

Force Spectroscopy and Two Photon Excited Luminescence in an Optical Tweezers System

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ABSTRACT

Up to now optical spectroscopies have analyzed the scattered light or the heat generated by absorption as a function of the wavelength to get information about the samples. Among the light matter interaction phenomena one that has almost never been used for spectroscopy is the direct photon momenta transfer. Probably because the forces involved are very small, varying from hundreds of femto to tens of pico Newtons. However, the nowadays very popular Optical Tweezers can easily accomplish the task to measure the photon momenta transfer and may be the basis for the Optical Force Spectroscopy. We demonstrate its potential as such a tool by observing more than eight Mie resonance peaks of a single polystyrene microsphere, and showed the capability to selective couple the light to either the TE, TM or both microsphere modes depending of the beam size, the light polarization and the beam positioning. The Mie resonances can change the optical force values by 30–50 %. Our results also clearly show how the beam polarization breaks the usually assumed azimuthal symmetry by Optical Tweezers theories. We also obtained the spectrum from the two photon excited luminescence using the Optical Tweezers to hold a single bead suspended and a femtosecond Ti:sapphire laser for the non-linear excitation. This spectrum shows the pair of peaks due to both TE and TM spherical cavity modes. We have been able to observe more than 14 Mie resonance peaks in the TPE luminescence. Our results are in good agreement with optical force calculations using Maxwell stress tensor and partial wave decomposition of the incident beam approximated to a 3th order gaussian beam.

Keywords: optical tweezers, Mie resonances, spectroscopy, force and polarization

Optical tweezers rely on the radiation pressure to trap and manipulate microscopic particles¹⁻². The recent review by David Grier³ shows its important use in many fields of research from physics to life sciences. The optical forces involved are very small, in the range of hundreds of femto to tens of pico Newtons, thus it has also been used as a mechanical properties measuring tool for microscopic phenomena where the forces involved are in the same scale. Typically, it has been used to measure forces, stiffness or elasticities of membranes or single DNA macromolecule⁴⁻⁸. Force measurements are usually performed with restorative systems like springs. The optical tweezers, being one of the smallest force stable trap, can be itself used to characterize optical forces involved in scattering of particles. Its ultrasensitiveness can be useful for experimental characterization of hard to measure very low force fundamental phenomena. The understanding of the optical scattering forces in dielectric microspheres under different incident beam conditions is very important as they have been used as the natural transducer for force and other mechanical measurements. Very few reports of levitation experiments observed Mie resonances directly through the optical force⁹. The Optical Tweezers system has the advantage and flexibility of a stable restorative force measurement system that do not exist in levitation experiments. Mie resonances, related to the modes of a dielectric cavity, are known as MDR (Morphology Dependent Resonances) because its position and width are very sensitive to particles shape and refractive index. Some pure liquids or liquid solutions, such as ethanol, glycerol, silicone oil and rhodamine have been used to generate droplets for MDR experiments¹⁰⁻¹², as well as Polystyrene microspheres¹³. These modes were observed by monitoring scattered light, Raman, radiation pressure and fluorescence¹⁴⁻¹⁷. The light confinement in a microcavity

enhances both linear and nonlinear spectral signal of substances bonded at the cavity surface and allows the construction of biosensors¹⁸⁻¹⁹.

In this work, we demonstrate the photonic force spectroscopy setup by using the optical tweezers to create a stable trap for a microsphere and another modulated tunable laser to disturb this equilibrium and observe the trapped particle displacement. This experiment was performed with the disturbing beam focused on the trapped particle or out of focus, with the beam size larger than the particle diameter, simulating an incident plane wave. The sensitivity of our technique was demonstrated by observing more than eight Mie resonance peaks of a single polystyrene microsphere. We also show the system capability to selective couple the light to either the TE, TM or both microsphere modes depending of the beam size, the light polarization and the beam positioning. These Mie resonances were also observed by the luminescence excited by two photon absorption (TPE – luminescence) using the Optical Tweezers to hold a 6 μm bead suspended and a femtosecond Ti:sapphire laser for two photon excitation. The use of two lasers, one as the optical tweezers that holds the single microcavity suspended and the other, cw or pulsed, disturbing laser to couple light into the cavity, provides enough freedom to perform all kinds of linear and nonlinear microcavity enhanced spectroscopies. The luminescence and plane wave spectra shows the pair of peaks due to both TE and TM spherical cavity modes and agree well with classical Mie scattering theory. However, the force spectroscopy results for focalized beam required a more complex Gaussian shaped beam partial wave decomposition theory²⁰.

