

# Single-molecule near-field optical energy transfer microscopy with dielectric tips

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## Summary

The fluorescence lifetime and the fluorescence rate of single molecules are recorded as a function of the position of a  $\text{Si}_3\text{N}_4$  atomic force microscopy tip with respect to the molecule. We observe a decrease of the excited state lifetime and the fluorescence rate when the tip apex is in close proximity to the molecule. These effects are attributed to the fact that the dielectric tip converts non-propagating near-fields to propagating fields within the dielectric tip effectively quenching the fluorescence. The spatial extension of the quenching area is of sub-wavelength dimensions. The results are discussed in terms of molecular fluorescence in a system of stratified media. The experiment provides surprising new insights into the interactions between a fluorescent molecule and a dielectric tip. The methodology holds promise for applications in ultra high-resolution near-field optical imaging at the level of single fluorophores.

## Introduction

Understanding the physics that rules the world of nano-optics is of fundamental interest for technology and basic research. Scanning near-field optical microscopy (SNOM) is an optical imaging technique that overcomes the limits of diffraction by requiring close proximity between a subwavelength optical probe and a sample. Aperture SNOM (Pohl *et al.*, 1984; Betzig & Trautman, 1992; Hecht *et al.*, 2000) has been successful in demonstrating the potential of near-field optical (NFO) techniques in various applications ranging from ultra-high density data storage (Betzig *et al.*, 1992) to super-resolution imaging of single fluorescent molecules (Betzig & Chichester, 1993). However, the application of such techniques is com-

pllicated by the difficult fabrication and the low light throughput of aperture probes (Hecht *et al.*, 2000). Apart from that, the spatial resolution of aperture SNOM is fundamentally limited to about 20 nm, because of the finite depth of penetration of light into metals.

Tip-enhanced (scattering-type) SNOM constitutes an alternative approach to near-field optical imaging. Tip-enhanced SNOM has been applied to image polymer mixtures with infra-red light in vibrational absorption at a resolution of  $\lambda/100$  (Knoll & Keilmann, 1999). In the visible spectrum, tip-enhanced two-photon fluorescence microscopy (Sanchez *et al.*, 1999) and tip-enhanced fluorescence (Martin *et al.*, 1996; Hayazawa *et al.*, 1999; Azoulay *et al.*, 2000; Hamann *et al.*, 2000) have been reported. For tip-enhanced SNOM the spatial resolution is not limited by the skin depth of metals, which opens the road to optical imaging and spectroscopy at sub-10 nm resolution. Experimental (Novotny, 1996; Gersen *et al.*, 2000) and theoretical (Girard *et al.*, 1995) studies of the interaction of sharp tips with well-defined objects, i.e. single dipolar emitters, can contribute to a deeper understanding of tip-enhanced SNOM. Here, the ability to detect single fluorescent markers with ultra-high resolution is of special interest because of its potential applications in life science. However, despite of all achievements, the implementation of tip-enhanced SNOM schemes with single-molecule sensitivity remains a challenge. Quenching of molecular fluorescence by the proximity of the metallic probe tip was thought to be a major disadvantage of tip-enhanced SNOM techniques when extended to ultra-high resolution (Ambrose *et al.*, 1994; Xie & Dunn, 1994).

Here we present a study of the nano-optical interaction between a sharp dielectric tip and a single fluorescing molecule embedded in a 20 nm polymer film on glass. We simultaneously record changes in the fluorescence rate (Yang *et al.*, 2000) and in the excited state lifetime (Pedarnig *et al.*, 1995; Pastré *et al.*, 1998) as a function of the tip position relative to the molecule. The resulting fluorescence and lifetime maps

