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Plasmonic Nanorod Arrays for Enhancement of Single-Molecule Detection

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Abstract

We fabricated silver nanorod arrays producing enhanced fluorescence and evaluated the photophysical behaviors of single probes immobilized on nanorods. The observation of bright emission indicated that a highly enhanced field was created near the nanorod. A considerable enhanced fluorescence up to a factor of two orders of magnitudes was observed as compared to the emission on the controlled substrate.

Collective electron oscillations known as localized surface plasmons (LSP) can be excited in noble metal nanostructures along a dielectric surface resulting in strong amplification of the local electromagnetic field and appearance of surface plasmon absorption bands[1–6]. These enhanced fields are confined to a distance of within 300nm from the nanostructure and decay significantly beyond it. Upon appropriate optimization, the coupling of fluorophores with surface plasmons in metallic nanostructures increase the excitation and/or emission rates of the fluorophores, and results in a strong enhancement of the fluorescence[4,7–9]. In last couple of decades there is a surge of interest in plasmon-enhanced fluorescence approaches. In general, these investigations were based on coupling of fluorophores with surface plasmons generated in randomly distributed metal nanostructures or nanoscale roughness in metallic films[10–12]. For an array of nanostructures, the individual properties are modified by the inter-particle and particle-substrate interactions. The near-field hybrid coupling among particle pairs can lead to shifts in the resonant wavelength and anomalously large fields. State-of-art lithographic techniques provide tools for tailoring the interaction of nanostructures with light and offer precise control of size and spacing for the fabrication of a wide variety of complex shapes. These nanostructures can be used to control and modify electromagnetic field of the incident light for applications in subwavelength optics, biophotonics, detection and sensor development. Hence, continuous efforts have been devoted in studying the plasmonic effects on these nanosystems[8,9,13–17].

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[†] Electronic Supplementary Information (ESI) available: Silver nanoarray fabrication procedures, DNA oligo hybridyzation& immobilization, and single molecule experiments. See DOI: 10.1039/b000000x/

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The development of well-defined nanoarrays creates new opportunities to dramatically improve the fluorescence sensitivity. Herein, we report on the fabrication of silver nanorod arrays and nanoscale fluorescence characterization of a single fluorophore labelled DNA immobilized on the silver nanoarray. We observed considerable enhancement of fluorescence intensity for the fluorophore immobilized on nanoarray compared to that of fluorophore in the absence of patterned silver nanostructure. When large scales of single biological molecules are immobilized on each nanostructure of the substrate, massively parallel single-molecule analysis can be performed. Furthermore, increased sensitivity with nanoscale control could allow us to further retrieve useful information on kinetic processes between biomolecules.

Highly regular nanorod arrays were fabricated using electrodeposition method with AAO template (Supporting Information). A representative example of the periodic nanorod array is shown in Figure 1, showing the large area of silver nanorod arrays with roughly 40-nm inter-nanorod gaps. To elucidate the gap-induced localized plasmon coupling between the adjacent Ag-NRs for plasmon-enhanced fluorescence effect, we used Finite Element Method (FEM) modelling to investigate the localized electric field intensities of the arrays. Theoretical simulated result shows the electrical field strength distribution along the coordinate axis around the Ag nanorods as illustrated in Figure 1c. Strong field or “hot spots” are identified locally in the gap. A highly enhanced local electric field is present showing a larger field at the angle point of the nanorod than other positions, which is similar to those observed in a previous report and decays quickly along the axis[18,19]. The areas of high near-field enhancements in between the particles means that a labelled protein or any other biomolecules locating between the nanorods will experience a much higher excitation field than if it were isolated and directly excited only by the incident light. This will result in higher excitation rates of the fluorophore, which leads to greater excitation-emission cycles in a given time period. The use of double stranded DNA (Supporting Information) to covalently attach dye-labeled oligo to streptavidin/avidin disulfide functionalized nanorods allowed obtaining a relatively uniform fluorophore-metallic nanostructure distance, which was estimated to be ~8 nm (It is known that the biotin disulfide molecules are mostly attached to the ends at the point with high curvature[20]). When a fluorescent dye is placed around the nanorod array, it interacts with the dipolar plasmon mode of the nanoarray, and the enhanced fluorescence could be achieved. As a result, we observed noticeable differences in brightness of emission spots from confocal scanning fluorescence reflectance images (Figure 1c) recorded from the individual Cy5-tagged dsDNA dispersed on bare substrate in the absence of silver nanoarray (left) and immobilized probes on the Ag array under similar experimental conditions, indicating preliminary information of enhanced fluorescence emission in the presence of silver nanostructures.