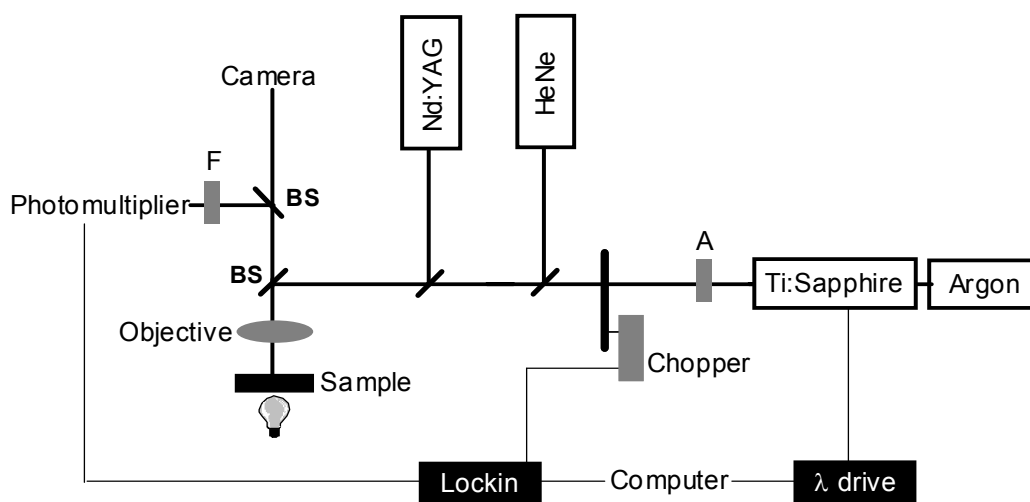


Figure 1. Experimental System to observe MDR modes using optical tweezers. The Nd:YAG, Ti:Sapphire and HeNe lasers are focused through a 100 \times oil immersion objective a microscope. The back-scattering signal of the HeNe laser is detected with a photomultiplier. In the sketch; F is filter, A is attenuator and BS is beam-splitter.

Figure 1 shows the experimental system for photonic force spectroscopy measurements. 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 disturbing beam in any desired position at the sphere and, therefore, to choose the MDR modes coupled to the disturbing beam. Polystyrene microspheres with diameter of 9 μm (Polysciences), were diluted in water and placed in a Neubauer chamber. The microspheres were trapped by the optical tweezers consisting of a Nd:YAG laser (1064nm, model 3800S, Spectra Physics) focused through a 100 \times oil immersion objective (SPlan) in an Olympus microscope (BH2, Olympus Optical CO., Ltd.). The MDR modes were excited by a cw Ti:Sapphire laser (700-900nm, model 3900S, Spectra Physics Lasers) highly attenuated (100 times) to guarantee the small displacement regime. The beam was modulated at 12 Hz by a mechanical 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. The signal was detected with a photomultiplier (Hamamatsu) coupled to the eyepiece of the microscope and a lock-in amplifier (Stanford Research Systems, model SR830 DSP). The resonances were observed by monitoring the amplitude of the displacements while changing the wavelength of the Ti:Sapphire laser. The wavelength was scanned by rotating the birefringent plate inside the laser cavity with a rotating step motor (Newport, Microcontrol, model ITL09) and calibrated with a monochromator (Acton Research Corporation) equipped with a CCD (Princeton Instruments). A labview program controls the step motor

movement and the lock-in amplifier signal collection. The MDR modes were observed in different manners. First the Ti:Sapphire beam out of focus, figure 2 (a), a situation where the disturbing beam can be well approximated by a plane wave. Second, the beam is focused at the edge of the microsphere at three different configurations (1), (2) and (3) as follows. In configuration (1), the x direction polarized beam is focused at $(R, 0, 0)$, where the electric field is radial and magnetic field transverse. In configuration (2) the beam is focused at $(0, R, 0)$, with a transverse electric and radial magnetic fields. Finally at configuration (3) the beam is focused at $((R, R, 0)/\sqrt{2})$, where there are radial and transverse components for both fields, as shown in figure 2 (b). The three measurements have been done with the same microsphere. To assure the microsphere stayed trapped for the time necessary to acquire the data, hours, a very diluted microsphere solution was used, to diminish collisions between the trapped and other suspended microspheres. A Gaussian shaped beam partial wave decomposition theory²⁰⁻²¹ was able to explain our results for the force magnitude and mode coupling as a function of the disturbing laser wavelength.

