Published in final edited form as:

Proc SPIE Int Soc Opt Eng. 2008 January ; 6862: . doi:10.1117/12.770381.

Single molecule photophysics near metallic nanostructures

Jian Zhang, Yi Fu, Krishanu Ray, Mustafa H. Chowdhury, Henryk Szmazinski, Kazimierz Nowaczyk, Joseph R. Lakowicz*

Center for Fluorescence Spectroscopy, University of Maryland School of Medicine, Department of Biochemistry and Molecular Biology, 725 West Lombard Street, Baltimore, MD 21201

Abstract

Metal-enhanced fluorescence (MEF) is useful in single molecule detection (SMD) by increasing the photostability, brightness and increase in radiative decay rates of fluorophores. We have investigated MEF from an individual fluorophore tethered to a single silver nanoparticle and also a single fluorophore between a silver dimer. The fluorescence lifetime results revealed a near-field interaction mechanism of fluorophore with the metal particle. Finite-difference time-domain (FDTD) calculations were employed to study the distribution of electric field near the metal monomer and dimer. The coupling effect of metal particles on the fluorescence enhancement was studied. We have also investigated the photophysics of FRET near metal nanoparticles and our preliminary results suggest an enhanced FRET efficiency in the presence of a metal nanoparticle. In total, our results demonstrate improved detectability at the single molecule level for a variety of fluorophores and quantum dots in proximity to the silver nanoparticles due to the near-field metal-fluorophore interactions

Keywords

metal-enhanced fluorescence (MEF); single molecule detection (SMD); metal nanoparticle; FRET; fluorescence intensity; lifetime; photostability; photoblinking; finite-difference time-domain (FDTD); Mie theory

1. INTRODUCTION

The fluorescence enhancement of a fluorophore near a metal particle is known to be caused by a near-field interaction of fluorophore with a metal plasmon resonance [1–4]. The metal plasmon resonance can be described as arising from the collective oscillations of a group of electrons that migrate freely on the surface of metal particle (Figure 1) [5–8]. This energy resonance expresses as absorbance, excitation, and scattering when the metal particle is in the sub-wavelength size. The wavelength of metal plasmon resonance is principally dependent on the metal core factor including metal species, core size, and core shape [9,10]. The core of metal particle can be prepared to be composed of different noble metals including gold, silver, platinum, palladium, and copper, etc., or alloy [10–12]. The size of metal core can be controlled in a range of one – hundreds nm upon the experiment conditions in the

* lakowicz@cfs.umbi.umd.edu; phone 1 410 706-8408; fax 1 410 7065-8409.

preparation [3]. The metal particles can be made in different shapes of sphere, rod, wire, shell, triangle, prism, and tetrapod, etc [13–15]. The metal particles are stabilized with organic monolayers or inorganic thin layers coated on their metal cores. The radiating energy from the fluorophore is dramatically altered through coupling with the metal plasmon resonance to cause a change of the emission property. This concept is defined as Radiative Decay Engineering (RDE) [16,17]. An local electric field is believed to concentrate around the metal particle when it is exposed to incident light [18–25]. Actually, the metal particles are typically large enough to accurately apply this classic electric field theory, so the local electric field can be estimated theoretically by discrete dipole approximation (DDA) mode [18,19]. Accordingly, a large metal particle corresponds to a more insensitive electric field nearby. According to Schatz and our recent result, the electric field in the space between the coupled metal particles is more intense than that of individual metal particles due to the field coupling [23–25].

During the past several years, some calculation techniques, such as Finite-Difference Time-Domain (FDTD) method, have been developed to study the electric field near the metal particle [26–32]. The FDTD technique is an implementation of Maxwell's time-dependent curl equations for solving the temporal variation of electromagnetic waves within a finite space that contains a target of arbitrary shape, and has recently become the start-of-the-art method for solving Maxwell's equations for complex geometries. Since FDTD is a direct time and space solution, it offers a unique insight into all types of issues in electric and photonic. Furthermore, FDTD can also be used to obtain the frequency solution by exploiting Fourier transforms, thus a full range of useful quantities in addition to fields around particles can be calculated, such as the complex Poynting vector and the transmission / reflection of light. In the FDTD technique, Maxwell's curl equations are discretized by using finite-difference approximations in both time and space that are easy to program and are accurate. In order to achieve high accuracy for realizing the spatial derivatives involved, the algorithm positions the components of the electric field about a unit cell of the lattice that constitutes the FDTD computational domain. The electric field near a metal particle can be described well by FDTD calculations.

