

Two-photon fluorescence imaging and spectroscopy of nanostructured organic materials using a photon scanning tunneling microscope

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Photon scanning tunneling microscopy and spectroscopy using femtosecond two-photon excitation are demonstrated. The measurement of both intensity dependence and spectral dependence is performed on a two-photon chromophore. A subdiffraction-limited resolution is obtained, and the domain-size dependence of spatial and spectral features is observed, which indicates the high degree of molecular order in the isolated nanoparticle. It is shown that the light confinement due to a quadratic dependence of the fluorescence intensity leads to an optical contrast enhancement with a coated probe. © 2000 American Institute of Physics. [S0003-6951(00)02401-3]

Photon scanning tunneling microscope (PSTM) has been demonstrated to overcome optical diffraction limits in the past few years.¹⁻⁴ The principle of PSTM is to detect the evanescent field above a sample that is illuminated under total internal reflection with a nanoscopic probe. Due to the fact that the depth of penetration for PSTM is limited, the resolution of PSTM is not as good as that of near-field scanning optical microscope (NSOM),⁵⁻⁸ but it is better than that of the conventional optical microscope, and this noninvasive technique shows potential benefit in thin-film analysis.

Two-photon excitation microscopy is a nonlinear optical imaging technique,⁹ which has advantages of effective rejection of background, reduced volume of photobleaching, and depth discrimination. Some of these advantages can benefit PSTM. Since many new organic compounds are synthesized for application in recently developed multiphoton microscopy, and some two-photon fluorophores are incorporated in a multifunctional nanoparticle, two-photon PSTM can be used as a promising tool in analysis and control of such nanoparticle synthesis. In this letter, we present the extension of two-photon excitation to photon scanning tunneling microscopy and localized spectroscopy to probe thin organic films with nanoparticles. We obtain a subdiffraction-limited resolution and observe the domain-size dependence of the spatial and spectral features. We show that two-photon PSTM provides advantage in signal-to-noise improvement and optical contrast enhancement.

The schematic of the experimental setup is shown in Fig. 1. A self-mode-locked Ti:sapphire laser is used as an excitation source at 800 nm with an average power set at 12 mW. The pulse width is 80 fs at a repetition rate of 90 MHz. The laser beam is focused by a lens ($f=25$ cm) and illuminates the sample that is mounted with an index-matching oil on a

fused silica prism under total internal reflection. The dispersion length of the prism is only 5 mm, and pulse broadening and nonlinear effects are negligible. The fluorescence signal is collected by an aluminum-coated fiber probe (Topometrix) in the near field above the sample, passed through a bandpass filter to remove scattered excitation light, and detected by a cooled photomultiplier tube (PMT) (Hamamatsu R943-02) to generate an optical image. The probe with an apex diameter of ~ 200 nm is attached to a piezotube scanner and oscillated at its resonance frequency. A shear-force feedback that uses tuning fork detection keeps the probe-sample separation constant and produces a topographic image simultaneously with the optical image as the probe is rastered across the sample surface. The fluorescence spectrum is recorded with a combination of a spectrograph (Kaiser Optical Systems) and

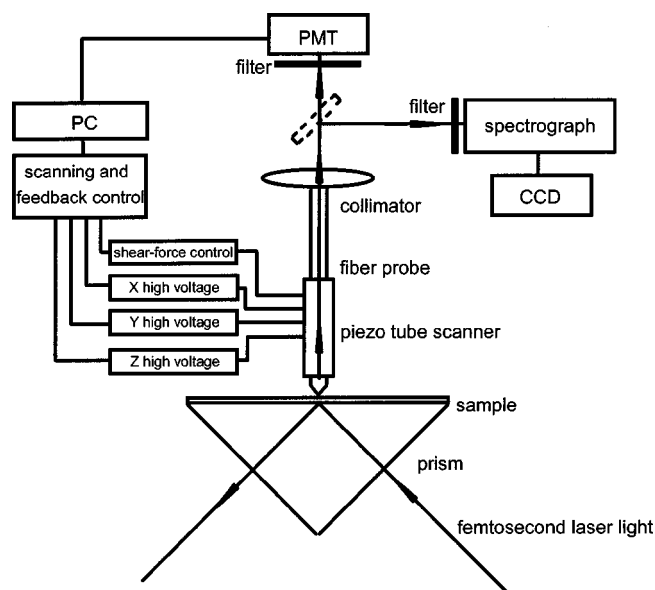


FIG. 1. Schematic of photon scanning tunneling microscopy and spectroscopy with two-photon excitation.

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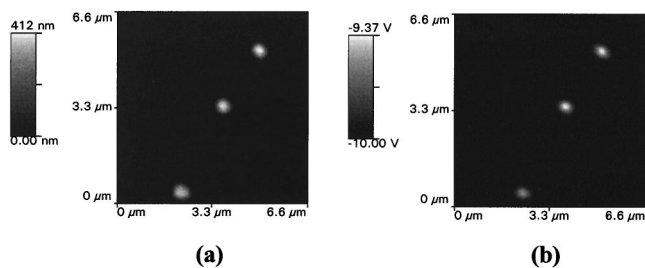


FIG. 2. (a) shear-force image and (b) two-photon fluorescence PSTM image of an AF-390 nanoparticle.

a charge-coupled device (CCD) camera (Princeton Instruments).

The sample is prepared by spreading a dilute chloroform solution of AF-390 across a cover slip previously spin coated with a 60 nm polymethylmethacrylate (PMMA) film. AF-390 is a two-photon chromophore, synthesized at the Polymer Branch of the U.S. Air Force Research Laboratory. It exhibits photostability and a large two-photon excitation cross section ($9.1 \times 10^{-20} \text{ cm}^4/\text{G W}$) in the 760–840 nm range.