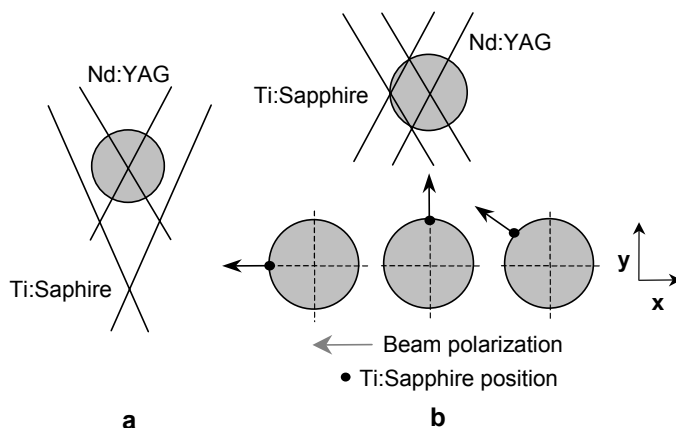


Figure 2. Scheme of the measurements of MDR modes with a plain wave (a) and focused beam incidence (b). The sketch (b) shows the beam polarization direction and the three focused beam position of the Ti:Sapphire laser for a point of view of the axes z.

The micro-spectroscopy & Optical Tweezers system to observe the Mie resonances by TPE luminescence used the same cw Nd:YAG laser for trapping and a femtosecond Ti:Sapphire laser (700-900 nm, Tsunami, Spectra Physics) for the two photon excitation. The laser beams were also focused by the 100× oil immersion in the Olympus microscope. The images and spectra were collected with a digital camera while the back-scattered spectroscopic signals were collected with a 30 cm monochromator equipped a CCD, as shown in Figure 3. A super notch filter was used not only as a filter for the backscattered light but also as a mirror to reflect both, the femtosecond and the cw Ti:Sapphire laser beams to the microscope. We also used a short pass color filter to transmit the visible and to cut the infrared light.

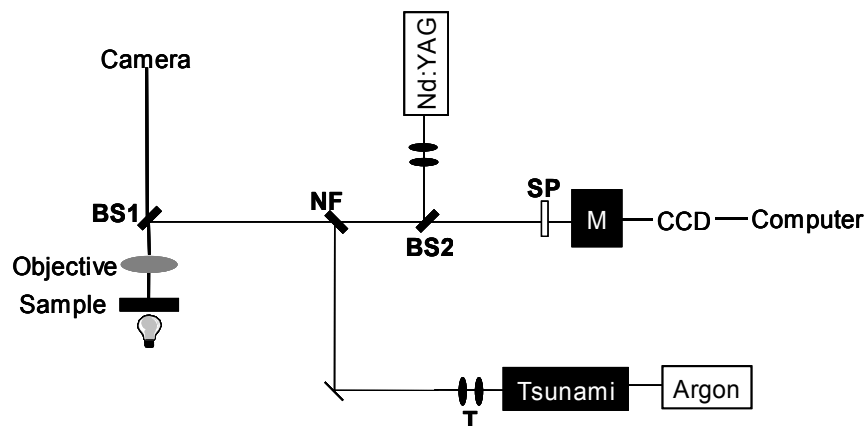


Figure 3. System for micro-spectroscopy (TPE luminescence) plus optical tweezers. BS1 is a metallic beamsplitter, BS2 is a dichroic mirror of Nd:YAG, SP is the short pass, T are the telescopes and M is the monochromator.

