Scanning particle trapped optical microscopy based on two-photon-induced morphology-dependent resonance in a trapped microsphere

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We report on scanning particle trapped optical microscopy based on two-photon-induced morphology-dependent resonance (MDR) in a trapped microsphere. In this imaging mode, a femtosecond pulsed laser is adopted for simultaneous laser trapping and two-photon excitation. Due to the localized excitation, MDR is significantly enhanced. As a result, an image contrast enhancement of 9.3% is achieved by the spectral detection of a single on-resonance MDR mode compared with that obtained by the off-resonance MDR mode without compromise in transverse resolution. © 2006 American Institute of Physics. [DOI: 10.1063/1.2186076]

Laser trapping or laser tweezing of a microparticle, based on the radiation pressure, was employed as a sensing probe for particle trapped microscopy. In this case, a trapped particle is positioned at the sample surface and acts as an imaging probe. Based on this concept, there have been two main imaging modes, force microscopy and optical microscopy. The principle of the first imaging mode is the force sensitivity of a trapped particle, which can be used for surface profiling and position tracking. The second imaging mode of particle trapped microscopy utilizes optical signals generated by various interaction processes between a trapped particle and the incident light. First, scattering of a trapped dielectric particle with an evanescent field at the sample surface converts the localized photons to a far-field region, providing a tool for high resolution near-field optical microscopy. The second optical imaging method is based on the scattering of a trapped particle with the trapping beam, which is determined by the surface profile and the material of a sample. The third type of optical signal is the radiation induced by the trapping beam in a trapped particle, including second harmonic generation or fluorescence.

A fluorescent Mie microsphere is an excellent candidate for use as a laser trapped optical imaging probe rather than the particle acting solely as a scatter. First, a laser trapped imaging probe allows multiple imaging and sensing modes by the excitation of fluorescence resulting from morphology-dependent resonance (MDR) due to the total internal reflection of the internal field within the microcavity. Second, the fluorescence process can be localized if two-photon excitation is used, enhancing the visibility of MDR spectra. Further, the use of a femtosecond laser exhibits the enhancement of the two-photon-induced MDR effect. The strength and position of the enhanced and localized MDR spectra are highly sensitive to the environment with which a trapped particle interacts. In this letter, based on the two-photon-induced MDR effect from a microsphere trapped by a femtosecond laser, we report on particle trapped optical microscopy of a sample surface profile of several hundred nanometers in height.

The physical principle of this imaging method is the sensitivity of two-photon-induced MDR with respect to the position of the trapping focus within a trapped particle. When a trapped particle scanned at a given velocity interacts with a topological feature of a sample, the trapping position initially produced by the balance between trapping force and viscous drag force is displaced further from the center of the particle to the particle edge. This feature leads to the fluorescence imaging contrast resulting from the two-photon excited MDR effect in a trapped particle.

The laser trapped imaging performance of a femtosecond pulsed laser beam was demonstrated via the experimental system shown in Fig. 1(a). A train of linearly polarized 86 fs pulses of wavelength 870 nm (Spectra-Physics Tsunami) was directly coupled into an inverted trapping microscope so that the back aperture of the trapping objective was filled. The trapping objective used was a high numerical-aperture (NA) water immersion objective (Olympus Up-
lanXW60, NA=1.2). The sample cell consisted of yellow-green fluorescent microspheres of 10 μm in diameter (Polysciences), which has an absorption peak close to the laser wavelength for two-photon excitation. The microspheres were suspended in water within a sealed sample cell. The scanning of a trapped particle was achieved by a computer-controlled scanning stage on which the sample cell was attached.

An s-polarized trapping beam was employed, such that the polarization direction of the trapping beam was parallel to the direction of the transverse displacement of a trapped particle [Fig. 1(b)]. The resonance fluorescence emission from an excited microsphere was analyzed by a high-resolution spectrograph (ARC, ΔΛ=0.1–0.3 nm). The spectral properties of the return signal from the laser trapped fluorescent probe were monitored via the high-resolution spectrograph acting as a monochrometer, so that a spectral bandwidth of 1 nm was incident at the detector. This spectral window of detection was then shifted so that the fluorescence intensity from either a single MDR maxima (peak) or minima (valley) was measured [Fig. 1(c)]. The terms peak, valley, and integrated correspond to the signal from the aforementioned maxima, minima, and entire fluorescence MDR spectra, respectively.