Figure 2 shows the simultaneously collected shear-force image and fluorescence image, respectively, of isolated AF-390 nanoparticles, in which the intensity variations of the fluorescence are correlated with the topographic features over the entire sample surface. The strong correlation is due to the optical contrast originating from local height variations of the AF-390 particles. The full width at half maximum (FWHM) of the optical intensity profile is 340 nm, which is better than $\lambda/2$, where λ is the illumination wavelength. The FWHM of the topographic line profile is 420 nm. The difference originates from the fact that the force probe consisting of the optical fiber plus aluminum coating is larger than the optical probe confined to the width of the aperture. It is noted that the emission patterns in the fluorescence image are oriented in the same direction for all isolated particles. Such an effect is most likely due to the polarization-dependent dipolar interaction.¹⁰ Since the incident light is linearly polarized, once excited, the molecules act as electric dipoles, and dipole orientations approximately follow the excitation polarization. Therefore, the spatial fluorescence feature indicates the high degree of molecular order in the isolated nanoparticle. Another measurement is performed (not shown) on a microaggregate that has a FWHM of $\sim 1.8 \mu\text{m}$. In contrast, the symmetric profile of fluorescence intensity suggests that the molecular arrangement in the microaggregate is inhomogeneous and the average orientation of the large ensemble of molecules is polarization independent.

The dependence of the fluorescence intensity on excitation power is shown in Fig. 3. The slope of the logarithmic fitted line is 2.02, which indicates two-photon excitation. No two-photon-induced fluorescence is observed from the pure prism, cover slip, and the PMMA film. The quadratic dependence of light intensity limits the effective excitation to a small volume, which enables a light confinement at the aperture of the probe, and leads to image contrast enhancement with the coated probe, as can clearly be seen in Fig. 2(b). Coated probes with an aperture smaller than 100 nm detect no fluorescence, even if the particles could be resolved on

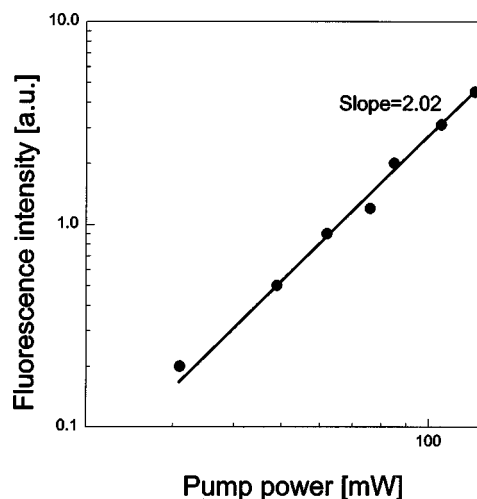


FIG. 3. Logarithmic plot of fluorescence as a function of laser power for an AF-390 nanoparticle.

topographic images. This is due to the fact that the number of photons collected is too low. It is believed that a strongly emitting sample could be optically imaged with a smaller aperture.

The fluorescence spectrum is obtained by first locating an isolated particle in the fluorescence image, then holding the probe stationary above the particle and switching the fluorescence signal from the photomultiplier tube to a spectrograph. Figure 4(a) shows the near-field fluorescence spectrum for an isolated AF-390 nanoparticle. The spectra of nanoparticles in different locations show a slight inhomogeneity. Figure 4(b) shows the near-field fluorescence spectrum of an AF-390 microaggregate of $\sim 12.4 \mu\text{m}$, which is similar to that obtained in the far field (not shown). The spectra show a slight variation among the microaggregate. No hole burning and photobleaching are observed, which indicates the photostability of the specimen. The spectrum of an isolated nanoparticle exhibits a slight shift relative to the bulk spectrum, and is narrower. The inhomogeneous spectral broadening observed for the microaggregate is due to the average effect of many domains each with its own local energy in a dynamic surrounding. An isolated nanoparticle, on the other hand, exists in a rigid environment with a higher

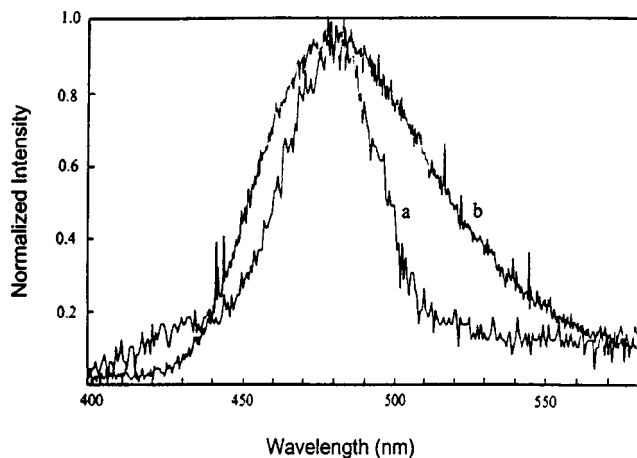


FIG. 4. (a) Near-field fluorescence spectrum of an AF-390 nanoparticle (acquisition time: 90 s). (b) Near-field fluorescence spectrum of an AF-390 microaggregate (acquisition time: 30 s).

degree of molecular order and a smaller energy distribution,^{11,12} which leads to spectral line narrowing.

In summary, we have successfully employed two-photon excitation for photon scanning tunneling microscopy and spectroscopy. We show that two-photon PSTM has advantages of reduced alignment constraints, minimized background interference, and enhanced light confinement. We demonstrate that two-photon PSTM can be used as a valuable tool to spatially and spectrally probe emitting regions on a nanometer scale.

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