Nanocrystal fluorescence

DOI: 10.1002/smll.200600130

Fluorescence-Emission Control of Single CdSe Nanocrystals Using Gold-Modified AFM Tips**

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In single-molecule-manipulation techniques the physical concepts of optical switching[1–3] and optomechanics,[4] as well as the local energy transfer (i.e., quenching and fluorescence resonance energy transfer (FRET)[5]) between single nano-objects, open fascinating means of controlling and manipulating matter on the nanometer scale.[6–8] Single semiconductor nanocrystals (quantum dots) exhibit remarkable resistance to photobleaching, can be derivatized in a biocompatible way, and allow tuning of their spectroscopical properties.[9,10] Furthermore, these nanosystems are not only isolated position markers but can be regarded as active reporters that interact with their microenvironment and carry information about their local vicinity, for example, in their blinking frequency.[11,12] In recent years, nanocrystal fluorescence markers for use in biological applications have been designed based on these qualities and new assays based on their energy-transfer properties have been developed.[13–16] In this context, the external control and switching of single-nanocrystal fluorescence emission is a significant step towards a better control of the functional properties of matter on the single-molecule level. In this Communication, we report on the external but local emission control of a single nano-object by means of a gold-functionalized tip. It was possible to fully control the light-emission state of a single nanocrystal from emitting (blinking) to quenched (dark) by mechanically approaching and retracting the tip. Moreover, we were able to measure and quantify the fluorescence emission as a function of distance between the nanocrystal and the tip.

The experimental setup[17] combines total-internal-reflection fluorescence microscopy (TIRFM)[18,19] with the piezo-controlled nanometer-sensitive movement of an atomic force microscope (AFM)[20] (Figure 1). Previously, the lifetimes of single dyes in the presence of an AFM tip have been investigated with a combination of AFM and confocal microscopy.[21] A similar confocal setup using an AFM tip functionalized with quantum dots has recently been used for a fluorescence-energy-transfer-based microscopy of dye molecules.[22] In contrast to these experiments, our focus is on the effective local switching of a single fluorophore; we use CdSe/ZnS nanocrystals, the emission of which can be recovered after quenching, and an AFM tip covalently functionalized with gold nanoparticles serves as an appropriate and effective quenching agent. The nanocrystals are immobilized in submonolayer coverage on a cover glass so that

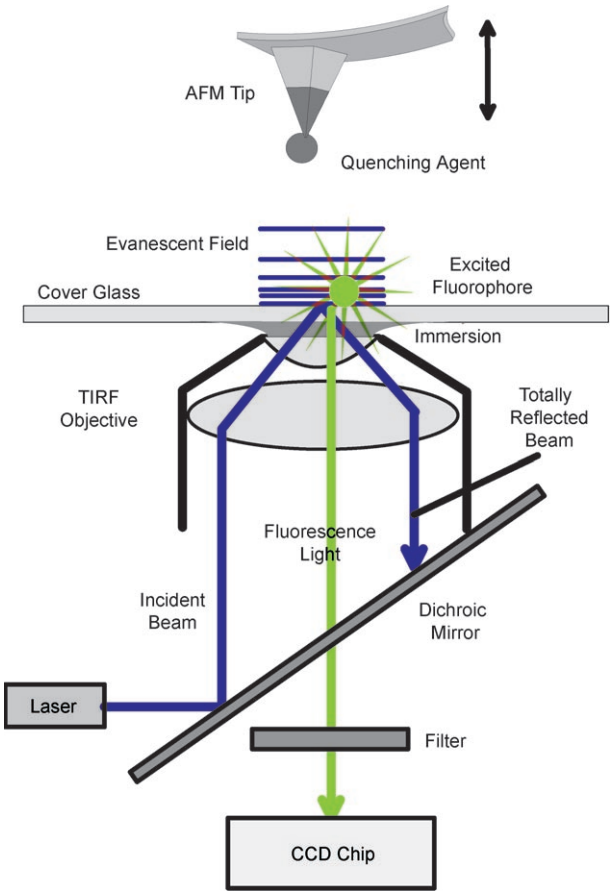

Figure 1. Schematic image of single light-emission control experiment. The complete setup is placed on an inverted microscope. A laser beam is directed via a TIRF objective lens at an angle of total reflection onto the cover glass. The evanescent wave protruding less than 50 nm beyond the interface excites fluorophores immobilized on the sample surface. Surface occupancy allows for the addressing of individual fluorophores. Fluorescence emission from the surface is recorded by a CCD camera. An AFM head is positioned on top of the microscope. The AFM tip is functionalized with nanoparticles known to effectively quench the fluorescence emission of the fluorophore when brought into close proximity.