Compared with the free-space emission of the excited fluorophore, the photophysical properties of fluorophore on the metal particle can be interpreted by a modified Jablonski diagram (Figure 2) [16,17]. The fluorophore-induced plasmons can radiate to the far-field and create observable emission. The usefulness of an increase in the radiative decay rate can be understood by considering the definitions of the quantum yield (Q_0) and the lifetimes (τ_0). The lifetime and quantum yield (Q_0) of a fluorophore in the free state can be given by Equations 1 and 2,

$$Q_o = \Gamma / (\Gamma + k_{nr})$$

(1)

$$\tau_o = (\Gamma + k_{nr})^{-1}$$

(2)

In classical fluorescence experiments, the change in quantum yield or lifetime is due to the change in the non-radiative decay rate (k_{nr}), which results from the change in environment of a fluorophore, quenching, or FRET. The values of Q_0 and τ_0 can both increase or both decrease, but do not change in the opposite direction. Unique spectral changes are possible for fluorophores near metal particles or surfaces. Suppose this radiative decay rate near the metal is increased and is given by $\Gamma + \Gamma_m$, when Γ_m is the additional rate due to the metal substrate, the quantum yield Q_m and lifetime τ_m of the fluorophores near the metal substrate thus become

$$Q_m = \frac{\Gamma + \Gamma_m}{\Gamma + \Gamma_m + k_{nr}}$$

(3)

$$\tau_m = (\Gamma + \Gamma_m + k_{nr})^{-1}$$

(4)

The metal-induced change to $\Gamma + \Gamma_m$ results in unusual effects, the quantum yield increases and the lifetime decreases, which is different from the change in k_{nr} where both Q and τ change in the same direction. A decrease in lifetime has several favorable consequences. Fluorophores can become more photostable because they spend less time in the excited state where the photochemistry occurs. Hence a fluorophore can undergo more excitation and emission cycles prior to photobleaching. The maximum emission rate of a fluorophore is approximately equal to the inverse of the lifetime. Hence, a fluorophore with a shorter lifetime will be less prone to optical saturation and have higher maximum emission rates. These changes result in an increasing detectability of single or multiple fluorophores. The interactions of fluorophores with metal particles have been studied in the 1970s and 1980s [33,35]. However, the practical usefulness of these interactions has only recently been recognized. It provides a fundamental approach to develop the metal particle associated imaging agents with brighter signal, short lifetime, and more photostable for this proposal.

Mie theory is an alternative tool to describe the interaction of fluorophore with metal particle. According to Mie theory, the extinction coefficient of metal particles is due to both

light absorption and light scattering [36–38]. The absorption component C_A represents the cross-section due to absorption. The scattering component C_S represents the ability of a metal particle to be an oscillating dipole that radiates light and propagates to far-field. The extinction cross-section C_E is given by $C_E = C_A + C_S$. To an approximation, these spectra can be used to predict the effect of a nearby particle on a fluorophore. If a fluorophore interacts with the absorption component C_A , it will result in the metal plasmons. The scattering component C_S allows the fluorophore-induced plasmons to radiate to far-field showing as fluorescence. The lifetime of fluorophore-metal particle complex is decreased because of high extinction coefficient. We also discuss the dependence of fluorescence enhancement on the size of metal particle [17]. The extinction properties of metal particles with the sub-wavelength sizes can be expressed by a combination of both absorption (C_A) and scattering (C_S) factors,

$$C_E = C_A + C_S = k_1 \text{Im}(\alpha) + \frac{k_1^4}{6\pi} |\alpha|^2$$

(5)

where $k_1 = 2\pi\nu_1 / \lambda_0$ is the wavevector of the incident light in medium 1 and α is the polarizability of the sphere of radius r ,

$$\alpha = 4\pi r^3 (\epsilon_m - \epsilon_1) / (\epsilon_m + 2\epsilon_1)$$

(6)

and ϵ_m is the complex dielectric constant of metal. The first term represents the cross section due to absorption and the second term represents the cross section due to scattering. According to our recent approach, we expect C_A to cause quenching and C_S to cause enhancement. The quenching term increases as the r^3 factor, and the enhancement term increases as the r^6 factor. Hence, a small particle can quench fluorescence because the absorption dominates over the scattering. Conversely, a large particle can enhance fluorescence because the scattering component is the dominant factor. This result is partially in accordance with FDTD calculations, but cannot be used to explain why the enhancement efficiency decreases significantly after the intermediate size of metal particle as the observation in our experiment. It means the theory we give in this proposal is not perfect. However, we note that it is valuable to use both Mie theory and FDTD calculations for completeness. Mie theory can only be practically used with spherical geometry to predict the local fields directly on the metal surface. In contrast, FDTD calculations can be used to predict the local electric fields of metal particles with any shape and at all distances from the metal cores.

