Adsorbed Sites of Individual Molecules on Ag Nanoparticles in Single Molecule Sensitivity−Surface-Enhanced Raman Scattering

Masayuki Futamata*,† Yoshihiro Maruyama,‡,|| and Mitsuru Ishikawa§

Nanoarchitectonics Research Center (NARC) - National Institute of Advanced Industrial Science and Technology (AIST), Tsukuba 305-8562, Japan, Research Consortium for Synthetic Nano-Function Materials Project - AIST, Tsukuba 305-8568, and Single-molecule Bioanalysis Lab.-AIST, Takamatsu 761-0395, Japan

The blinking of surface-enhanced Raman scattering (SERS) signal was observed for Ag touching particles with an extremely small number of molecules progressively extinguished in 10−30 min probably because of thermal diffusion to marginal sites from the junction or complete desorption of adsorbates. Interestingly, we found a significant change in elastic scattering spectra observed with a white light source during the inactivation process of SERS. Furthermore, it turned out exploiting numerical simulations for the local electric field and scattering spectra that such spectral changes result from the distinct electromagnetic coupling between the localized surface plasmon (LSP) and absorption of dye according to the on/off features of SERS activity. This is induced by thermal diffusion of individual molecules in and out of the junctions in accordance with the experimental observations on the vast enhancement in SERS. Thus, the critical importance of the junctions of Ag particles was confirmed concerning the origin of the blinking and mechanism of the enormous enhancement in SERS.

Introduction

To realize a bottom-up nanotechnology, for example, a single molecule device or a nanochemical factory, it is indispensable to establish analytical techniques to elucidate the natures of the components at an atomic or molecular scale. Recently, advanced technologies such as inelastic tunneling spectroscopy attracted attention to induce and monitor single molecule reaction process[1] or scanning tunneling emission spectroscopy featuring the local electronic state at an atomic scale.[2] Indeed, these techniques provide extremely detailed information on the target molecules or atoms at an atomic scale, while they are used under ultrahigh vacuum or under extremely low temperatures for precise control of the mechanical drift or diffusion of the samples. In contrast, surface-enhanced Raman scattering (SERS) is available under ambient conditions, even at the solid/liquid interfaces. It attracts increasing emphasis because of single molecule sensitivity and also because of potential usefulness when combined with near-field microscopy to improve the spatial resolution into nanometer scale.

In fact, SERS has been well-established phenomena[3,4] for more than 30 years, and Raman signal from adsorbates on roughened metal surfaces is enhanced by a factor of 10^4-10^6 because of excitation of the surface plasmon polariton (SPP) of roughened metals or because of the "first-layer" enhancement including charge-transfer resonance between adsorbates and metals. These enhancement factors are population-averaged values for numerous Ag particles with various shapes and sizes and thus obviously are insufficient to yield single molecule detection in SERS. Recent progress in SPM (scanning probe

microscopy) in addition to a highly sensitive CCD (charge-coupled device) detector enables us to observe extremely weak spectral signal from an individual metal particle or even from a single molecule. In fact, several groups reported vast enhancement in SERS, for example, 10^{14}, corresponding to single molecule sensitivity with probing only "hot" particles with appropriate shapes and sizes,[5−9] whereas the mechanism of the enormous enhancement is still obscure. As is well known, valuable information can be extracted from SERS experiments such as a molecular structure, orientation, or interaction with neighboring species. In contrast, fluorescence spectroscopy precedent as a single molecule detection method yields less informative broad spectra albeit useful to identify individual molecules. Thus, it is challenging to establish a single molecule detection method (SMD) with SERS in terms of application to various fields, such as elucidation of elementary reaction process at solid/liquid interfaces. The following is commonly observed concerning the vast enhancement in SERS:[5−9] (1) only small numbers of hot particles emerge with prominent enhancement when surface coverage of adsorbates is ca. several tens to one hundred per each Ag particle and (2) at lower surface coverage <ca. 1 molecule/particle, the "blinking" of the Raman signal that intensity suddenly and repeatedly changes with time is observed for merely trace amounts of Ag particles with dye or other biomolecules such as hemoglobin.

The blinking is attributed to a single molecule phenomenon, since it is not plausible for enormous molecules to move or to change their orientation at the same time. Recently, Weiss and Haran[10] reported that the rate of spectral fluctuation is proportional to laser intensity and suggested a nonthermal process such as photoinduced desorption of adsorbates. Bosnick et al.[11] reported the SERS scattering intensity fluctuates because of motions of the molecule in and out of the hot spot and is highly localized around it. These endeavors would provide us insight into the origin of the blinking and the vast enhancement in SERS

* Corresponding author. E-mail: m.futamata@aist.go.jp.
† Nanoarchitectonics Research Center.
‡ Research Consortium for Synthetic Nano-Function Materials Project.
§ Single-molecule Bioanalysis Lab.
|| Permanent address: Tsukuba Research Laboratory, Hamamatsu Photonics K. K., 300-2635 Tsukuba, Japan.