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[**] Financial support from the Deutsche Forschungsgemeinschaft (SFB 613) is gratefully acknowledged. R.E. and V.W. contributed equally to this work.

Supporting information for this article is available on the WWW under http://www.small-journal.com or from the author.

single emitters can be clearly discriminated and resolved. The mechano-optical setup combines a home-built AFM head mounted on a three-dimensional (3D) piezo stage (Physik Instrumente AG) with an inverted optical microscope (Axiovert S100, Carl Zeiss) equipped with a TIRFM lens (100×, numerical aperture (NA) = 1.45; Olympus). The setup is controlled via home-built software. The TIRFM mode significantly reduces optical background noise. For excitation, the sample is illuminated using an Ar^+ laser beam (488 nm). A dichroic mirror and an optical bandpass filter (580/75 nm) separate excitation and emission light; in order to prevent optical crosstalk between the exciting and the AFM laser an appropriate bandpass filter (670/20 nm) is mounted on the quadrant diode position detector. Fluorescence detection is realized with a sensitive CCD camera (I-PentaMAX, Roper) equipped with a 512×512 pixel chip, a microchannel plate image intensifier, and a 5 MHz, 12 bit A/D converter.

Luminescent CdSe/ZnS nanocrystals were prepared according to a previously published procedure[23] and exhibited an emission maximum at 585 nm. Briefly, a solution of Cd stearate and trioctylphosphineselenide in trioctylphosphine (TOP) was swiftly injected into a hot mixture of trioctylphospineoxide (TOPO) and hexadecylamine. After several minutes of reaction, the mixture was quenched by the addition of cold butanol. The yielded nanocrystals were redispersed in TOPO. Diethylzinc and hexamethyldisilathiane in TOP were injected dropwise at elevated temperature. The reaction was terminated after one day and the resulting CdSe/ZnS nanocrystals were purified and resuspended in chloroform. The nanocrystals were immobilized by dilution in heptane and subsequent incubation and drying on a clean (treated with caroic acid, UVO cleaner) glass coverslip. Spherical gold nanoparticles (average diameter 5 nm) were synthesized by the reduction of $HAuCl_4$ in the presence of citric acid in water.[24] The gold nanoparticles were used as synthesized. Silicon nitride AFM tips (Microlevers, Veeco) were amino functionalized with 3-mercaptopropyltriethoxysilane and incubated with a suspension of the gold nanoparticles. It was verified that these gold particles effectively quench the nanocrystal emission when applied to the sample in aqueous suspension.[25] This quenching is most likely induced by energy or electron transfer between the gold nanoparticles and the CdSe nanocrystals. In distance-controlled AFM experiments, hard Si cantilevers (BS-Tap300Al, BudgetSensors, ν_{res} = 250–280 kHz) coated with a 20-nm gold layer were used.

Figure 2a shows the general experimental setup when the AFM tip is withdrawn from the sample surface. Nanocrystals immobilized on the glass coverslip surface are excited by the evanescent field emanating from a totally reflected laser beam, and exhibit fluorescence emission. When the AFM tip is brought into near proximity of the nanocrystal (Figure 2b), significant quenching of the single fluorophore emission induced by the quenching agent at the AFM tip is expected. The fluorescence-emission time trace of a single nanocrystal and the corresponding z position of the AFM tip is shown in Figure 2c (cf. the video in the Supporting Information); an AFM-tip travel of $z = 0$ is equivalent to physical contact between the AFM tip and the sample surface