The plane wave results for force spectroscopy and TPE luminescence can be well explained by the conventional Mie scattering theory. For the force spectroscopy results, the radiation force cross-section is given by²²⁻²³:

$$Q_{pr} = \frac{2}{x^2} \sum_{n=1}^{\infty} Re \{ (2n+1)[a_n + b_n] \} - \left(\sum_{n=1}^{\infty} \frac{(2n+1)}{n(n+1)} Re [a_n b_n^*] + \sum_{n=1}^{\infty} \frac{n(n+2)}{(n+1)} Re [b_n b_{n+1}^* + a_n a_{n+1}^*] \right) \quad (1)$$

where the size parameter $x = ka$ with $a =$ microsphere radius and $k = 2\pi n_1 / \lambda$, ($\lambda =$ 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:

$$a_n = \frac{m^2 j_n(m x) [x j_n(x)]' - j_n(x) [m x j_n(m x)]'}{m^2 j_n(m x) [x h_n^1(x)]' - h_n^1(x) [m x j_n(m x)]'} \quad b_n = \frac{j_n(m x) [x j_n(x)]' - j_n(x) [m x j_n(m x)]'}{j_n(m x) [x h_n^1(x)]' - h_n^1(x) [m x j_n(m x)]'} \quad (2)$$

where 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 resonances happen at the minimum of the Mie coefficient denominators, which do not have real roots. The peaks for the TE (b_n) is slightly shifted to the right respect to the TM (a_n) peaks for the same n value and the resultant spectrum usually shows a pair of peaks for each n . On the other hand, the theoretical peaks for TPE luminescence were calculated using the efficiency function Q for backscattering light:

$$Q_b = \left| \sum_n (2n+1)(-1)^n (a_n - b_n) \right| / x^2 \quad (3)$$

Mie scattering theory was developed for plane waves only and cannot explain the 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 more at the origin of the coordinate system all the beam azimuthal symmetry is lost. The resonance peak positions are satisfactorily described by any well localized incident beam, thus we used a first order Davis Gaussian description²⁴ instead of the more precise Born & Richards beam description²⁵. There is a ready to use prescription to calculate the beam shape coefficients $g_{n, TE}^{m, TM}$ for the Davis description²⁶⁻²⁷. The scattered beam coefficients are given by $g_{n, TM}^m a_n$ and $g_{n, TE}^m b_n$, and the force cross sections for the x, y and z directions by²⁰⁻²¹:

$$Q_{pr, z} = \frac{4}{x^2} \sum_{n=1}^{\infty} \sum_{p=-n}^n \left(\frac{1}{(n+1)^2} \frac{(n+1+|p|)!}{(n-|p|)!} \times Re \left[(a_n + a_{n+1}^* - 2a_n a_{n+1}^*) g_{n, TM}^p g_{n+1, TM}^{p*} \right. \right. \\ \left. \left. + (b_n + b_{n+1}^* - 2b_n b_{n+1}^*) g_{n, TE}^p g_{n+1, TE}^{p*} \right] + p \frac{2n+1}{n^2 (n+1)^2} \frac{(n+|p|)!}{(n-|p|)!} \times Re \left[i (2a_n b_n^* - a_n - b_n^*) g_{n, TM}^p g_{n, TE}^p \right] \right) \\ Q = \frac{2}{x^2} \sum_{p=1}^{\infty} \sum_{n=p}^{\infty} \sum_{m=p-1 \neq 0}^{\infty} \frac{(n+p)!}{(n-p)!} \times \left[(S_{m,n}^{p-1} + S_{n,m}^{-p} - 2U_{m,n}^{p-1} - 2U_{n,m}^{-p}) \times \left(\frac{1}{m^2} \delta_{m,n+1} - \frac{1}{n^2} \delta_{n,m+1} \right) + \right. \\ \left. \frac{2n+1}{n^2 (n+1)^2} \times \delta_{n,m} (T_{m,n}^{p-1} - T_{n,m}^{-p} - 2V_{m,n}^{p-1} + 2V_{n,m}^{-p}) \right] \quad (4)$$

where, $Q_{pr, x} = Re[Q]$ and $Q_{pr, y} = Im[Q]$ for the x and y components. The other quantities that appear in the expressions are:

$$U_{n,m}^p = a_n a_m^* g_{n, TM}^p g_{m, TM}^{p+1*} + b_n b_m^* g_{n, TE}^p g_{m, TE}^{p+1*}; \quad V_{n,m}^p = i b_n a_m^* g_{n, TE}^p g_{m, TM}^{p+1*} - i a_n b_m^* g_{n, TM}^p g_{m, TE}^{p+1*} \\ S_{n,m}^p = (a_n + a_m^*) g_{n, TM}^p g_{m, TM}^{p+1*} + (b_n + b_m^*) g_{n, TE}^p g_{m, TE}^{p+1*}; \quad T_{n,m}^p = i (b_n + a_m^*) g_{n, TE}^p g_{m, TM}^{p+1*} - i (a_n + b_m^*) g_{n, TM}^p g_{m, TE}^{p+1*} \quad (6)$$