In order to characterize the imaging potential of a laser trapped probe, a sample with a surface profile as shown in the inset of Fig. 1(a), was used. The sample is a polymer grating produced by the two-photon polymerization method in Norland NOA63 resin on the surface of a cover slip. It has a spacing of 10 μm, a thickness between 0.1 and 3 μm, and a height <1 μm, respectively. An atomic force microscope (AFM) (NT-MDT, Moscow) in a semicontact mode was used to take a profile of the grating, showing a typical full width at half maximum (FWHM) of 0.6 μm and a peak height of approximately 600 nm [Fig. 1(d)].

Figure 2 shows the one-dimensional integrated image obtained by using the laser trapped probe scanning over the grating structure (I). A one-dimensional reflection confocal laser scanning image of the grating structure is also shown in Fig. 2 (I'). The respective image cross sections obtained under the laser trapping microscope and the reflection confocal microscope is also illustrated in Fig. 2 (T and T'). A FWHM of approximately 1.00 μm is obtained in the confocal image trace while a FWHM of approximately 0.60 μm is achieved by laser trapped particle microscopy. The laser trapped particle microscopy trace produces transverse resolution comparable to that of AFM, demonstrating that the resolution is not necessarily limited to the particle size but to the interaction region between the probe and the sample.

The laser trapped particle probe trace shows three regions as the probe scans over the structure (T and T'). This is attributed to the fact that MDR is highly dependent on the change of the fluorescence excitation to resonance modes. As the trapped particle crests the top of the structure, and then down the slope, the trapping position moves to the center, resulting in a sharp decrease in mode coupling and signal intensity. The signal then increases as the scanning of the trapping beam restores the focal position to the stable transverse trapping position.

The laser trapped image contrast of the grating structure under the spectral detection of the MDR peak, valley, and integrated signals is examined over a range of translation velocities of a trapped probe. The average image intensity profiles over the relative displacement of the trapped particle for translation velocities of 8.7, 10.8, 12.9, and 15.0 μm/s for integrated, peak, and valley signals are shown in Fig. 3. The sharpest image profile was spectrally detected from the MDR peak intensity shown in Figs. 3(e)–3(h). This is attributed to the fact that MDR is highly dependent on the change in the laser trapping focal position within the scanning microcavity/microsphere probe. Correspondingly, the MDR valley signal has little modulation as it is essentially a measure of the fluorescence background [Figs. 3(i)–3(l)]. The spectral detection of the integrated MDR fluorescent spectrum shown in Figs. 3(a)–3(d) approximates the average effect of the peak and valley signals. The respective contrast
enhancement of MDR peak and integrated signal images compared with the valley signal images is illustrated in Fig. 4(a), showing an approximate contrast increase of 2.7% and 9.3% at a scanning velocity of 8.7 μm/s. At high transverse trapping velocity, the rapid ascent of a structural element can result in the trapped particle breaking contact and leaving the sample surface. This results in a loss of image contrast due to the decreased displacement of the trapping spot within the trapped microcavity.

The spectrally resolved images of the grating can be obtained with a cooled charge-coupled device (CCD) camera attached to the spectrograph for a given exposure time. The resultant spectrally resolved image of a one-dimensional scan in the x direction is given in Fig. 4(b), where the graphic inset along the x axis schematically depicts the grating structure atop the coverslip. The spatial resolution of the image is 1 μm every sampling point according to the scanning speed (10 μm/s). It is clear to see in Fig. 4(b) that the MDR effect is enhanced at the position of each grating element as the trapping spot is pushed further toward the edge of the particle, resulting in a stronger MDR signal. The fully spectrally resolved image reveals the different coupling of the excitation to the resonance modes as the probe is scanned across the sample due to the variation of the surface profile of the sample.

In conclusion, the principle of particle trapped optical microscopy based on two-photon-induced MDR from a trapped microsphere has been experimentally demonstrated. It has been shown that images in this system can be constructed with three spectral modalities: integrated, peak, and valley signals. The integrated image trace of a grating structure exhibits transverse resolution comparable to that obtained with AFM, while the contrast from the peak imaging modality shows a considerable enhancement compared with the integrated and valley imaging modalities. A spectrally resolved image demonstrates that this technique could provide an alternative imaging mechanism for near-field optical microscopy and surface tomography via MDR.

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