

Single-molecule near-field optical energy transfer microscopy

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The nano-optical interaction between a sharp tip and a single dipolar emitter is investigated. Changes of the excited state lifetime and the fluorescence rate of single molecules are recorded simultaneously as a function of the tip position relative to the molecule. A subdiffraction-limited area of decreased fluorescence and shortened lifetime is observed for gold-coated Si_3N_4 tips. The results are discussed in terms of molecular fluorescence in a system of stratified media. The outlined methodology holds promise for applications in ultrahigh-resolution near-field optical imaging at the level of single fluorophores. © 2002 American Institute of Physics. [DOI: 10.1063/1.1506952]

Tip-enhanced (scattering-type) scanning near-field optical microscopy (SNOM) constitutes an alternative approach to near-field optical imaging with aperture probes.^{1,2} Tip-enhanced SNOM has been applied to image polymer mixtures with infrared light in vibrational absorption at a resolution of $\lambda/100$.³ In the visible spectrum, tip-enhanced two-photon fluorescence microscopy⁴ and tip-enhanced fluorescence^{5–8} have been reported.

Experimental^{9,10} and theoretical studies¹¹ of the interaction of sharp tips with well-defined objects, i.e., single dipolar emitters, can contribute to a deeper understanding of nano-optics. However, despite of all achievements, the implementation of tip-enhanced SNOM schemes with single-molecule sensitivity remained a challenge. Quenching of molecular fluorescence by the proximity of the metallic probe tip was thought to be a major disadvantage of SNOM techniques when extended to ultrahigh resolution.^{12,13}

In this letter, we present a study of the nano-optical interaction between a sharp metallized tip and a single-dipolar emitter. We simultaneously record changes in the fluorescence rate¹⁴ and lifetime changes^{15,16} as a function of the tip position relative to the molecule. Respective maps show distinct features with lateral dimensions well below the diffraction limit. The lifetime maps clearly demonstrate a shortening of the lifetime, compatible with the appearance of additional molecular decay channels in the proximity of the tip. By simultaneous recording of both, tip-position dependent lifetime and intensity, effects of field enhancement, and energy transfer close to a tip can be disentangled. By exploiting selective quenching of individual molecules in small clusters, the method holds promise for applications in ultrahigh-resolution tip-enhanced SNOM of single molecules.

The setup is a sample-scanning confocal optical microscope based on an inverted microscope (Zeiss Axiovert 135) in combination with a tip-scanning atomic force microscope

(AFM) (Digital Instruments, Bioscope) (Fig. 1). An actively mode-locked Nd:YAG laser (Coherent Antares), frequency-doubled to 532 nm with 150 ps pulse width and 76 MHz repetition rate is intensity-stabilized (Cambridge Instruments, LS 100) and coupled into a single-mode optical fiber. A small fraction of the excitation light is directed onto a fast photodiode to provide a time reference. After exiting the fiber, the circularly polarized light is collimated by a lens and reflected via a dichroic mirror onto the back aperture of an oil immersion microscope objective (Zeiss, Plan-Apochromat, $\times 63$, 1.4 NA, ∞) which focuses the light to a diffraction-limited spot on the sample. Emission from the sample is collected by the objective and transmitted by the dichroic mirror. A set of two holographic notch filters (Kaiser Optical Systems) and a set of suitable cutoff and shortpass filters remove Rayleigh-scattered excitation light and the 675 nm line of the AFM laser. The transmitted fluorescence (550–625 nm, $\geq 60\%$) is focused onto the 200 μm diameter active area of a single-photon counting avalanche photodiode