Figure 2 illustrates time traces of single dye-labeled dsDNA molecules suffered photobleaching as evidenced by the discrete drop in fluorescence intensity to the background level. Most of spots investigated show constant fluorescence emission and suffered permanent photobleaching in an abrupt step, ensuring a single binding event had been observed. Using the same incident excitation power, we observed significantly more fluorescence from the single molecules on the silver nanorod array, which is approximately more than 50-fold greater than that observed in the absence of silver nanostructure. The time

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profiles presented in Figure 2a is representative of more than half of those emission spots in the scanned images and illustrates the overall trend observed from more than 30 single molecules in each environment. The intensity is fairly constant until it drops abruptly to a lower level in a single step. The eventual photobleaching dynamics reveal that most of the fluorescence from this spot is due to a single probe. The distribution of brightness is presented in Figure 2b. In fact, different sites on the nanopatterned surface in which the probe molecules were tethered are moderately varied. The magnitude of the enhancement depends on the location of the fluorophore around the nanorod and the orientation of its dipole moment relative to the metal surface. The width of the distribution can be fit well with two Gaussian functions, indicating the presence of two sub-populations of nanoscale environments. We observed some extremely high enhancement of more than 200-fold which could contribute to the molecules residing in “hot spots”. The distribution of the enhancement in brightness is wider than that estimated by simulation. The position variation of the dye molecule attached on the nanostructure may also influence the wide distribution of the fluorescence enhancement factor. Those molecules aligned parallel to the electric field could experience the highest probability of excitation. Despite the fact, the overall large enhancement in fluorescence intensity was observed for the fluorophores located on nanopatterned silver surfaces.

In our experiment, we further performed time-resolved single-photon counting measurements on dye labeled DNA randomly immobilized on a two-dimensional nanorod array. A time-correlated single photon counting repeatedly measures the time interval between the excited laser pulse and the detected photons. The fluorescence interacting with nanorod shows a reduced lifetime compared with the reference signal. The reference signal is fitted with a single exponential decay with a lifetime of 2.3 ns. The decay of free fluorophore is almost monoexpontial, with only small deviations associated to slight differences in the dielectric nanoenvironment. The fluorescence decay changes dramatically in the presence of metallic nanoarray. The decay indicates that the intrinsic emission properties of the excited molecules are drastically modified in the presence of near-field coupling to metallic nanoarray. While in the presence of nanoarrary the signal requires a double exponential decay to give a reasonable fit with a long component of 1.5 ns and a short one of 0.3 ns. The lifetime values of the double exponential fit indicate that some of the dye interacts with the nanorod array, leading to a reduced lifetime, whereas another portion of the dye does not interact much with the nanorod and decays at roughly at the same rate as the control fluorescence. The plasmon interaction results in an approximately 10-fold increase in the decay rate. Shorter lifetime corresponds to interaction with the nanorod and a slow lifetime corresponds to the adjacent regions with background fluorescence.

The strongly confined fields near metallic nanostructures can profoundly alter the light emission properties of nearby optical emitters by increasing optical excitation rates and modifying radiative and nonradiative decay rates. For optical pumping below saturation, the fluorescence emission rate γ_{em} can be expressed as $\gamma_{em} = \gamma_{exc}[\gamma_{rad}/(\gamma_{rad} + \gamma_{nonrad})]$, where γ_{exc} is the excitation rate and γ_{rad} and γ_{nonrad} are the radiative and nonradiative decay rates, respectively. In general fluorescence experiments, without nearby noble metals, the changes in quantum yields (Q_0) and lifetimes (τ_0) are due to changes in the non-radiative decay rates

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γ_{nonrad} which result from changes in a fluorophore's environment, quenching or FRET. The values of Q_0 and τ_0 either increase or both decrease, but do not change in opposite directions because the radiative rate is mostly unchanged. Assuming an emitter's internal quantum structure remains unchanged, the electric fields are concentrated around the nanoarrays because of collective electron oscillations known as plasmons. These plasmons create enhanced local fields which extend short distances from the surface. These fields result in increased rates of excitation (γ_{exc}) of fluorophores proximity to the metal surfaces, which is where labeled DNA molecules located. These structures can also affect the relaxation of excited emitters back to their ground state by introducing new electromagnetic decay channels. According to the Purcell effect, spontaneous emission is not an intrinsic property of an emitter and can be modified by the surrounding electromagnetic environment. Thus, the metal-induced changes to radiative decay rate result in unusual effects: increases in the emission rate increases and decreases in the lifetimes.