On the basis of above theoretical description and simulation, we have designed single molecule fluorescence measurements near the metal nanoparticle. All experiments were achieved using scanning confocal microscopy. The results were achieved and reviews as following.

2. RESULTS AND DISCUSSION

2-1. Synthesis of metal particle and functionalization

We have extensive experiences on synthesis, functionalized, and characterization of metal particles (Figure 3), which are composed of different metal cores, core sizes, and shapes [39–51]. These metal particles were coated with organic monolayers (citrate, amine, or thiolate, etc). In which, the thiolate monolayers were mostly employed due to their chemical stability in solution. They were dispersed in organic solvent and /or water solution principally depending on the polarity of coating layers. These metal particles were found to display their metal plasmon resonance principally dependent on the metal core factor such as metal specie, core size, and shape, etc. Abundant surface chemistries on the metal particles have been verified to occur well including ligand exchange and chemical reaction, supporting that these metal particles can be bound definitely by the fluorophores and biological functionalities (e.g. DNA or peptide) as required in the experiments.

We also synthesized the metal particles that were immobilized on the glass substrate by chemical [16,17] or vapor deposition method [52,53], so called silver islands. These silver islands are used widely in our single molecule studies.

2-2. Metal-enhanced fluorescence (MEF) on monomer or dimer metal particle.

The emission intensity was observed to enhance efficiently up to 10 – 100 times when localizing the fluorophores near the metal particle with an optimal distance from the metal surface [54]. We studied the dependence of single molecule fluorescence on the size of nearby metal particle. The silver particles were synthesized with average diameters of the metal cores 5, 20, 50, 70, and 100 nm, respectively. A single-stranded oligonucleotide was chemically bound to a single silver particle and a Cy5-labeled complementary single-stranded oligonucleotide was hybridized with the particle-bound oligonucleotide (Figure 4). The space between the fluorophore and metal core was separated by a rigid hybridized DNA duplex of 8 nm length. The single fluorescence images and intensity traces were recorded by scanning confocal microscopy. The single fluorophore-labeled 50 nm silver particles were observed to display the most enhanced intensity of 17-fold increase relative to the free fluorophores in the absence of metal. Numerical simulations by FDTD calculation and results from Mie theory were used to compare with the experimental results (Figure 5). The 50 nm silver particles were also labeled by multiple fluorophores/per particle. The fluorescence intensity of multiple fluorophore-labeled metal particle increases dramatically with the loading number and reached 400-fold relative to the free single fluorophore when the loading number was 50. The fluorophores bound on the metal particle also displayed better photostability than the free fluorophore. The lifetime of bound fluorophore was shortened with increasing the core size of metal particle from 5 to 50 nm.

We also prepared the 20 nm silver particle dimers with single Cy5 molecules localized between coupled metal particles to discuss the coupling effect of metal particle on the fluorescence enhancement (Figure 4) [55]. In this case, the dimers were formed by hybridization with double-length single-stranded oligonucleotides that contained single Cy5 molecules. The image analysis revealed that the single molecule fluorescence was enhanced 7-fold on the metal monomer and 13-fold on the metal dimer relative to the free Cy5-labeled oligonucleotide in the absence of metal. The lifetimes were shortened on the silver monomers and further shortened on the silver dimers, demonstrating the near-field interaction mechanism of fluorophore with the metal substrate. FDTD calculations were employed to study the distribution of electric field near the metal monomer and dimer (Figure 6). The correlation between the emission enhancement and the field density was used to interpret the mechanism. The metal particle affects the intrinsic decay rate of fluorophore. The near-field interaction between the fluorophore and metal particle was reflected by a change of lifetime [16]. The results revealed that the lifetimes of all samples with the metal were shortened significantly relative to that of free Cy5 in the absence of metal, indicating the near-field interactions for all metal particles. The lifetimes of fluorophore on the smallest silver particle (5 nm) and largest silver particle (100 nm) were obviously longer than those on the intermediate size of metal particles such as 20, 50, and 70 nm, supporting our theoretical calculations and experimental results that the fluorophore could interact most efficiently with the intermediate size metal particles at an optimized distance from the metal core.