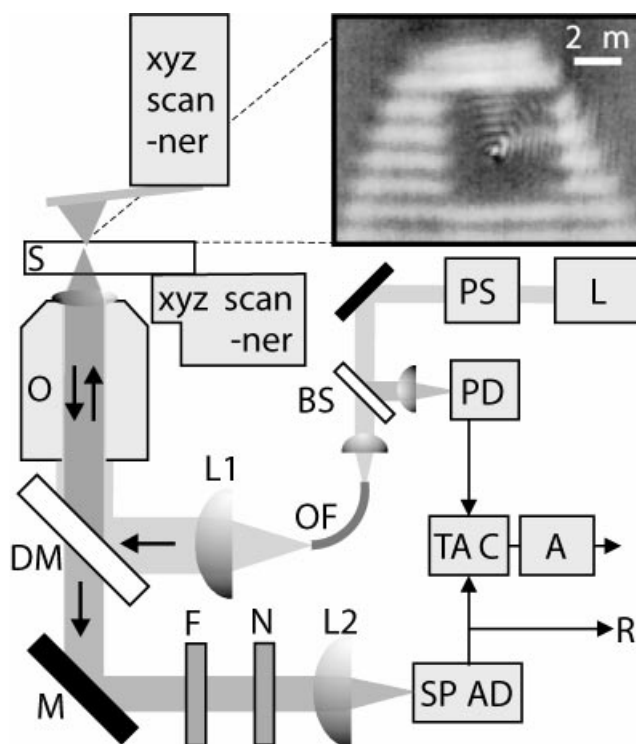
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show distinct features with lateral dimensions well below the diffraction limit. The lifetime maps clearly demonstrate a shortening of the lifetime, compatible with the appearance of additional decay channels for the molecular excited state in the proximity of the tip. The conversion of non-propagating molecular near-field to modes propagating in the dielectric tip is a likely explanation for this effect. In a previous study similar results were obtained using dielectric tips coated with 50 nm of gold (Trabesinger *et al.*, 2002b). In this system also a decreased fluorescence and lifetime in presence of the tip was observed, which was attributed to non-radiative energy transfer and heat dissipation in the gold layer.

The results reported here may trigger a whole series of near-field optical experiments that aim at exploiting the interaction of single molecules with dielectric tips.

## Experimental

The set-up is a combination of a sample-scanning confocal optical microscope based on an inverted microscope (Zeiss Axiovert 135, Carl Zeiss, Feldbach, Switzerland) and a tip-scanning AFM (Digital Instruments, Bioscope, Veeco Instruments, Woodbury, NY, U.S.A.) used in contact mode. The set-up is shown schematically in Fig. 1. An actively mode-locked Nd:YAG laser (L, Coherent Antares, Santa Clara, CA, U.S.A.) with 150 ps pulse width and 76 MHz repetition rate was frequency-doubled to 532 nm, intensity-stabilized (PS, Cambridge Instruments, LS 100, Woburn, MA, U.S.A.), and coupled into a single-mode optical fibre (OF). The single mode fibre provides excellent spatial filtering and at its exit a spatially stable point-like light source. A small fraction of the excitation light is directed onto a fast photodiode (PD) to provide a time reference. After exiting the fibre the polarization is set to circular. The light is collimated using a lens (L1) and reflected via a dichroic mirror (DM) to overfill the back aperture of an oil immersion microscope objective (O, Zeiss, Plan-Apochromat,  $\times 63$ , 1.4 NA,  $\infty$ ). The light is focused to a diffraction-limited spot on the upper surface of the sample (S) to a spot with a diameter below 250 nm. Emission from the sample is collected by the objective (O) and transmitted by DM. A set of two holographic notch filters (N, Kaiser Optical Systems, Ecully, France) and a set of suitable cut-off and short-pass filters (F) remove Rayleigh-scattered excitation light and the 675 nm line of the AFM beam deflection laser. Removing the AFM laser light by a holographic notch alone is not possible because the laser diode has a broad luminescence background. The transmitted fluorescence (550–625 nm,  $\text{ge}60\%$ ) is focused via another lens (L2) onto the 200  $\mu\text{m}$  diameter active area of a single-photon counting avalanche photodiode (SPAD, SPCM-ARQ 14, Perkin-Elmer, Fremont, CA, U.S.A.), 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