In conclusion, the electric field intensity is highly localized between the gaps of the nanorods with the maximum intensity around the edge of nanorod. Our single molecule studies confirm a remarkable increase in fluorescence of single emitters controllably linked to ends of Ag nanorods. The fluorescence enhancement to nanorod end-linked fluorophore is due to a combination of processes including the localized electric field contour around the nanorods and the modification of the radiative decay rate. We have immobilized dye-labelled DNA molecules on the fabricated nanoarrays, and demonstrate the capability to detect enhanced fluorescence at a single-molecule level on the substrate. The result suggests a novel approach to perform DNA analysis and would benefit many applications to research as clinical sensing and imaging, proteomics and genomics, and other prospective applications in nanophotonics.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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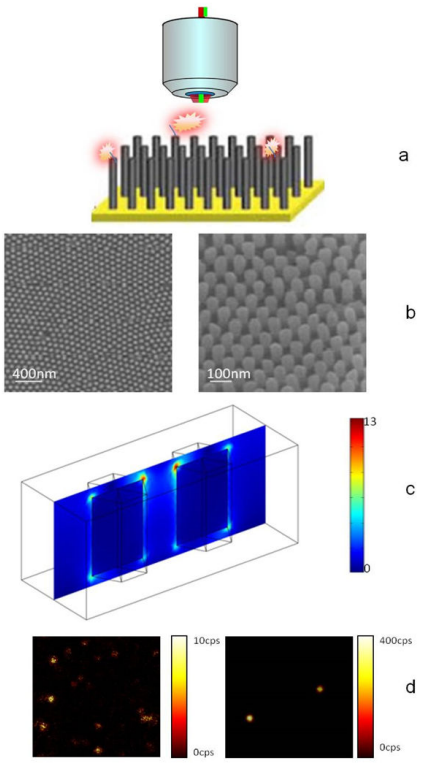

Figure 1.

(a) A scheme of the experimental setup; (b) SEM images of silver nanorod arrays; (c) simulated field intensity contour around the nanorods; (d) confocal scanning images of single emitters immobilized on controlled substrate (left) and on silver nanorod arrays (right).

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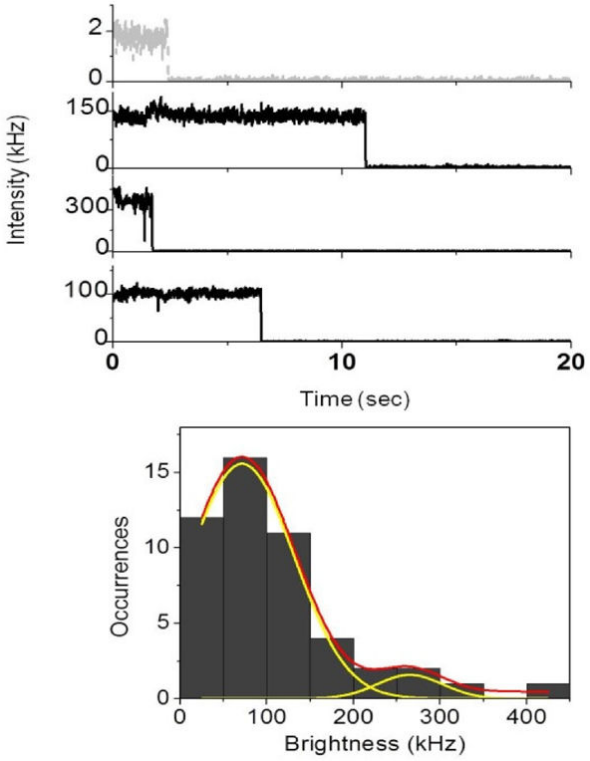

Figure 2.

(top) Representative time traces of single molecules on the substrate (gray) and on silver nanorod arrays (black); (bottom) Histograms of brightness of single molecules immobilized on silver nanoarrays, the red and yellow lines illustrate Gaussian fittings.

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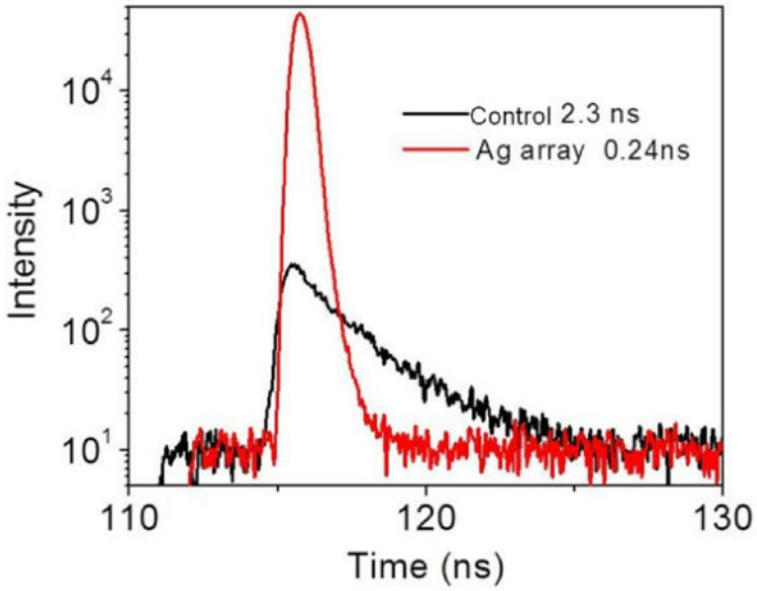

Figure 3.

Typical fluorescence decay curves of single molecules (control: black; silver nanrod array: red).