Figure 4 shows the experimental results together with the Mie theoretical calculation for the plane wave experiment in the force spectroscopy experiment. Figure 5 (a) shows the fluorescence emission in the blue region for the trapped polystyrene microspheres, curve 1 for a 2.5 μm microsphere and curve 2 for a 6 μm microsphere. One can see

peaks in the blue fluorescence spectra of the larger microsphere that are not seen in the spectra of the smaller microsphere. These are the MDR mode peaks of the single trapped microsphere excited by TPE luminescence. In figure 5 (b) we show the experimental results of the MDR modes compared with the theoretical calculation using the Mie Theory. The experimental MDR results were obtained by removing the background fluorescence from curve 1 of figure 5 (a). The expressions for the Mie coefficient involve the refraction indexes of the microsphere and water, which changes with the wavelength. We performed the theoretical calculations using $n_2 = 1.5683 + 10.087 \times 10^3 / \lambda^2$ for the dispersion relation of the polystyrene sphere and $n_1 = 1.324 + 3.046 \times 10^3 / \lambda^2$ for the water²⁸. The best agreement for the force spectroscopy (plane wave) was found for the microsphere diameter of 9 μm , very close to the nominal diameter of $(9.126 \pm 0.5) \mu\text{m}$ provided by the manufacturer, while the best agreement of TPE luminescence results was found for the microsphere diameter of 5.44 μm , that is well within the nominal diameter range, of $(6 \pm 0.6) \mu\text{m}$. The difference between the theoretical and experimental data is smaller than the peak width which is given by the imaginary part of the Mie coefficients. The fact that more than 20 experimental and theoretical double peaks shown in these figures coincide shows how well these resonances are described by Mie scattering model and also shows the sensitiveness of the measurement.

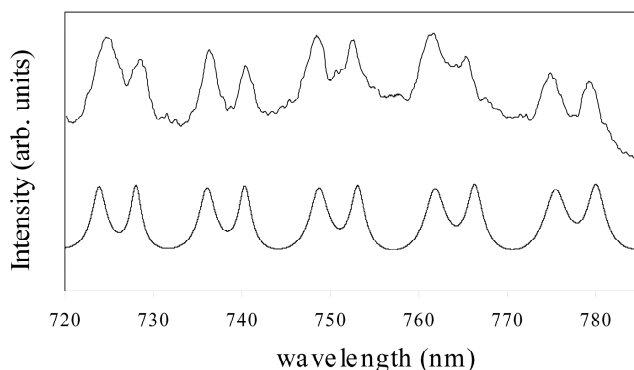


Figure 4. Experimental (above) and Theoretical (below) spectra of MDR. Modes for a 9 μm dielectric sphere excited by a plane wave.

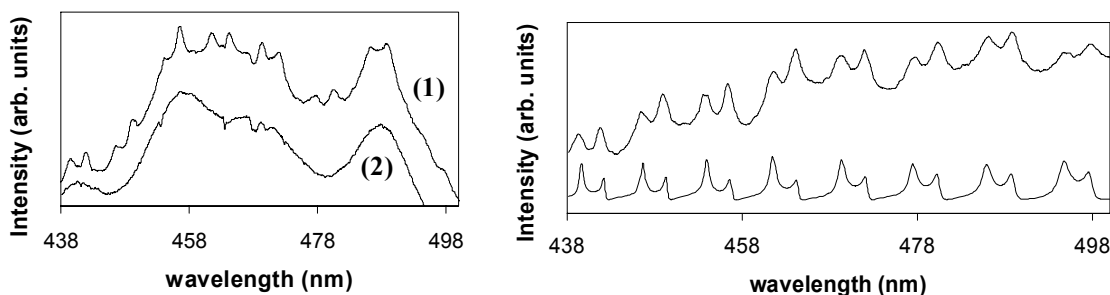


Figure 5. (a) Spectra of fluorescence excited by TPE for trapped polystyrene microspheres, curve (1) diameter of 6 μm and curve (2) diameter of 2.5 μm . Curve (1) also shows the microsphere MDR modes. **(b)** Comparison between the theoretical and experimental MDR data.