2-3. Enhanced Förster energy transfer near metal particle.

Beside the fluorescence intensity, we found that Förster Resonance Energy Transfer (FRET) could be enhanced significantly when the donor-acceptor pair was localized near the metal particle [56,57]. This result could be utilized as a ruler to measure the energy transfer distance. In the experiment, a donor-labeled DNA was chemically bound to a single 20 nm silver particle and then an acceptor-labeled DNA was coupled by hybridization (Figure 7). The photophysical behavior of FRET between the donor-acceptor pair was studied using single molecule time-resolved spectroscopy. Both the emission intensity and lifetime obtained from the bulk emission measurement and single molecule fluorescence measurement indicated that FRET was enhanced on the metal particle, which resulted in increasing the efficiency and lengthening the distance of energy transfer to 1.4 times. The metal enhanced rate constant for FRET was 21 times faster than free donor-acceptor pair in solution.

The metal-enhanced FRET was also found to depend on the size of metal core. The Förster distance was extended to 1.4 times for the donor-acceptor pair was bound on 20 nm silver particle, 1.6 times on 50 nm silver particle, and 1.7 times on 100 nm silver particle. The enhancement of FRET was dependent on the distance of spacer between the donor-acceptor pair and metal particle. The Förster distance was noted to lengthen from 1.4 R_0 to 1.6 R_0 when the spacer increased from 2 to 10 nm. The studies included the effect of donor-acceptor distance, silver particle size, and distance from the metal surface. These results will aid in the design of metal particle for using FRET to determine biomolecule proximity at

distances beyond the usual Förster distance. This result is in consistence with the simulation by FDTD technology.

2–4. Reduced blinking and long-lasting fluorescence of single fluorophores on silver nanoparticles.

The fluorescence signal of single organic fluorophores was characterized by the random blinking and the irreversible photobleaching [58–59]. Photoinduced blinking of Cy5 has posed various limitations of this popular near infrared (NIR) probe in biological applications. Here we showed that fluorophore-metal nanoparticles (NP) complexes greatly suppress Cy5 blinking and noticeably reduced photobleaching events. The blinking behaviors of single Cy5 molecules were investigated and compared in the absence and the presence of silver nanoparticles. A power-law distribution of off time population was observed for single Cy5 molecules. Average off times was compared to evaluate the plasmon effect of silver nanoparticles on the triplet decay rates. We furthermore demonstrate that enhanced photostability and brightness in the presence of silver particles. The results show that plasmon-controlled fluorescence can lead to a novel physical mechanism to enhance fluorescence intensity.

2–5. Metal-enhanced fluorescence from quantum dots.

We described the metal-enhanced fluorescence from the CdTe nanocrystals instead of organic fluorophores spin coated on silver island films (SIFs) [60]. CdTe nanocrystals showed 5-fold increase in fluorescence intensity, 3-fold decrease in lifetime, and reduction in blinking on the SIF surfaces that were observed by ensemble and single-molecule fluorescence studies (Figure 8). The single-molecule study also provided further insight on the heterogeneity in the fluorescence enhancement and lifetimes of the CdTe nanocrystals on both glass and SIF surfaces, which was otherwise not possible to observe using ensemble measurements.

2–6. Metal-enhanced fluorescence by fluorophore-metal distance control.

Methods that increase the total emission per fluorophore would provide increased sensitivity and a wider dynamic range for chemical analysis, medical diagnostics, and in vivo molecular imaging [61]. The use of fluorophore-metal interactions has the potential to dramatically increase the detectability of single fluorophores for bioanalytical monitoring (Figure 9). The fabrication and single-molecule analysis of fluorophore-labeled DNA molecules tethered to silver island films were described in single molecules. Analysis of fluorescence images, intensity profiles, total emitted photons, and lifetime distributions revealed some of sample heterogeneities. Investigations of time-dependent emission characteristics of single molecules indicated that the total number of emitted photons on the silvered surface was more than 10 times greater than on free labeled DNA molecules on a glass substrate. In addition, time-correlated single-photon counting results revealed the reduced lifetimes of single molecules tethered to silver island films.

