory for the opposite. However, when the particles are of the same order of the wavelength these approximations are no longer valid and full vectorial electromagnetic theory is necessary. Mie resonances are typical of this size regime. Moreover, just the description of a vectorial electromagnetic field of a very high numerical aperture beam is still an open field ${ }^{5-7}$. Therefore good sensitive optical force measurements are
necessary to discriminate among the several models. In previous work we have shown that the double optical tweezers can be used to perform an ultrasensitive force spectroscopy by observing forces due to the light scattering and selectively coupling the light to either the TE, the TM or both TE and TM microsphere modes in a single isolated particle as function of the beam polarization and position ${ }^{8}$. Our results showed how careful one has to be when using optical force models for mechanical properties measurements. The microsphere modes can change the force values by more than $30-50 \%$. Also it clearly shows how the usually assumed azimuthal symmetry in the horizontal plane no longer holds because the beam polarization breaks this symmetry. In this article we continue the investigation of the forces in an optical tweezers by using this ultrasensitive experimental technique to measure whole optical force curves as a function of the 3D beam position and polarization. The importance of understanding the optical scattering forces of dielectric microspheres under different incident beam conditions comes from the fact that they have been used as the natural transducer for force and other mechanical measurements. Because optical tweezers itself is an ultrasensitive technique, we used another optical tweezers to characterize the optical force of microspheres.


Figure 1: Experimental setup to control the gradient optical trap in $3 D$. Nd:YAG laser is used as trap, the Ti:Sapphire is used to disturb the trap and the HeNe laser to detect the backscattered light due to the induced perturbation.

Figure 1 shows the experimental setup used, the beam from a Nd:YAG cw laser (denoted as trapping beam) is used to keep the particle trapped while the beam from a tunable Ti:Sapphire cw laser, which is modulated and highly attenuated (denoted as perturbing beam), is used to perturb the particle from its equilibrium position. Some advantages of this setup are that it allows: 1. the measurement to be performed in suspended and isolated particles
trapped for hours; 2. to observe the same particle submitted to different conditions; 3. to localize the perturbing beam in any desired position at the sphere. The Ti:Sapphire cw laser beam was modulated at 12 Hz by a chopper. A signal proportional to the displacement was measured using the back-scattering of a HeNe laser after passing through two short pass filters to reject the Nd:YAG and Ti:Sapphire laser beams and detected with a photomultiplier (Hamamatsu) coupled to the eyepiece of the microscope and a lock-in amplifier (Stanford Research Systems, model SR830 DSP). Polarization was also controlled by a $\lambda / 2$ waveplate. The axial and radial forces were observed by monitoring the amplitude of the displacements while changing the position of the Ti:Sapphire laser. The translation of the focal spot in the focal plane of the objective is accomplished by the use of a gimbal mount as shown in the experimental setup. A gimbal mount is a mirror mount that allows the rotation along the axis at the center of the mirror face, while typical optical mounts rotate about axes that don't lie in the mirror plane. This is very important for the axial control since the surface of the gimbal mount is imaged at the objective aperture. The distance between the laser spot on the mirror and the objective pupil is constant, in conjugate planes, so a change in beam angle at the mirror corresponds to a change of angle at the objective. Conjugate planes are formed by placing two lenses, separated by the sum of their focal lengths, between the gimbal mount and objective. This is also useful to overfill the objective aperture so that an efficient gradient trap can be obtained. The axial control of the focal spot in the objective focal plane is obtained adjusting the beam divergence without changing the diameter at the objective aperture. This is accomplished by constraining the beam diameter at the gimbal mirror independent of the changes in beam divergence. A movable telescope with the lens nearer to the gimbal mount fixed. By translating the lens from the telescope (as shown in Figure 1) the laser focal spot can be controlled in the axial direction. With these two techniques our setup is capable of a full 3D translation of our laser spot inside of a Neubaur chamber. The rotation and translation are computer controlled by a step motor. To relate the motor steps to microns a previous calibration run was executed. For the radial calibration, the motor steps where related to the microscopes translating stage by moving the Neubauer chamber and recovering the spot position with the gimbal mount, this was done for $40 \mu \mathrm{~m}$. For the axial calibration, we used a microscope slide covered with a thin film of $0,5 \mu \mathrm{~m}$ fluorescent spheres that was evenly translated in the Z direction using the microscope focus micrometer readings. The fluorescence signal is maximized by moving the telescopic system with the step motor, motor position is them mapped for each
microscope focus position for $100 \mu \mathrm{~m}$. This system is computer controlled using Labview software, a lock-in amplifier and a photomultiplier tube to detect the fluorescence signal.