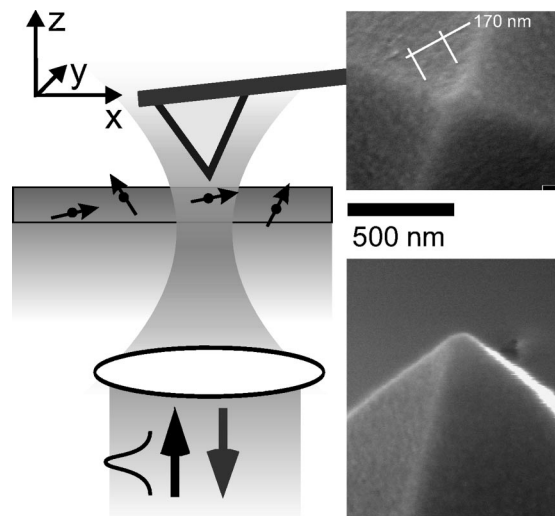


FIG. 1. Left-hand side panel: Principle of the experiment. Right-hand side panel: Shape of the gold-coated tip used in the experiment of Fig. 3. Top view and side view. The thickness of the gold layer is 50 nm. The tip ends in a knife edge of 170 nm in width.

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(SPCM-ARQ 14, Perkin-Elmer) which serves as confocal pinhole. For online acquisition of fluorescence lifetime data during scans, time-correlated single-photon counting in combination with an averaging scheme is employed. Specifically, the output of a time-to-amplitude converter is converted into a continuous step function and averaged by a low-pass filter. Details are described elsewhere.¹⁷

AFM measurements are performed in contact mode with commercial cantilevers (Digital Instruments, DNP) with Si_3N_4 tips in the shape of a quadratic pyramid (base length $4\ \mu\text{m}$, height $3.3\ \mu\text{m}$) coated with $\sim 20\ \text{nm}$ of gold. In conventional contact mode AFM scans, only the very tip apex (i. e., a microtip) interacts with the sample. In fluorescence experiments, also regions of the tip, which are not in immediate contact with the sample, can affect image formation. Scanning electron microscopy images showed that the four corners of pyramidal tips in general do not meet in a single point but often form a knife-edgelike structure (see Fig. 1, right-hand side panel). The particular tip of Fig. 1 was used in one of the experiments discussed later in this letter.

Samples were prepared by spincoating (9600 rpm) of a $10\ \mu\text{l}$ droplet of a solution of poly(methylmethacrylate) (PMMA) in toluene that contained the dye *1,1'-dioctadecyl-3,3,3',3'-tetramethylindocarbocyanine* at a concentration of $\approx 10^{-9}\ \text{M}$ onto cleaned standard glass cover slips. The difference in refractive indices between PMMA and glass are neglected in the following. AFM of the polymer film revealed a smooth surface and a thickness of about $20\ \text{nm}$. Standard confocal single-molecule fluorescence microscopy without AFM tip showed typical diffraction-limited fluorescence spots of molecules with random dipole orientation. During the experiments, the sample was subject to a laminar flow of nitrogen to exclude oxygen.

Prior to the experiment, the scan window has to be centered around the confocal volume¹⁸ containing a single molecule. While scanning the tip over the surface of the PMMA film, the molecule is continuously illuminated and fluorescence rate and lifetime are recorded as a function of the tip position.

In order to locate a molecule, the tip is held at a fixed position and the sample is scanned using an actively linearized piezoelectric *xyz*-scanning stage (PI, P-517.3CL), until the fluorescence exceeds a threshold, indicating the presence of a molecule. Once a molecule is found, the position of the scan stage is frozen for continuous monitoring of the molecular fluorescence during tip action. The excitation intensity is typically $500\ \text{nW}$, resulting in fluorescence emission rates of $1-3 \times 10^4$ photons/s.