We also investigated fluorescence enhancements and lifetime reductions of Cy5 probe molecules at various distances from the deposited silver island film surface using single molecule spectroscopic methods [62]. The proximity of fluorophore molecules to the surface

was controlled by alternating layers of biotinylated bovine serum albumin (BSA-biotin) and avidin, followed by binding of Cy5-labeled oligonucleotides to the top of a BSA-biotin layer structure (Figure 9). We observed dramatically varied brightness of fluorophores with distances from metal structures as well with reduced blinking in the presence of silver island films. In addition, distributions of fluorescence lifetimes and apparent emission intensities from individual molecules indicate an inhomogeneous nature of local matrix surface near metallic nanostructures. These studies illustrate the exclusive information that is otherwise hidden in ensemble measurements.

3. CONCLUSION

In this paper, we review our recent results on the photophysical properties, e.g. intensity, lifetime, photostability, photoblinking, as well as FRET, of single fluorophore near the metal particles. It is found that these properties are dependent on the metal particle size, fluorophore and its excitation and emission wavelengths, distance from fluorophore to surface of metal particle, etc. FDTD calculation and a model developed from Mie theory were used to understand the experimental results. These results can aid us to develop novel fluorescence detection technology for clinical diagnostics and bioassay.

REFERENCES

- [1]. Haynes CL; McFarland AD; Zhao L; Van Duyne RP; Schatz GC; Gunnarsson L; Prikulis J; Kasemo B; Kall M "Nanoparticle optics: the importance of radiative dipole coupling in two-dimensional nanoparticle arrays" *J. Phys. Chem. B* 107, 7337(2003).
- [2]. Charnay C; Lee A; Man SQ; Moran CE; Radloff C; Bradley RK; Halas NJ "Reduced symmetry metalodielectric nanoparticles: chemical synthesis and plasmonic properties" *J. Phys. Chem. B* 107, 7327(2003).
- [3]. Foss CA; Feldheim DL [Metal nanoparticles: synthesis, characterization, and applications], Marcel Dekker, New York, 2001.
- [4]. Barnes WL; Dereux A; Ebbesen TW "Surface plasmon subwavelength optics" *Nature* 424, 824(2003). [PubMed: 12917696]
- [5]. Ferrel TL; Callcott TA; Warmack RJ "Plasmons and surfaces" *American Scientist*, 73, 344(1985).
- [6]. Link S; Wang ZL; El-Sayed MA "Alloy "Formation of gold-silver nanoparticles and the dependence of the plasmon absorption on their composition" *J. Phys. Chem. B* 103, 3529(1999).
- [7]. Charnay C; Lee A; Man SQ; Moran CE; Radloff C; Bradley RK; Halas NJ "Reduced symmetry metalodielectric nanoparticles: chemical synthesis and plasmonic properties" *J. Phys. Chem. B* 107, 7327(2003).
- [8]. Ingram RS; Hostetler MJ; Murray RW "Poly-hetero- -functionalized alkanethiolate-stabilized gold cluster compounds" *J. Am. Chem. Soc* 119, 9175(1997).
- [9]. Templeton AC; Wuelfing WP; Murray RW "Monolayer-protected cluster molecules" *Acc. Chem. Res* 33, 27(2000). [PubMed: 10639073]
- [10]. Ribbe AE; Okumura A; Matsushige K; Hashimoto T "Element spectroscopic imaging of poly(2-vinylpyridine)-block-polyisoprene microdomains containing palladium nanoparticles macromolecule" *Macromol.* 34, 8239(2001).
- [11]. Vitulli G; Bernini M; Bertozzi S; Pitzalis E; Salvadori P; Coluccia S; Martra G "Nanoscale copper particles derived from solvated Cu atoms in the activation of molecular oxygen" *Chem. Mater* 14, 1183(2002).
- [12]. Jin R; Chao YW; Mirkin CA; Kelly KL; Schatz GC; Zheng JG "Photoinduced conversion of silver nanospheres to nanoprisms" *Science* 294, 1901(2001). [PubMed: 11729310]