Measurements to characterize the optical force as a function of polarization were done in the radial and axial position for $3 \mu \mathrm{~m}, 6 \mu \mathrm{~m}$ and $9 \mu \mathrm{~m}$ polystyrene spheres (Polysciences), diluted in water and placed in a Neubauer chamber. The Generalized Lorenz-Mie theory (GLMT) was used to explain the results ${ }^{5-6,9-10}$. Classical Mie scattering theory was developed for plane waves and cannot explain the measurements obtained by this focused beam experiment. In this case, it is necessary to decompose the incident beam in plane waves relative to the center of the microsphere. As the beam focus is no longer at the origin of the coordinate system all the beam azimuthal symmetry is lost. There is a ready to use prescription to calculate the beam shape coefficients $g_{n T E T M}^{m}$ for the Davis description ${ }^{11}$. The scattered beam coefficients are given by $g_{n T M}^{m} a_{n}$ and $g_{n T E}^{m} b_{n}$, and the force cross sections for the $\mathrm{x}, \mathrm{y}$ and z directions by ${ }^{12-13}$ :

$$
\begin{gather*}
Q_{p r, z}=\frac{4}{x^{2}} \sum_{n=1}^{\infty} \sum_{p=-n}^{n}\left(\frac{1}{(n+1)^{2}} \frac{(n+1+|p|)!}{(n-|p|)!} \times \operatorname{Re}\left[\left(a_{n}+a_{n+1}^{*}-2 a_{n} a_{n+1}^{*}\right) g_{n, T M}^{p} g_{n+1, T M}^{p^{*}}\right.\right.  \tag{1}\\
\left.\left.+\left(b_{n}+b_{n+1}^{*}-2 b_{n} b_{n+1}^{*}\right) g_{n, T E}^{p} g_{n+1, T E}^{p^{*}}\right]+p \frac{2 n+1}{n^{2}(n+1)^{2}} \frac{(n+|p|)!}{(n-|p|)!} \times \operatorname{Re}\left[i\left(2 a_{n} b_{n}^{*}-a_{n}-b_{n}^{*}\right) g_{n, T M}^{p} g_{n, T E}^{p}\right]\right) \\
Q=\frac{2}{x^{2}} \sum_{p=1}^{\infty} \sum_{n=p}^{\infty} \sum_{m=p-1 \neq 0}^{\infty} \frac{(n+p)!}{(n-p)!} \times\left[\left(S_{m, n}^{p-1}+S_{n, m}^{-p}-2 U_{m, n}^{p-1}-2 U_{n, m}^{-p}\right) \times\left(\frac{1}{m^{2}} \delta_{m, n+1}-\frac{1}{n^{2}} \delta_{n, m+1}\right)+\right.  \tag{2}\\
\left.\frac{2 n+1}{n^{2}(n+1)^{2}} \times \delta_{n, m}\left(T_{m, n}^{p-1}-T_{n, m}^{-p}-2 V_{m, n}^{p-1}+2 V_{n, m}^{-p}\right)\right]
\end{gather*}
$$

where, $Q_{p r, x}=\operatorname{Re}[Q]$ and $Q_{p r, y}=\operatorname{Im}[Q]$ for the x and y components, the size parameter $x=k a$ with $a=$ microsphere radius and $k=2 \pi n_{1} / \lambda,\left(\lambda=\right.$ wavelength and $n_{1}=$ fluid refractive index $)$, and $a_{n}$ and $b_{n}$ are the Mie coefficients for the TM and TE modes respectively, given by:

$$
\begin{equation*}
a_{n}=\frac{m^{2} j_{n}(m x)\left[x j_{n}(x)\right]^{\prime}-j_{n}(x)\left[m x j_{n}(m x)\right]^{\prime}}{m^{2} j_{n}(m x)\left[x h_{n}^{1}(x)\right]^{\prime}-h_{n}^{1}(x)\left[m x j_{n}(m x)\right]^{\prime}} \quad b_{n}=\frac{j_{n}(m x)\left[x j_{n}(x)\right]^{\prime}-j_{n}(x)\left[m x j_{n}(m x)\right]^{\prime}}{j_{n}(m x)\left[x h_{n}^{1}(x)\right]^{\prime}-h_{n}^{1}(x)\left[m x j_{n}(m x)\right]^{\prime}} \tag{3}
\end{equation*}
$$