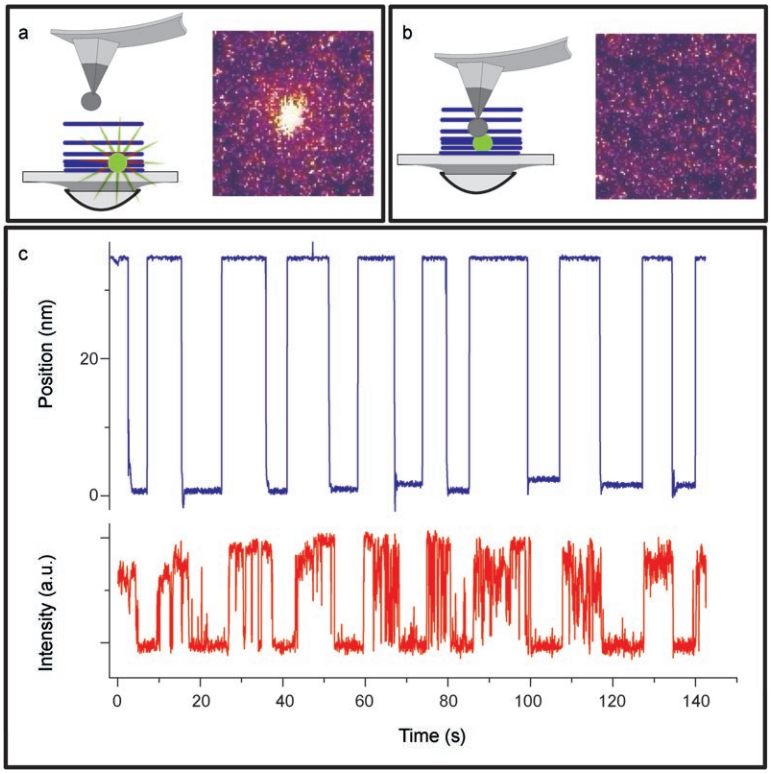

Figure 2. a) Schematic image of tip position and TIRFM image of a single nanocrystal. The AFM tip is withdrawn to ≈35 nm from the sample surface. The fluorophore at the surface is excited and emits fluorescence light. b) Schematic image of tip position and TIRFM image of the same nanocrystal when the quenching agent is positioned in close proximity to the nanocrystal. The fluorescence from the individual fluorophore is quenched. c) Traces of the AFM piezo stage (upper part) and the concomitant fluorescence emission (lower part) of an individual nanocrystal fluorophore. Each data point corresponds to a 50-ms video frame. A low position value signifies proximity to the sample surface. When the tip is positioned close to the nanocrystal on the surface, the fluorescence emission is almost totally quenched. When the tip is withdrawn, the blinking fluorescence emission typical of single nanocrystals is restored. As can be inferred from the traces, it was possible to repeatedly switch the fluorescence emission of a single dot from the emitting (bright) state to the quenched (dark) state simply by approaching and retracting the tip.

(total piezo travel: ca. 35 nm). Two regimes can clearly be distinguished: when the tip is removed from the surface-immobilized nanocrystal, the system shows the well-known blinking characteristics typical of a single nanocrystal; if brought into near proximity, the fluorescence emission of the nanocrystal is effectively quenched. The corresponding on- and off-time (cutoff threshold at 50% of maximum intensity) distributions are shown in Figure 3a and b for the withdrawn and approaching tip, respectively. Figure 3c and d shows the cumulated probabilities (step functions) for the on and off times of each of the two cases. Both the on-time distributions and the step functions are significantly different with respect to the AFM-tip position. For the withdrawn tip (Figure 3c), the total on-time sums to 79.8% of the total time, that is, the probability of finding the nanocrystal in the bright state is 79.8% as opposed to only 1% for the experiment when the tip is approaching (Figure 3d). Therefore, a "negative" control of the nanocrystal emission was confirmed, that is, an external stimulus (the quenching agent) brought into close contact with the nanocrystal causes effective quenching, whereas the typical blinking behavior is re-established upon mechanical removal of the quenching agent.

In order to investigate the dependence of the nanocrystal luminescence on the distance of the tip, fluorescence-intensity measurements were performed at several z distances between the tip and a single CdSe nanocrystal. Figure 4a shows the mean fluorescence-emission intensity of the quantum dot averaged over 600 frames as a function of tip–surface distance z; Figure 4b shows the probability of finding the nanocrystal in the on state. At close proximity to the surface ($z \approx 0$ nm), the quantum dot is quenched, in full agreement with the findings stated above. In order to prove that the tip is really in contact with the nanocrystal, Figure 4c shows a superposition of the fluorescence images of a single nanocrystal before (position 1) and after contact (position 2) with the tip. Control experiments using an unmodified Si cantilever produced no effect and proved that the quenching is not a mechanical artifact but due to interaction with the gold (cf. Supporting Information). Measurements with unmodified Si_3N_4 cantilevers suffered from an intense fluorescence background signal from the tips.[26]

