

# Morphology-dependent resonance induced by two-photon excitation in a micro-sphere trapped by a femtosecond pulsed laser

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**Abstract:** We report on the measurement of morphology-dependent resonance within a laser-trapped micro-sphere excited under two-photon absorption. Both trapping and two-photon excitation are simultaneously achieved by a single femtosecond pulsed laser beam. The effect of the laser power as well as the pulse width on the transverse trapping force is first investigated. The dependence of two-photon-induced morphology-dependent resonance on the scanning velocity of a trapped particle is then experimentally determined.

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**OCIS codes:** (260.5740) Resonance; (190.4180) Multiphoton processes; (140.7010) Trapping

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## 1. Introduction

Laser trapping has led to a strong impact on biological studies and chemical scientific research at a single molecule level [1-3] and as well as scanning near-field optical microscopy (SNOM) [4, 5]. Laser trapping SNOM utilizes a trapped micro-particle as a scanning probe for near-field imaging and has some advantages over conventional SNOM which uses a tapered fiber tip as a scanning probe. One of the most significant advantages is that controlling the distance between a probe and a substrate is not required in laser trapping SNOM [4, 5]. In addition, the concern associated with a fragile probe is also not an issue in laser trapping SNOM. However, due to the low signal strength, image contrast in laser trapping SNOM requires a significant improvement. One solution to this problem is the utilization of morphology-dependent resonance (MDR) [6-10] in a trapped micro-sphere.

A dielectric sphere possesses natural internal modes of oscillation at characteristic frequencies corresponding to specific ratios of size to wavelength, which are called MDR [6-8]. This feature provides a useful tool for enhancing the signal strength in sensing and imaging with a trapped micro-sphere [10]. MDR can be induced in fluorescent micro-spheres under single-photon [9, 10] and two-photon [11] excitation. To induce MDR in a trapped micro-sphere, one normally needs two beams; one is for trapping a micro-sphere and the other for fluorescence excitation. It is, however, difficult to dynamically control the two focal spots with high accuracy. In this paper, we introduce a novel trapping and excitation technique, which utilizes only one femtosecond pulsed laser beam for resonance excitation and trapping simultaneously. The induction of MDR is achieved under two-photon excitation. MDR induced by two-photon excitation [11] has shown advantages of separating excitation and MDR wavelengths and confining excitation illumination precisely.

Although various laser sources has been used to induce two-photon fluorescence from a dye-doped micro-sphere under a laser trapping system [12-15], it is difficult to achieve both excitation of MDR and trapping by a single CW laser beam simultaneously. The physical reason for this difficulty is that the requirement of the power density is different for particle trapping and MDR under two-photon excitation. For example, under a tight focus of a high numerical aperture objective, the power required for steady trapping is usually at least one order magnitude higher than the photo-bleaching level. To overcome this problem, a femtosecond pulsed laser beam is introduced to perform simultaneous trapping and two-photon fluorescence excitation.

## 2. Femtosecond illumination

The effect of a femtosecond pulsed laser beam on laser trapping performance was demonstrated in the experimental system shown in Fig. 1. A train of linearly polarized 86 fs pulses of wavelength 870 nm (Spectra-Physics Tsunami) is coupled directly into an inverted trapping microscope objective so that the back aperture of the trapping objective is filled. A high numerical aperture (NA=1.2) water immersion objective (Olympus UplanXW60) is used to focus the pulse beam into a sample cell. The sample cell consists of Yellow-Green fluorescent micro-spheres of 10  $\mu\text{m}$  in diameter (Polysciences), which has an absorption peak close to the laser wavelength for two-photon excitation [10]. The micro-spheres are suspended in water within a sealed sample cell. The displacement of a trapped particle is achieved by a computer-controlled scanning stage on which the sample cell is attached. Throughout this paper we employ an *s*-polarized trapping beam, meaning that the polarization direction of a trapping beam perpendicular to the direction of the transverse displacement of a trapped particle. The fluorescence emission from an excited micro-sphere, which exhibits the MDR feature [11], is analyzed by a high-resolution spectrograph (ARC,  $\Delta\lambda=0.1-0.3$  nm). A series of dispersive pulse-stretch SF-6 glass rods (supplied the Max Planck Institute for Biophysical

Chemistry at Goettingen, Germany) of lengths 25 mm and 65 mm are placed at the output of the laser, so that the laser pulse width on a trapped particle can be stretched to 206 fs and 436 fs, respectively.