The rather complex geometry of a single molecule embedded in a thin PMMA layer on glass with a gold-coated Si_3N_4 AFM tip may be modeled to first-order approximation by a system of stratified layers.¹⁹⁻²⁴ This is justified by the fact that on molecular length scales, the radius of curvature of the tips is still very large. However, all details about the spatial extension and directionality of the interactions parallel to the layered system are neglected. Nevertheless, the influence of the air gap, which is of major importance for high-resolution optical imaging, can still be studied in detail. Figure 2 shows the lifetime of a dipolar emitter calculated for a system of stratified layers of glass/air/gold/ Si_3N_4 as a

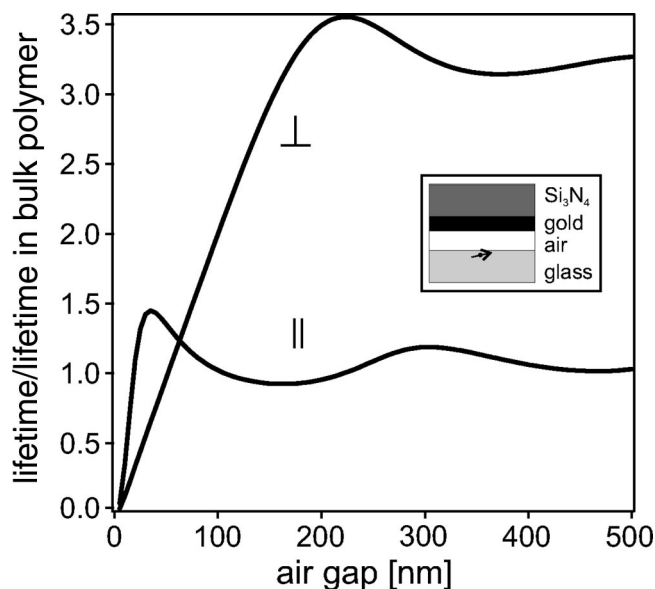


FIG. 2. Molecular lifetimes in a systems of stratified media indicated in the inset as a function of the air gap. Wavelength of emission is $570\ \text{nm}$. The lifetimes are expressed in units of the lifetime in a homogeneous polymer.

function of the air gap for the two fundamental orientations of the molecular dipole. The molecule is located $1\ \text{nm}$ below the polymer surface. For a large air gap, corresponding to the situation where the tip is absent, there is a strong orientation dependence of the fluorescence lifetime. Molecules with emission dipoles oriented perpendicular to the interface have a lifetime more than three times longer than that of molecules with parallel orientation. This effect has been verified experimentally.^{25,26} Decreasing the air gap leads to some interference undulations before, at around $200\ \text{nm}$, for the perpendicular dipoles, the lifetime drastically decreases¹¹ down to zero. For the dipoles oriented parallel to the interface, the lifetime decreases even more sharply to zero over the last $25\ \text{nm}$. This decay indicates strong nonradiative energy transfer to the gold.

We have investigated a large number of molecules using gold-coated Si_3N_4 tips. Figure 3 (a) shows a typical fluorescence rate map obtained with a gold-coated tip (top-to-bottom scan). The molecule stays stable throughout the scanning process until finally, after 60% of the image, photobleaching occurs. Undulations and a pronounced local decrease of the fluorescence rate can be observed. The fringes are likely due to far-field interferences due to the presence of the AFM tip in the vicinity of the molecule. Depending on the relative position of molecule and tip, the molecule experiences an effectively increased or decreased excitation intensity due to reflection by the tip. Also, the probability that a fluorescence photon is emitted in the solid angle covered by the detection optics is altered.

In contrast to the far-field fringes the sharp depression at the left-hand side center of Fig. 3 (a) is of subwavelength dimensions, clearly indicating a near-field optical origin, presumably due to local quenching of the molecular fluorescence by the tip.

The occurrence of a depression in the fluorescence is consistent with the fact, that a strong field enhancement at the tip is not to be expected: (i) the illumination conditions are such that the polarization component along the tip axis is