The integrated intensity does not, however, increase strictly monotonically if the tip is moved away from the surface; instead, at $z \approx 22$ nm, the total emission reaches a relative maximum followed by a minimum at $z \approx 45$ nm. This luminescence enhancement has been found and discussed previously in the distance-dependent interaction of metal nanoparticles and organic dye molecules[27] and can be attributed to resonant exciton–plasmon coupling. At $z \approx 120$ nm, the mean emission of the nanocrystal reaches a maximum.[28] The coupling efficiency, however, is rather small (a factor of two, if the values at 120 and 220 nm are compared), a finding that can also be inferred from the overlap between the emission spectrum of the CdSe nanocrystal and the absorption spectrum of the gold nanocrystal (cf. Supporting Information). The results of the distance dependence of the nanocrystal luminescence nicely complement the data obtained by Govorov et al., who studied the interaction between gold and CdTe nanocrystals bridged by polymer linkers in the ensemble as a function of the linker length.[28,29]

The second minimum can be explained in the framework of two competing processes: the quenching, which is due to the dipolar interaction and hence shows a strong distance dependence ($\approx R^{-6}$), and a luminescence enhancement. Calculations were performed in order to gain a deeper insight into the processes involved, especially with a view to explain the minimum in fluorescence emission observed in the experiments. These calculations were made for a simplified,

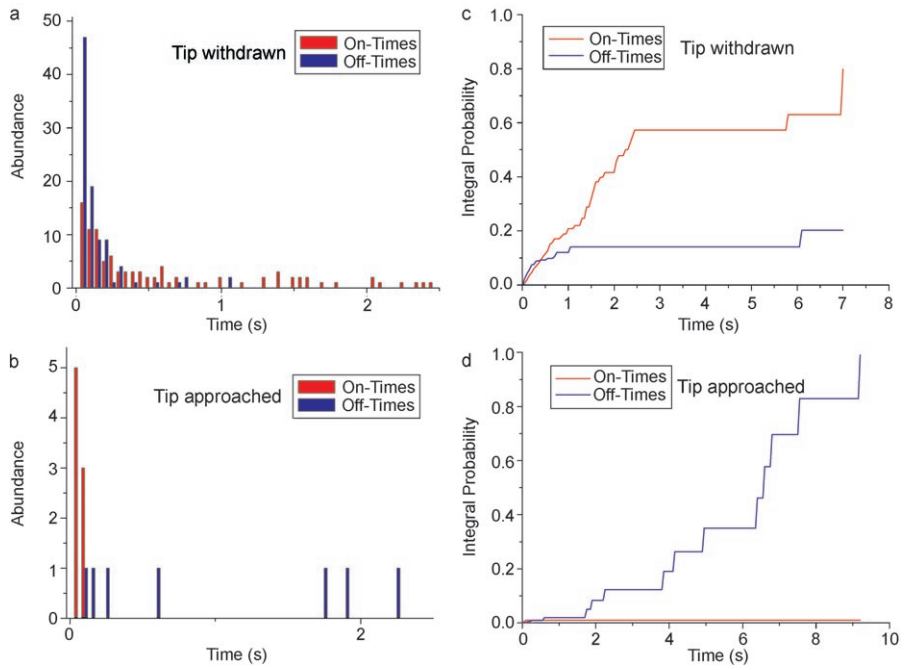

Figure 3. Statistics for single-nanocrystal quenching experiments. a) Part of the on- and off-time histogram (threshold: 50% of maximum intensity) for the experiment with the AFM tip withdrawn. The distribution of the off-times is much narrower than that of the on-times; most off-times have durations of only 50 or 100 ms. The situation is reversed for the experiment with the tip approached (b); no on-times longer than 100 ms were detected. c, d) Cumulated probabilities (total duration times normalized to one) as step functions for the cases with the tip withdrawn and approached, respectively. The probability of the nanocrystal being in the dark state is 20.2% for the tip withdrawn and 99.0% for the tip approached.