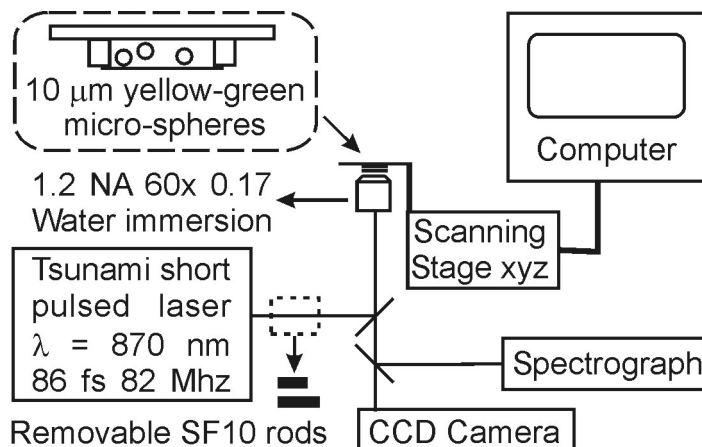


Fig. 1. Schematic diagram of the experimental setup.

For a given pulse width of 86 fs, the maximum transverse trapping force [16] as a function of the average laser power is depicted in Fig. 2. As expected, the maximum transverse trapping force linearly increases with the laser power initially ( $<2$  mW), then under goes a non-linear increase and subsequently saturates due to the increased two-photon absorption when the trapping power becomes large. To determine the range of the trapping power within which a particle can be trapped without damage, we monitored the trapping performance at a constant velocity of  $10 \mu\text{m/s}$  as the trapping power increases. The observed power range for the pulse widths of 86 fs, 206 fs and 436 fs is marked in the inset of Fig. 2. This power range defines a region where the two-photon-induced MDR phenomenon can be observed from a particle trapped by a pulsed beam.

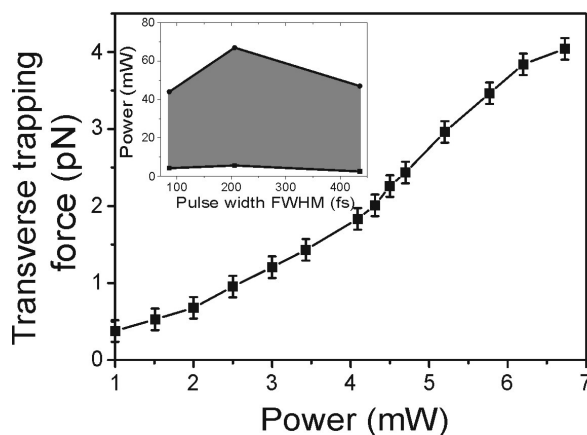


Fig. 2. The dependence of the maximum transverse trapping force on laser power. The inset gives the power range within which a particle can be trapped and scanned without damage.

The inset of Fig. 2 also implies that a trapped particle can be translated at different velocities when one selects a trapping power level within the marked range. As a result of the balance between the Stokes force and the transverse trapping force, which depend on the translation velocity of a trapped particle and on the transverse displacement of a trapped

particle, respectively, the trapping spot will be located at different transverse positions of a trapped particle. It has been previously demonstrated that the strength of the two-photon-induced MDR is highly dependent on the location of the excitation spot within a micro-sphere because of the highly localized nature of two-photon absorption [11]. Therefore, the strength and the visibility of the MDR signal varies with translation velocity of a trapped particle.

### 3. Resonance visibility

In order to quantify the MDR feature in relation to the translation velocity of a trapped particle, we introduce the measurable quantity, the visibility  $V$  defined as  $V = (I_{peak} - I_{background}) / (I_{peak} + I_{background})$ , where  $I_{peak}$  and  $I_{background}$  are the intensity of MDR peaks and the background fluorescence, respectively [11]. The MDR signal induced in a stable laser trapped particle for various translation velocities is shown in Fig. 3. It is shown that the MDR effect is greatly enhanced when the translation velocity increases. This phenomenon can be explained as follows. At a given laser power, the greater the translation velocity, the greater the transverse trapping force is required. This means that the trapping spot moves towards the edge of a trapped particle because the transverse trapping force increases with the displacement of the trapping beam [17]. It has been previously demonstrated that the two-photon-induced MDR effect becomes more significant when the excitation spot moves closer towards the edge of a trapped particle [11]. Therefore, the visibility of the MDR signal becomes pronounced when a particle is scanned fast. Note that the spectral change of the background fluorescence is determined by the coupling efficiency of the excitation beam and the collection efficiency of the two-photon fluorescence at different velocities.