**Fig. 1.** Experimental set-up with tip and sample xyz scanner: L: pulsed laser, PS: power stabilizer, BS: beamsplitter, PD: photodiode, OF: single-mode optical fibre, L1, L2: lenses, DM: dichroic mirror, O: microscope objective, S: sample, M: mirror, F: cut-off filters, N: holographic notch filters, SPAD: single-photon counting avalanche photodiode, R: fluorescence count rate,  $\tau$ : fluorescence lifetime. Inset: AFM cantilever with tip imaged by recording the reflected light (notch slightly tilted) while scanning the tip through the focus. For principle of operation see text.

low-pass filter (A). This scheme outperforms standard online lifetime determination methods at low count rates. Fifty photons are enough to determine the lifetime with a relative standard deviation of better than 0.2: details are given elsewhere (Trabesinger *et al.*, 2002a). AFM measurements are performed in contact mode with commercial cantilevers (Digital Instruments, DNP) with  $\text{Si}_3\text{N}_4$  tips in the shape of a quadratic pyramid (base length 4  $\mu\text{m}$ , height 3.3  $\mu\text{m}$ ).

## Sample preparation

Samples were prepared by spincoating (1500g) of a 10  $\mu\text{L}$  droplet of a solution of polymethylmethacrylate (PMMA) in toluene that contained the dye 1,1'-dioctadecyl-3,3',3'-tetramethylindocarbocyanine (DiI) at a concentration of  $\sim 10^{-9}$  M onto cleaned standard glass cover slips. The difference in refractive indices between PMMA and glass are disregarded in the following. AFM of the polymer film revealed a smooth surface and a thickness of about 20 nm. Standard confocal single-molecule fluorescence microscopy without AFM tip showed typical diffraction-limited fluorescence spots.

### Alignment procedures

Prior to the experiment, the scan window must be centred around the confocal volume. This is achieved by recording the reflected excitation light as a function of the tip position during a tip scan. To this end, the holographic notch filter is slightly tilted to transmit a small amount of the excitation light. If the tip is positioned correctly an image such as that in the inset of Fig. 1 is obtained, in which the front part of the cantilever and the pyramidal tip is outlined by interference fringes. The tip is usually not centred on the cantilever.

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-517.3 CL), 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 nW, resulting in fluorescence emission rates of  $1\text{--}3 \times 10^{-4}$  photons  $\text{s}^{-1}$ .

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. During the experiments, the sample was subject to a laminar flow of nitrogen in order to reduce photobleaching due to the presence of oxygen.

### Theory

The rather complex geometry of a single molecule embedded in a thin PMMA layer on glass with a  $\text{Si}_3\text{N}_4$  AFM tip on top of it may be modelled to first-order approximation by a system of stratified layers (Drexhage, 1969; Kuhn, 1970; Lukosz & Kunz, 1977a,b; Chance *et al.*, 1978; Novotny, 1997). This is justified by the fact that on molecular length scales the radius of curvature of the tips is still very large. This approximation, however, neglects all details about the spatial extension and directionality of the interactions parallel to the layered system. Nevertheless, the influence of the size of the air gap on the signals, which is of major importance for judging the method's potential 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/ $\text{Si}_3\text{N}_4$  as a function of the air gap for the two fundamental orientations of the molecular dipole. In the simulation, the molecule is located 1 nm below the polymer surface in order to observe the maximum effect when closing the air gap. In reality, the molecules are distributed randomly within the polymer film and may exhibit a range of behaviours because of the dispersion in the effective gapwidth.

For a large air gap, corresponding to the situation where the tip is absent, there is a strong dependence of the fluorescence lifetime on the orientation of the molecular emission dipole