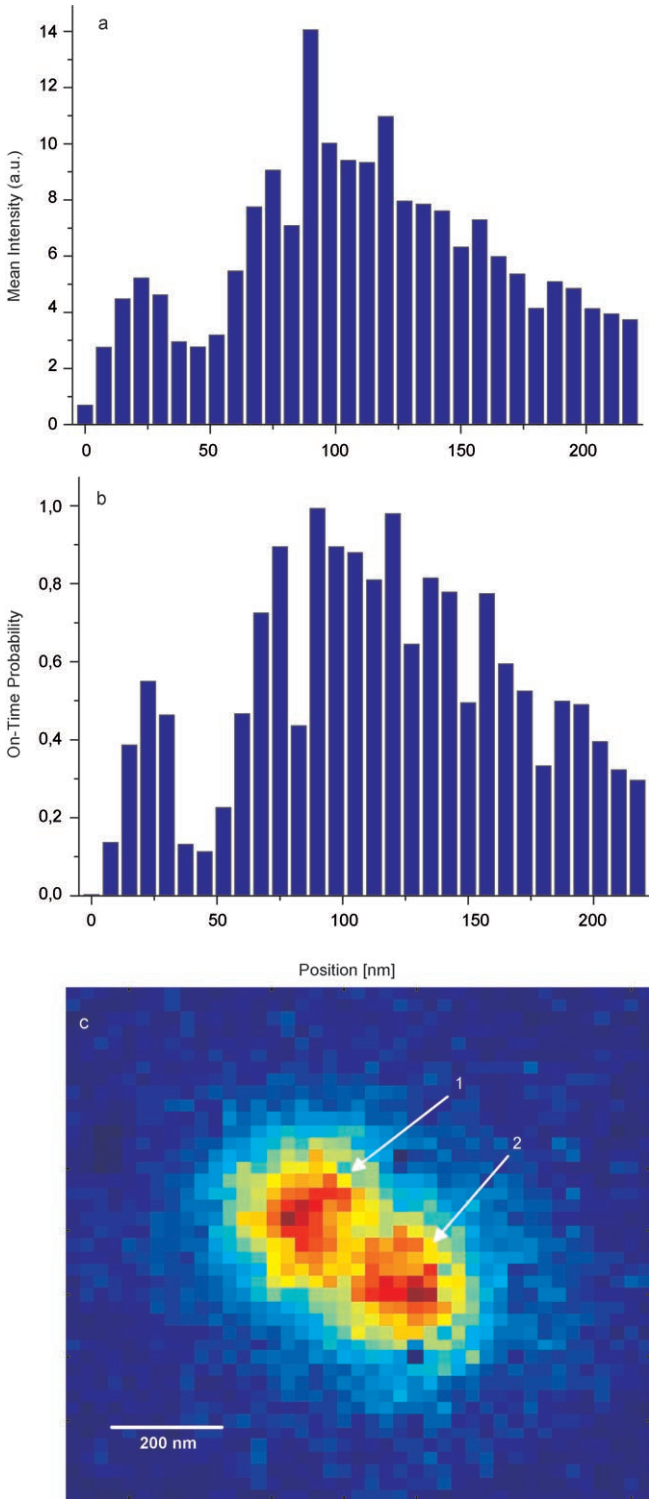

Figure 4. a) Mean intensity and b) probability of finding a single CdSe nanocrystal in the on-state as a function of distance from a gold-coated Si tip (binning size: 7.5 nm, averaged over 3 × 200 frames). c) Overlay of two TIRFM images of the same single CdSe nanocrystal before (position 1) and after (position 2) contact with the tip. After contact, the single dot is shifted by ≈ 200 nm.

yet analytically solvable setup, namely a planar system consisting of a glass support with ideal electric-dipole emitters on top, followed by an air gap of variable width d and capped by a 20-nm gold layer on silicon. Fluorescence excitation was assumed to be the result of a plane wave incident from the glass side with the angle of incidence above the angle of total internal reflection; fluorescence detection was also assumed to be due to the glass side with an oil-immersion objective of 1.45 NA. The electric-field distribution upon TIR excitation on the glass surface was first calculated using standard Fresnel theory[30] for planar systems. Second, the excitation rate of the dipole emitters was assumed to be proportional to the square of the scalar product of the local electric-field amplitude with the dipole vector. We then calculated the angular distribution of radiation of an emitting dipole into the glass half-space using a semiclassical electrodynamics approach, as explained in detail in Refs. [31–33]. Integrating this angular distribution of emission over the collected-light cone of the objective finally yields the amount of detectable fluorescence. In these calculations, the excitation- and peak-emission wavelengths were chosen to be 488 and 585 nm, respectively; the refractive indices of gold for these wavelengths are equal to $1.00+i$ 1.79 and $0.28+i$ 2.75, respectively[31] ($i=\sqrt{-1}$). The refractive index of silicon was chosen to be $3.9+i$ 0.017 for both wavelengths, and the refractive indices of glass and air were set to be 1.52 and 1, respectively. Figure 5a shows the detectable fluorescence as a function of air-gap width for a vertical dipole orientation (dipole oriented perpendicular to the glass surface), of which the different curves correspond to different angles of incidence of the plane-wave TIR excitation. Figure 5b shows the analogous results for a parallel dipole orientation (the dipole lies within the plane of the glass surface). The common feature of the curves at steeper incidence angles is a point of inflection at $d \approx 100$ nm. The main difference between this analytically solvable setup and the real experiment is that in the experiment the gold surface is not infinitely extended in the x and y directions but given by the tip, which could be more adequately modeled by a point source. A point source at a gap distance d from the surface would imply the multiplication of a monotonically decreasing reciprocal function, giving rise to a minimum at a certain d and thus explaining the experimentally obtained characteristics.