- [13]. Jana NR; Gearheart L; Murphy CJ “Wet chemical synthesis of high aspect ratio cylindrical gold nanorods” J. Phys. Chem. B 105, 4065(2001).
- [14]. Ahmadi TSW, Z. L.; Green TC; Henglein A; ElSayed MA “Shape-controlled synthesis of colloidal platinum nanoparticles” Science 272, 1924(1996). [PubMed: 8662492]
- [15]. Wiley B; Sun Y; Xia Y “Synthesis of silver nanostructures with controlled shapes and properties” Acc. Chem. Acc. Chem. Res 40, 1067(2007). [PubMed: 17616165]
- [16]. Lakowicz JR “Radiative decay engineering: Biophysical and biomedical applications” Anal. Biochem 298, 1(2001). [PubMed: 11673890]
- [17]. Lakowicz JR “Radiative decay engineering 5: metal-enhanced fluorescence and plasmon emission” Anal. Biochem 337, 171(2005). [PubMed: 15691498]
- [18]. Hubert C; Rumyantseva A; Lerondel G; Grand J; Kostcheev S; Billot L; Vial A; Bachelot R; Royer P; Chang S.-h.; Gray SK; Wiederrecht GP; Schatz GC “Near-field photochemical imaging of noble metal nanostructures” Nano Lett. 5, 615(2005). [PubMed: 15826096]
- [19]. Gunnarsson L; Rindzevicius T; Prikulis J; Kasemo B; Kall M; Zou S; Schatz GC “Confined plasmons in nanofabricated single silver particle pairs: experimental observations of strong interparticle interactions” J. Phys. Chem. B 109, 1079(2005). [PubMed: 16851063]
- [20]. Hao E; Li S; Bailey RC; Zou S; Schatz GC; Hupp JT “Optical properties of metal nanoshells” J. Phys. Chem B108, 1224(2004).
- [21]. Kelly KL; Coronado E; Zhao LL; Schatz GC “The optical properties of metal nanoparticles: the influence of size, shape, and dielectric environment” J. Phys. Chem. B 107, 668(2003).
- [22]. Pinchuk AO; Kalsin AM; Kowalczyk B; Schatz GC; Grzybowski BA “Modeling of electrodynamic interactions between metal nanoparticles aggregated by electrostatic interactions into closely-packed clusters” J. Phys. Chem. C 111, 11816(2007).
- [23]. Kelly KL; Lazarides AA; Schatz GC; Van Duyne RP “Nanosphere lithography: effect of the external dielectric medium on the surface plasmon resonance spectrum of a periodic array of silver nanoparticles” J. Phys. Chem. B 103, 9846(1999).
- [24]. Haynes CL; McFarland AD; Zhao L; Van Duyne RP; Schatz GC; Gunnarsson L; Prikulis J; Kasemo B; Kall M “Nanoparticle optics: the importance of radiative dipole coupling in two-dimensional nanoparticle arrays” J. Phys. Chem. B 107, 7337(2003).
- [25]. Haynes CL; McFarland AD; Smith MT; Hulteen JC; Van Duyne RP “Angle-resolved nanosphere lithography: manipulation of nanoparticle size, shape, and interparticle spacing” J. Phys. Chem. B 106, 1898(2002).
- [26]. Taflove A, Hagness SC [Computational Electrodynamics: The Finite-Difference Time-Domain Method], Artech House, Boston, 2000.
- [27]. Yee KS, “Numerical solution of initial boundary value problems involving Maxwell’s equations in isotropic media”, IEEE Trans. Antennas Propagat, vol. AP-14, 302–307, 1966.
- [28]. Yang P, Liou NK “Finite-difference time domain method for light scattering by small ice crystals in three-dimensional space” J. Opt. Soc. Am. A 12, 162(1995).
- [29]. Yang P, Liou NK “Application of the symplectic finite-difference time-domain method to light scattering by small particles” J. Opt. Soc. Am. A 13, 2072(1996).
- [30]. Gray SK, Kupka T “Propagation of light in metallic nanowire arrays: Finite-difference time-domain studies of silver cylinders” Phys. Rev. B 68, 045415(2003).
- [31]. Chang S-H; Gray SK; Schatz GC “Surface plasmon generation and light transmission by isolated nanoholes and arrays of nanoholes in thin metal films” Optics Express 13, 3150(2005). [PubMed: 19495214]
- [32]. Sullivan DM [Electromagnetic simulation using the FDTD method], IEEE Press, New York, 2000.
- [33]. Chance RR, Prock A, and Silbey R “Molecular fluorescence and energy transfer near interfaces” Adv. Chem. Phys 37,1(1973).
- [34]. Ford GW, and Weber WH “Electromagnetic interactions of molecules with metal surfaces” Phys. Reports 113, 195(1984).
- [35]. Gersten J, and Nitzan A “Spectroscopic properties of molecules interacting with small dielectric particles” J. Chem. Phys 75, 1139(1984).