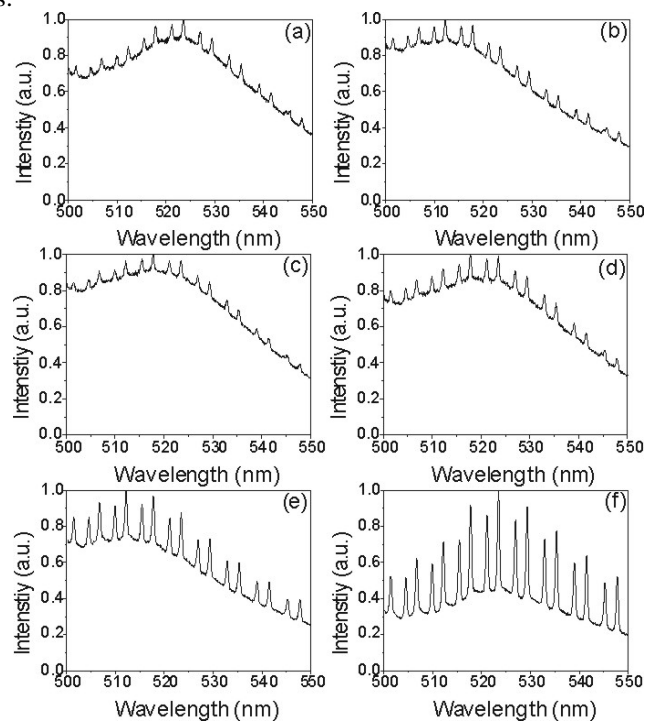


Fig. 3. MDR spectra of a laser trapped micro-sphere at velocities 4, 9, 14, 19, 26 and 29  $\mu\text{m/s}$  (a) to (f), respectively.

The two adjacent peaks in the MDR fluorescence spectrum represent two cavity modes, the transverse electric (TE) and transverse magnetic (TM) modes [11]. It is shown in Fig. 4 that both modes share a similar increase in visibility with increasing the translation velocity.

This feature enables the potential multi-modality imaging or sensing including polarization-dependent signals.

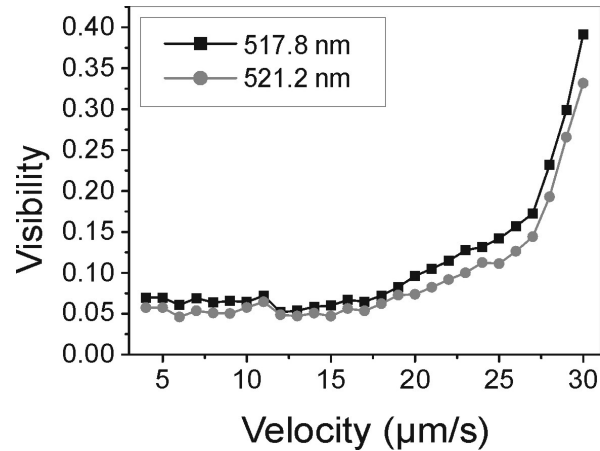


Fig. 4. Visibility of peaks 517.8 nm (squares) and 521.2 nm (circles) as a function of the translation velocity of a trapped particle.

#### 4. Conclusion

In conclusion, we have demonstrated the achievement of simultaneous two-photon induced MDR and trapping of a micro-sphere by a single ultrashort-pulsed beam. The use of a femtosecond-pulsed beam allows for localized two-photon excitation while a trapped particle can be scanned at different velocities. The measured dependence of the visibility of the MDR signal on the translation velocity indicates that a high sensitivity and a high scanning velocity of a trapped particle can be achieved simultaneously. This result implies that a better sensitivity in laser trapping SNOM [4] could be achieved based on the two-photon induced MDR signal, which will be an invaluable tool for mapping tomography, topography and force.

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