from Ag nanoparticles to establish SMD−SERS as an analytical tool. Similarly, thermal activation of the blinking was evidenced by the temperature dependence when the intensity fluctuation is suppressed at 77 K.[12] In addition, local electric field intensity evaluated by a numerical method, for example, finite difference time domain (FDTD) method, yields vast enhancement of >10[10] at the junction of touching Ag particles with various sizes and shapes in contrast to modest enhancement of 10[4]−10[5] at other ordinary sites or on isolated particles. These results strongly suggest that the blinking is due to a thermal process, most probably because of a thermal diffusion of adsorbate molecules from the junctions to other ordinary sites on Ag particles.[12]

To verify these attributions, it is critical to specify the adsorbed site of individual dye molecules on Ag nanoparticles. Recently, we found the evidences exploiting the elastic scattering spectra that adsorbed molecules on Ag particles are sitting at the junction to provide vast enhancement as reported here.

Experiments

Details in sample preparation and optical measurements were the same as described previously.[5] Briefly, Raman microscopy was combined with atomic force microscopy (AFM) measurement to observe a scattered light image, Raman image, and Raman spectra for individual Ag particles, of which morphology is elucidated.[1,3,4] SERS images of Ag nanoparticles were obtained using a notch filter and CCD camera at a video rate (30 flames/s), while SERS spectra were measured with Renishaw spectrometer. Only a countable number of Ag nanoparticles prepared from AgNO$_3$ and sodium citrate are immobilized onto a Si wafer coated with 3-aminopropyltrimethoxysilane. A gridlike maker with a space of 30 μm along the two perpendicular directions enables us to identify each particle in the AFM and optical images. With tuning the concentration of adsorbates, that is, R6G or adenine, and Ag particle, we realized only single blinking particle exists in a sampled area of ca. 30 μm × 30 μm. Scattering spectra using white light source (Xe lamp) were measured with the same spectrometer under SERS active (hot or blinking) or inactive (dark or invariant intensity) conditions.

Scattering cross section of the Ag particles with or without R6G adsorbates was evaluated with the FDTD method. To our knowledge, Maxwell equations are solved for spheres or ellipsoids with or without substrate,[7,13,14] while analytical solutions have not been obtained for other complicated structures such as triangular, tetrahedral, or touching particles with or without unsymmetrical protrusions. It is because isolation of variables in the differential equations is intrinsically difficult in these cases. Therefore, the numerical simulations, for example, the FDTD method, are valuable, which transfer the differential equations to difference equations. Then, the numerical simulations provide the electromagnetic field at given positions for any sample systems consisting of Ag nanoparticles with adsorbates and surrounding media, when irradiated by the electromagnetic wave. Relevance of the FDTD method in this field was confirmed by Krug et al.[15] for designing the near-field probe or by our prior work to explore the optimum metal nanostructure[13] with the highest electric field under the localized surface plasmon (LSP) resonance. In this paper, both two-dimensional (2D) and three-dimensional (3D) calculations were employed to evaluate the scattering cross section, far-field response, from Ag nanoparticles in the absence and presence of R6G adsorbates at various wavelengths using dielectric constants of constituents.[16,24]

Results and Discussion

As reported in our previous paper,[12,13] the following results were observed in addition to the literature (vide supra): (1) Identical blinking features in the SERS signal were observed for adenine that has no electronic absorption bands in the visible wavelength at a bulk state. It suggests that the sufficient enhancement is obtained within the framework of the electromagnetic mechanism, although detailed study by electron energy loss spectroscopy (EELS), extremely fast spectroscopy, or near-field spectroscopy is required to yield definite consequence if such molecules yield an additional electronic resonance, for example, exploiting a charge-transfer state, upon adsorption on the metal surfaces at a single molecule level. These spectra were sequentially measured with an accumulation time of 1 s to give sufficiently high signal-to-noise ratio with the CCD, while actual blinking frequency is several Hz, slightly faster than the accumulation time. Nevertheless, intensity and peak frequency fluctuations were explicitly observed, while prominent intermittent features were observed by Emory et al.[1] using avalanche photodiode with much faster response. (2) Temperature increase by the excitation laser is negligible and thus the fluctuation of the LSP resonance does not take place. In addition, laser trapping power is much lower than thermal energy at room temperature under our experimental condition.[12] Also, photochemical decomposition is neglected, since SERS spectra only from the samples, R6G or DNA base molecules, were observed through the measurement. No contaminants such as amorphous carbon, possibly formed by photochemical decomposition of organic molecules, were detected. From these observations, the blinking in SERS signal is not due to photochemical reaction or relaxation via a triple electronic state. Probably, there are various adsorption sites on Ag particles with different interactions and enhancement, where adsorbed molecules possibly diffuse. (3) On the basis of the local electric field evaluation exploiting the FDTD method, junctions of the touching metal particles yield vast enhancement of >10[10] only with the polarization parallel to the touching direction. In contrast, isolated cylinder, sphere, or ellipsoidal particles give only modest enhancement of 10[4]−10[5], similar to the ordinary sites on the touching particles that are apart from the junction by >ca. 5 nm. These results accord well with the experimental observations that only touching metal particles exhibit the blinking with the polarization parallel to the touching axis.[12,13] If adsorbed molecules thermally diffuse on the metal surfaces, it can be frozen by decreasing the sample temperature. (4) Actually, explicit temperature dependences were found. The blinking observed at RT was suppressed at 77 K so that invariant bright or dark spots were observed through the measurement. Only a part, that is, one-third to one-fourth, of the whole blinking particles were frozen, indicating average activation energy for the diffusion is still much smaller than thermal energy at 77 K. The temperature dependences are reversible, since the blinking recovered after warmed to RT. Thus, these observations strongly suggest the blinking results from thermal diffusion of adsorbed molecules, most probably between the junction and other ordinary sites on the Ag nanoparticles.