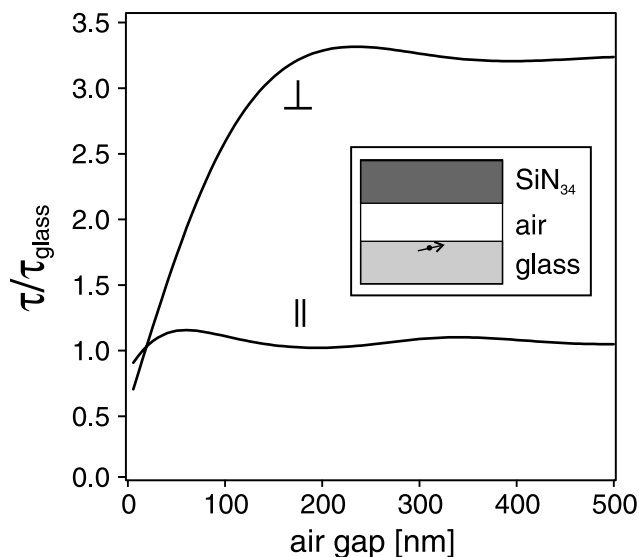
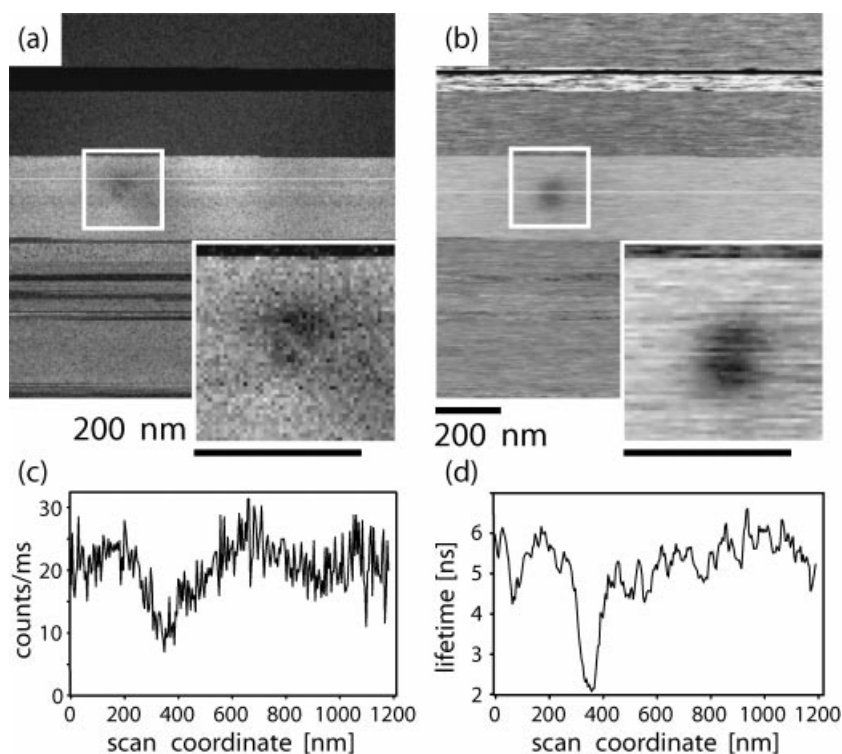


Fig. 2. Molecular lifetimes in a system of stratified media indicated in the inset as a function of the air gap. Wavelength of emission is 570 nm. The lifetimes are expressed in units of the lifetime in a homogeneous polymer.

with respect to the interface normal. Molecules located 1 nm below the interface with emission dipoles orientated 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 (Xie & Trautman, 1998; Kreiter *et al.*, 2002). Decreasing the air gap leads to some interference undulations before for both dipole orientations. At around 200 nm for the perpendicular dipoles the lifetime drastically decreases (Girard *et al.*, 1995). It decreases from about three times the unperturbed value in glass to about 0.75 of this value. For the dipoles orientated parallel to the interface, hardly any change in the lifetime can be observed that differs markedly from the far-field interference effects, even for very small gap widths.

### Results and discussion

Figure 3 summarizes the results of a typical successful experiment. We have investigated a large number of molecules using bare  $\text{Si}_3\text{N}_4$  tips. From the behaviour of the lifetime of a parallel dipole plotted in Fig. 2 hardly any distinct change in lifetime is expected for molecules orientated in the plane of the polymer film. However, for molecules with some out-of-plane orientation, there is a strong decay of the lifetime over a distance of about 100 nm (see Fig. 2). In accordance with the expected random orientations of the molecular dipole moments in the polymer film we found only very weak lifetime changes for the majority of investigated molecules. Figure 3 shows the result of an experiment with a molecule that presumably has an orientation perpendicular to the polymer film and thus shows a drastic decay of the fluorescence lifetime.