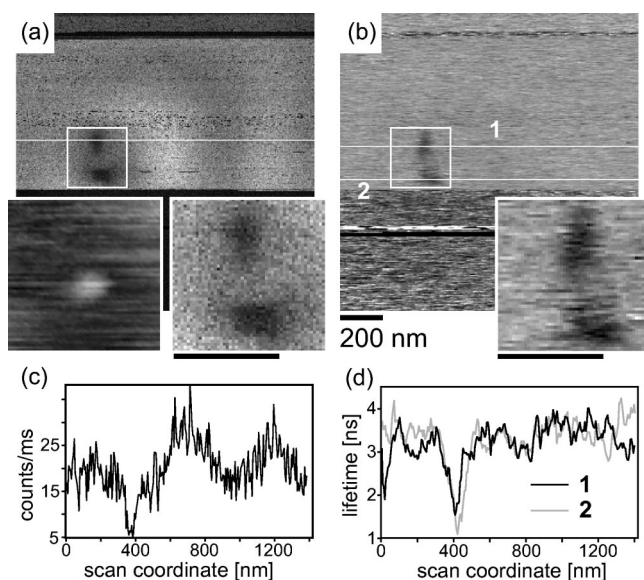


FIG. 3. Quenching experiment with gold-coated Si_3N_4 cantilever: (a) fluorescence rate as a function of the tip position. Insets show zooms of the area indicated by the white square: Left-hand side: topography, right-hand side: fluorescence. (b) fluorescence lifetime as a function of the tip position recorded simultaneously to (a). Inset: Zoom of the area indicated by the white square. (c) Line section at the white line through (a). (d) line section at the white lines (1, 2) through (b). All scale bars are 200 nm.

small. For this configuration, even a decreased intensity beneath the tip apex is predicted.²⁷ (ii) The wavelength of excitation (532 nm) is too far in the green to excite local plasmons at the tip. For a similar tip configuration, a plasmon resonance was observed for wavelengths around 588 nm.²⁸ For a similar wavelength (578 nm), using solid gold tips scanned over a single fluorophore, enhanced fluorescence was observed.²⁹

The depression exhibits a double-lobed shape. Such asymmetric patterns are expected when quenching dipolar emitters have symmetric tips. The shape of such patterns should be related to the dipole orientation. However, images acquired on several molecules (with random orientations) with the tip shown in Fig. 1 (right-hand side panel) exhibit identically oriented patterns. The shape of the depression is therefore likely due to the knife-edgelike shape of the tip. The orientation and the length of the knife edge fit very well to the dimension of the depression. The interruption of the depression is caused by a small particle on the surface of the polymer film [left-hand side inset of Fig. 3 (a)], which forces the tip to retract locally by ≈ 10 nm. A linecut across the upper lobe reveals a width of ≈ 70 nm and a local decrease of the fluorescence count rate by 10 kHz, with steep edges, also indicative of very short-ranged interactions. In the lower lobe, which is spatially more extended, almost complete suppression of the molecular fluorescence down to a count rate of 5 kHz is seen.

Mapping the molecular fluorescence rate alone does not permit one to conclude whether a change in the emission rate of the molecule is due to altered excitation or to a change of the excited-state decay rate. For this reason, simultaneous fluorescence lifetime maps were recorded. Changes of the (nonradiative) decay rate manifest in modified fluorescence lifetimes. Continuous monitoring of the fluorescence lifetime

therefore provides a measure for the strength of additional decay channels appearing due to the presence of the tip. Furthermore, lifetime measurements provide an intensity independent contrast mechanism for near-field optical imaging.

Figure 3 (b) shows a fluorescence lifetime map recorded simultaneously with the fluorescence rate map. The lifetime map reproduces the subdiffraction-limited dip, while no fringes are visible. The lifetime dip appears at the same position as in Fig. 3 (a) and is also 70 nm in width. The fluorescence lifetime of the unperturbed molecule is found to be 3.2 ns which is consistent with a molecule oriented in the plane of the sample. The dip depth corresponds to a local lifetime decrease of more than 1 ns.

At the position of the topographic protrusion, the fluorescence rate and the lifetime return close to their unperturbed values of 20 kHz and 3 ns, respectively. This is a proof for the short range of the interaction causing the depressions, consistent with the expected behavior of a dipole close to a gold layer oriented parallel to the interfaces sitting about 10 nm under the surface of the polymer film (see Fig. 2). Here, quenching leads to (i) a decreased fluorescence lifetime and (ii) a decreased fluorescence quantum yield.

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