The results obtained for the focalized beam are shown in figure 6 (a, b, c). The theoretical results are calculated considering all force components, $Q_{res} = \sqrt{Q_x^2 + Q_y^2 + Q_z^2}$, because, for the beam focused at the edge, the z force component is of the same order of the x and y force components. All the graphics for the focused beam were done with the spot size of 0.4 μm , estimated by Gaussian optics. For the focus at $(R, 0, 0)$ and $(0, R, 0)$, we observed only one peak instead of the usual pair of peaks. This is because only one mode, TM or TE, is excited in each case. This can be intuitively understood by noticing that only the radial component of the electric field will excite the TM mode and only the radial component of the magnetic field will excite the TE modes. When the focus is at $(R, 0, 0)$ position, the electric field, polarization, is radial, while the magnetic field is transverse, and only TM modes were excited. The opposite is true for the focus at

$(0, R, 0)$ position. Now there is no radial component of the electric field and only TE modes are excited, as clearly shown by the force spectra of figure 6 (a, b). On the other hand, for the focus at $((R, R, 0)/\sqrt{2})$ position, there are both, electric and magnetic, radial components of the fields. In this case both, TM and TE modes, are excited and the pair of peaks appear again, as shown in figure 6 (c). The particle diameter of $9.32 \mu\text{m}$ used to plot these graphics was obtained by fitting the $((R, R, 0)/\sqrt{2})$ spectrum with plane wave Mie theory. To excite the MDR modes we placed visually the beam focus at the microsphere edge and moved it looking at a continuous fast spectra of only one peak and maximized the intensity of this peak. The good agreement between the calculated and the experimental values could only be achieved because we obtained all three spectra with the same microsphere. Also the fact that the microsphere is suspended in the fluid by the optical tweezers is important. Any mechanical device used to support the microsphere will ruin out the boundary conditions on its surface.

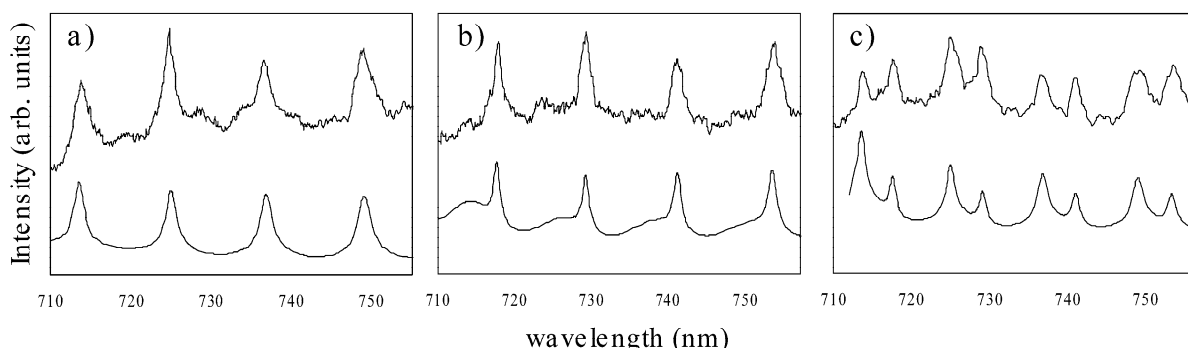


Figure 6. Experimental (above) and Theoretical (below) spectra of MDR. (a, b, c). Spectras obtained with a focused beam of a $9.32 \mu\text{m}$ dielectric sphere. (a) TM modes for the particle moving parallel to the incident beam polarization, (b) TE modes for the particle moving perpendicular to the incident beam polarization. (c) TM and TE modes for the particle moving a 45° of the beam polarization.

In conclusion we have shown that the double optical tweezers can be used to observe the forces due to the light scattering and Morphology Dependent Resonances (MDR) in a single isolated particle. The double tweezers was used to perform ultra sensitive force spectroscopy and by using the beam polarization and positioning to selectively coupled the light to either the TE, the TM or both TE and TM microsphere modes. The importance to understand 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. Our results show how careful one has to be when using optical force models for mechanical properties measurements. The MDR resonances 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. We also have presented a set-up of an optical tweezers combined non-linear micro-spectroscopy. With this system, we were able to observe more than 14 Mie resonance peaks excited by TPE luminescence in a single stained trapped microsphere of $6 \mu\text{m}$ diameter.

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