In summary, we have presented a new means of switching the fluorescence emission of a single nanocrystal by an external intervention of a gold-modified AFM tip. Furthermore, we were able to monitor the luminescence properties of a single nanocrystal as a function of distance between the single quantum dot and the tip. Significant luminescence enhancement due to exciton–plasmon coupling was detected. In future experiments, we plan to examine the distance dependence of the interaction in more detail, for example, using different nanocrystals and polarized light for excitation.

Generally, this technique represents an important step on the way to locally addressing and controlling molecular individuals with conceivable applications of the general concept ranging from microarrays to the manipulation of single reporter molecules. AFM yields important information about dynamic processes, for example, the force response of folded molecular systems to the application of an external

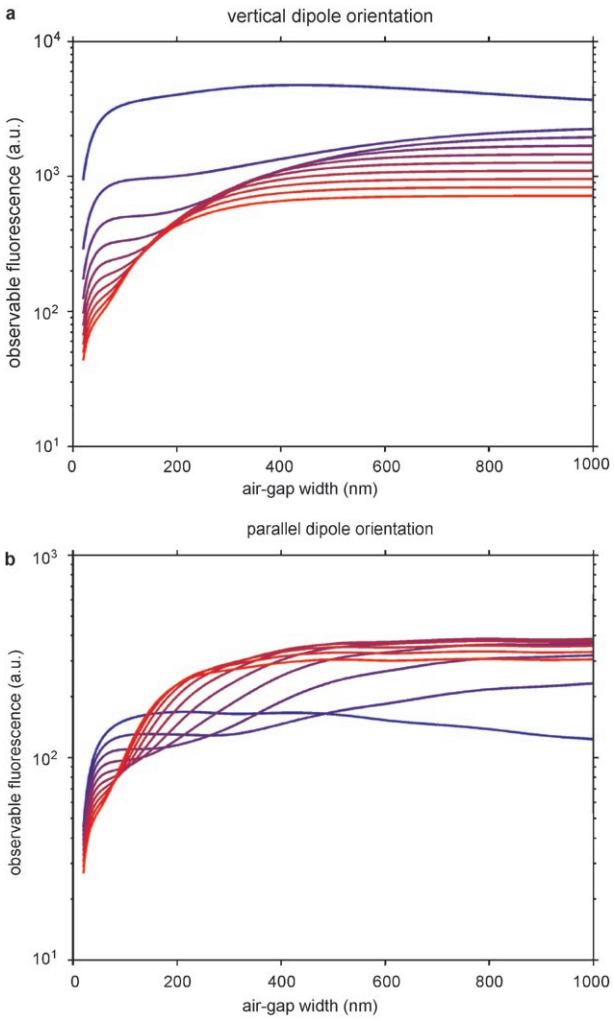

Figure 5. Calculated detectable fluorescence signal as a function of the gap size and incident angle for a simplified planar setup consisting of (a) vertical and (b) horizontal TIR excited dipoles on a glass surface with an air-gap size *d* and a 20-nm-thick gold layer on silicon. For both images the incidence angle is from 42° (blue) to 60° (red) in steps of 2°. $\lambda_{ex} = 488$ nm, $\lambda_{em} = 585$ nm.

stress[34] or the adhesion forces between individual ligand–receptor pairs.[35–40] Fluorescence resonance energy transfer yields complementary data in terms of molecular distances, orientations, and electrodynamical coupling.[41] The combination of both approaches promises to be a valuable tool for the investigation of the interplay of molecular individuals, their molecular recognition, folding pathways, or microenvironments.[22] The controlled, local switching of single fluorophores adds to this repertoire of single-molecule manipulation techniques.

Keywords:
fluorescence · luminescence · nanocrystals · single-molecule studies · total internal reflection

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Received: March 13, 2006
Revised: July 12, 2006
Published online on November 23, 2006