**Fig. 3.** Quenching experiment with bare  $\text{Si}_3\text{N}_4$  cantilever: (a) fluorescence rate as a function of the tip position; (b) fluorescence lifetime as a function of the tip position. Insets: zoom of the area indicated by the white squares. (c) Line section at the white line through (a); (d) line section at the white line through (b). All scale bars are 200 nm.

The intensity map Fig. 3(a) as well as the lifetime map Fig. 3(b) exhibit small depressions of non-circular shape. The corresponding topographic image shows a flat surface (data not shown). The images show raw data scanned from top to bottom. In the upper thirds of the images a weak or no molecule was present in the focal volume. The intensity is close to the background level (black) with some stripes due to the searching procedure. The corresponding lifetime value in Fig. 3(b) is fluctuating between its limiting values because of low numbers of photons per pixel. Then a bright and stable molecule is found and held fixed in the focal spot. In the lifetime map this shows up as a well-defined constant lifetime signal with small fluctuations. The intensity image shows a decrease of the fluorescence count rate by a factor of two when the tip is over the molecule. The depression has a diameter of about 100 nm, which is consistent with the decay length estimated in Fig. 2. It also shows some asymmetry similar to a double-lobed structure. Such asymmetric patterns are expected when quenching dipolar emitters with symmetric tips (Novotny, 2000). The shape of such patterns should be related to the dipole orientation. However, no systematic studies that could prove this effect have been performed up to now. In the vicinity of the depression, fringe-like structures are seen that probably originate from a variation in the effective excitation intensity at the position of the molecule as the tip moves through the focus. Also, the probability that a fluorescence photon is emitted in the solid angle covered by the detection optics may be altered.

The occurrence of a depression in the fluorescence count rate is consistent with the fact that a strong field enhancement

at the tip is not to be expected in the present experimental setting: (i) the illumination conditions are such that the polarization component along the tip axis is small. For this configuration even a decreased intensity beneath the tip apex is predicted (Novotny *et al.*, 1998). (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 (Ashino & Ohtsu, 1998).

Mapping the molecular fluorescence rate alone does not permit us to conclude whether a change in the emission rate of the molecule is due to altered excitation intensity or due to a change of the excited-state decay rate. For this reason, simultaneous fluorescence lifetime maps were recorded. Changes of the (non-radiative) 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.

The fact that the molecule has a strong out-of-plane orientational component is consistent with its long unperturbed lifetime of  $\sim 5.3$  ns (Lukosz & Kunz, 1977a,b). With the AFM tip over the molecule its lifetime is decreased to 2 ns, which is slightly smaller than the bulk lifetime value in solution of 2.9 ns. This is also consistent with the theoretical behaviour sketched in Fig. 2 for the perpendicular dipole. The structure in the lifetime image appears to be more sharp and well-defined due to the absence of fringes around it. The absence of the fringes in the lifetime image strongly indicates that they were due to variations in the molecular excitation rate that naturally cannot affect the lifetime. Additionally, in the lifetime

image, the spot indicates some double-lobe structure. It is interesting to note that in this configuration no dissipative structure is in the near-field of the molecule. The decrease in lifetime in this case is probably due to the opening of new radiative decay channels by the dielectric tip in the molecular near field. In the silicon nitride material, non-propagating photons in air can propagate and are thus guided away into the upper half space, in the present experiment without being detected. Future experiments may be devised in a way that they provide a simple method of detecting such radiation.

## Conclusion

Lifetime measurements provide a novel, intensity-independent contrast mechanisms in NFO imaging with single-molecule sensitivity. Our method promises to be suitable for systematic investigations of the optical contrast obtained in tip-enhanced NFO imaging schemes and for single-molecule imaging at a resolution only limited by the tip's radius of curvature.

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