- [36]. Lakowicz JR, Shen Y, D'Auria S, Malicka J, Fang J, Gryczynski Z, and Gryczynski I "Radiative decay engineering. 2. Effects of silver island films on fluorescence intensity, lifetimes, and resonance energy transfer" *Anal. Biochem* 301, 261(2002). [PubMed: 11814297]
- [37]. Lakowicz JR "Radiative decay engineering 3. Surface plasmon-coupled directional emission" *Anal. Biochem* 324, 153(2002).
- [38]. Gryczynski I, Malicka J, Gryczynski Z, and Lakowicz JR "Radiative decay engineering 4. Experimental studies of surface plasmon coupled directional emission" *Anal. Biochem* 324, 270(2004).
- [39]. Zhang J Malicka J; Gryczynski I; Lakowicz JR "Oligonucleotide-displaced organic monolayer-protected silver nanoparticles and enhanced luminescent of their salted aggregates" *Anal. Biochem* 330, 81(2004). [PubMed: 15183765]
- [40]. Aslan K; Zhang J; Lakowicz JR; Geddes CD "Saccharide sensing using gold and silver nanoparticles-A review" *J. of Fluorescence* 14, 391(2004).
- [41]. Zhang J; Geddes CD; Lakowicz JR "Complexation of polysaccharide and monosaccharide with thiolate boronic acid capped on silver nanoparticle" *Anal. Biochem* 332, 253(2004). [PubMed: 15325293]
- [42]. Zhang J; Roll D; Geddes CD; Lakowicz JR "Aggregation of silver nanoparticle-dextran adducts with concanavalin A and competitive complexation with glucose" *J. Phys. Chem. B* 108, 12210(2004).
- [43]. Zhang J; Gryczynski Z; Lakowicz JR "First observation of surface plasmon-coupled electrochemiluminescence" *Chem. Phys. Lett* 393, 483(2004). [PubMed: 19763232]
- [44]. Zhang J; Roll D; Geddes CD; Lakowicz JR "Aggregation of silver nanoparticle-Dextran adducts with concanavalin A and competitive complexation with glucose" *J. Phys. Chem. B* 104, 12201(2004).
- [45]. Zhang J Lakowicz JR "Enhanced luminescence of phenyl-phenanthridine dye on aggregated small silver nanoparticles" *J. Phys. Chem. B* 109, 8701(2005). [PubMed: 16852030]
- [46]. Zhang J; Malicka J; Gryczynski I; Lakowicz JR "Surface-enhanced fluorescence of fluorescein-labeled oligonucleotides capped on silver nanoparticles" *J. Phys. Chem. B* 109, 7643(2005). [PubMed: 16851886]
- [47]. Zhang J Lakowicz JR "A Model DNA detection by metal enhanced fluorescence from immobilized silver nanoparticles on solid substrate" *J. Phys. Chem. B* 110, 2387(2006). [PubMed: 16471829]
- [48]. Zhang J; Gryczynski I; Gryczynski Z; Lakowicz JR "Dye-labeled silver nanoshell – bright particle" *J. Phys. Chem. B* 110, 8986(2006). [PubMed: 16671705]
- [49]. Zhang J; Fu Y; Lakowicz JR "Emission behavior of Ru(bpy)₃²⁺ complex in silver nanoshell – enhanced self-quenching by metal nanostructure" *J. Phys. Chem. C*, 111, 1955(2007).
- [50]. Zhang J; Fu Y; Lakowicz JR "Luminescent images of single gold nanoparticles and their labeling on silica beads" *Optic Express* 15, 13415(2007).
- [51]. Zhang J; Fu Y; Lakowicz JR "Fluorescence images of DNA-bound YOYO between coupled silver particles" *Langmuir* 23, 11734(2007). [PubMed: 17914851]
- [52]. Zhang J; Lakowicz JR "Metal-enhanced fluorescence of an organic fluorophore using gold particles" *Optical Express*, 15, 2598(2007).
- [53]. Zhang J; Matveeva E; Gryczynski I; Leonenko Z; Lakowicz JR "Metal-enhanced fluoroimmunoassay on a silver film by vapor deposition" *J. Phys. Chem. B* 109, 7969(2005). [PubMed: 16851931]
- [54]. Zhang J; Fu Y; Chowdhury M; Lakowicz JR "Single molecule studies on fluorescently labeled silver particles: effects of particle size" *J. Phys. Chem C* 112, 18–26(2008).
- [55]. Zhang J; Fu Y; Lakowicz JR "Metal-enhanced single-molecule fluorescence on silver particle monomer and dimer: coupling effect between metal particles" *Nano Lett.* 7, 2101(2007). [PubMed: 17580926]
- [56]. Zhang J, Fu Y; Lakowicz JR "Enhanced Forester resonance energy transfer (FRET) on single metal particle" *J. Phys. Chem. C* 111, 50(2007).