$j_{n}$ and $h_{n}^{1}$ the spherical Bessel and Hankel functions and $m=n_{2} / n_{1}$ with $n_{2}=$ sphere refractive index. The other quantities in the forces expressions are:

$$
\begin{gather*}
U_{n, m}^{p}=a_{n} a_{m}^{*} g_{n, T M}^{p} g_{m, T M}^{p+1^{*}}+b_{n} b_{m}^{*} g_{n, T E}^{p} g_{m, T E}^{p+1^{*}} ; \quad V_{n, m}^{p}=i b_{n} a_{m}^{*} g_{n, T E}^{p} g_{m, T M}^{p+1^{*}}-i a_{n} b_{m}^{*} g_{n, T M}^{p} g_{m, T E}^{p+1^{*}} \\
S_{n, m}^{p}=\left(a_{n}+a_{m}^{*}\right) g_{n, T M}^{p} g_{m, T M}^{p+1^{*}}+\left(b_{n}+b_{m}^{*}\right) g_{n, T E}^{p} g_{m, T E}^{p+1^{*}} ; \quad T_{n, m}^{p}=i\left(b_{n}+a_{m}^{*}\right) g_{n, T E}^{p} g_{m, T M}^{p+1^{*}}-i\left(a_{n}+b_{m}^{*}\right) g_{n, T M}^{p} g_{m, T E}^{p+1^{*}} \tag{4}
\end{gather*}
$$

The experimental and theoretical results for the radial force for the $3 \mu \mathrm{~m}$ microsphere are shown in figure 2 . For the axial trapping the experimental results are illustrated in figure 3, and theoretical Generalized Lorenz-Mie theory (GLMT) in figure 4. It should be noted that no influence on the axial optical force due to polarization was observed. All measurements were with the particle trapped by the Nd:YAG laser. However as the TiSapphire laser is the perturbing beam, the results obtained for optical forces are for this laser. All the measeruments were done with the Ti:Sapphire laser at 785 nm . Any particle can be only trapped against gravity in the region of negative values of the axial force. Figures 3 and 4 show that there is no axial trapping for the $3 \mu \mathrm{~m}$ sphere at 785 nm . We checked this point back by turn of the Nd :Yag and the unsuccessful attempt to lift the particle using only the Ti:Sapphire. All the graphics for the focused beam were done with the spot size of $0.4 \mu \mathrm{~m}$, estimated by Gaussian optics.


Figure 2: GLMT theory (red curve) and experimental (black curve) for the radial force for a 3 3 m sphere moving (a) perpendicular to the polarization and (b) parallel to the polarization.


Figure 3: Experimental data for the axial force in (a) $9 \mu m$ (b) $6 \mu m$ and (c) $3 \mu m$ spheres


In conclusion we have shown that the double optical tweezers can be used to observe the forces due to the light scattering and perform an ultrasensitive force spectroscopy. The measurements of whole accurate curves of the force as a function of wavelength, polarization and incident beam 3D position with respect to the center of the microsphere is important to understand and evaluate the optical models for optical tweezers. The Localized Integral Approximation of the Generalized Lorenz-Mie theory assuming an incident Gaussian beam profile was able to explain the trends of the behaviour of the axial and radial forces, but not to explain all the undulations of the plot neither the force magnitude. We expect that the substitution of the Gaussian beam by a more accurate high numerical aperture incident beam can improve the results. We believe the experimental measurements are sensitive enough to discriminate different force models, or even point out the necessity to consider aberrations. Our results show how careful one has to be when using optical force models for mechanical properties measurements.

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