- [57]. Zhang J; Fu Y; Lakowicz JR “Enhanced Förster resonance energy transfer (FRET) on single metal particle 2: dependence on donor-acceptor separation distance, particle size, and distance from metal surface” J. Phys. Chem. C 111, 11784–11792(2007).
- [58]. Fu Y; Zhang J; Lakowicz JR “Suppressed blinking in single quantum dots (QDs) immobilized near silver island films (SIFs)” Chem. Phys. Lett 447, 96(2007). [PubMed: 18927603]
- [59]. Fu Y; Zhang J; Lakowicz JR “Reduced blinking and long-lasting fluorescence of single fluorophores coupling to silver nanoparticles” Langmuir, in print.
- [60]. Ray K; Badugu R; Lakowicz JR “Metal-enhanced fluorescence from CdTe nanocrystals: a single-molecule fluorescence study” J. Am. Chem. Soc 128, 8998(2006). [PubMed: 16834349]
- [61]. Fu Y; Lakowicz JR “Enhanced fluorescence of Cy5-labeled DNA tethered to silver island films: fluorescence images and time-resolved studies using single-molecule spectroscopy” Anal. Chem 78, 6238(2006). [PubMed: 16944907]
- [62]. Fu Y; Lakowicz JR “Enhanced fluorescence of Cy5-labeled oligonucleotides near silver island films: a distance effect study using single molecule spectroscopy” J. Phys. Chem. B 110, 22557(2006). [PubMed: 17092001]

Fig. 1.
Schematic of surface plasmons on a metal surface.

Fig. 2. Modified Jablonski diagram which includes metal fluorophore interactions. The thicker arrows represent increased rates of excitation and emission.

Fig. 3.
Preparation of organic monolayer protected metal nanoparticle, functionalization through ligand exchange, and binding of fluorophores by surface reaction.

Fig. 4. Scheme of succinimidylation of metal particle via ligand exchange, binding single-stranded DNA, hybridization with target DNA to form metal monomer and dimer, and labeling by hybridization.

Fig. 5. Left: electric field contours for different size Ag round particles with different two cross-sections. Right: dependence of enhancement efficiency of single Cy5-labeled metal particle and the electric field intensity at 8 nm away from the metal core on the core size.

Fig. 6. Left: histograms of emission intensity for free Cy5, labeled metal particle monomer, and dimer. Right: electric field contours for 20 nm Ag round monomer and dimer particles with different two cross-sections.

Fig. 7.

Left: succinimidyl-terminated silver particle was bound by aminated donor-oligonucleotide, and hybridized by complementary acceptor-labeled DNA. Right: histograms of FRET efficiency on the free oligonucleotide pairs and those bound to 15, 40, and 80 nm silver particles by 2 nm linker.

Fig. 8.

Intensity time trajectories (A and B), intensity decays (C and D) of individual CdTe on glass and SiFs. Fluorescence intensity (count rate) (E) and average fluorescence lifetime (F) histograms of CdTe on glass and SiF surfaces.

Fig.9.

Left: Schematic illustration of DNA immobilization process in our experiments. The Cy5-labeled oligonucleotide is hybridized to the complementary thiolated oligonucleotide. After incubation, labeled DNA molecules are tethered to silver island nanostructures on a APS-treated glass substrate. Right: Cy5-labeled oligonucleotides were bound to a BSA-biotin/Avidin multilayer deposited on silver island film.