At this stage, it is critically important to get definite evidences that adsorbed molecules are sitting at the junction when vast enhancement in SERS is observed. To our best knowledge, however, there are no such analytical tools to characterize the location of individual molecules on metal particles. For instance, single molecule detection is not so difficult a task using fluorescence spectroscopy or EELS (electron energy loss spectroscopy). However, extremely high spatial resolution at a

Single Molecule Sensitivity

J. Phys. Chem. B, Vol. 108, No. 35, 2004 **13121**
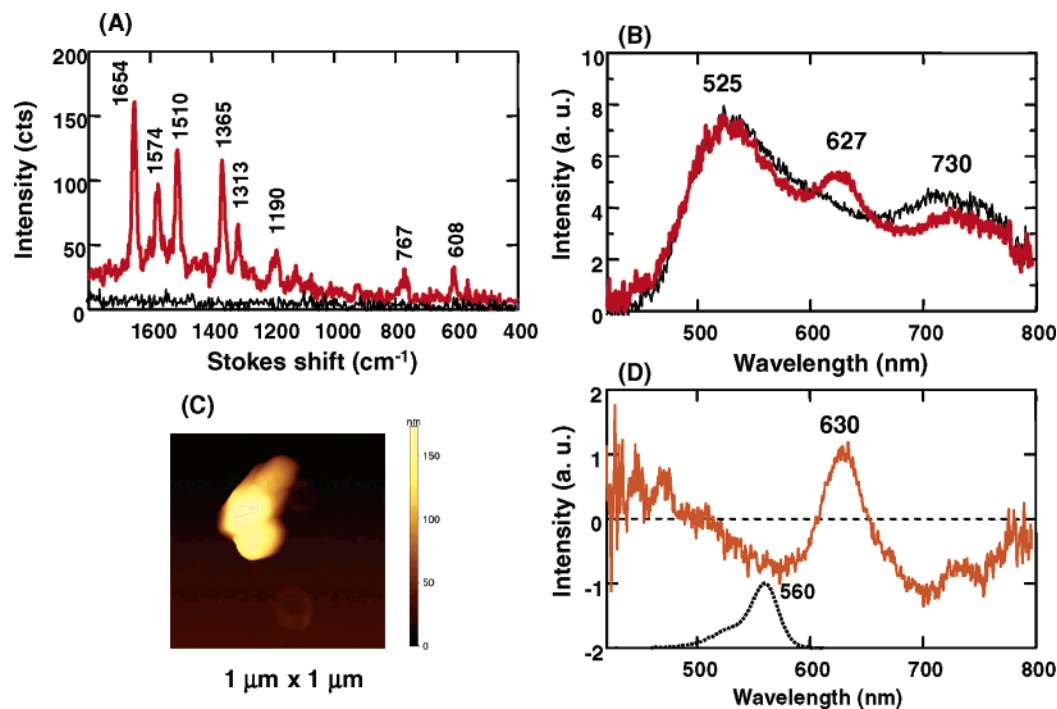
Figure 1. Topography and scattering spectra from the same hot Ag particles with a small amount of dye (30 molecules/Ag particle): (A) SERS spectra, (B) elastic scattering spectra, (C) differential scattering spectra in B between SERS active and inactive particles, and (D) AFM images of the hot particles. The bold (red) and thin (black) line show the spectra under SERS active and inactive states, respectively. The dotted line in D denotes the absorption of dye at bulk state.

single molecule level is prerequisite not only to obtain emission or Raman spectra but also to elucidate their position. Maybe STS (scanning tunneling spectroscopy) or near-field microscopy will be employed for this purpose because of their (potentially) excellent spatial resolution. However, it is not feasible even with these methods to approach the probes in the close vicinity of the junction of touching Ag particles because of geometrical hindrance of the probes and samples.

On this issue, critical evidences were obtained here to prove that individual molecules are located at the junction when enormous SERS signal is obtained on the basis of elastic scattering experiments together with the theoretical simulation.

(1) Experimental Results. It was found that the elastic scattering spectra from the touching particles provide marked differences according to the on/off feature of SERS activity. For example, the Ag particles consisting of a few ellipsoids as in the AFM image (Figure 1C) gave the elastic scattering peaks at 530 and 730 nm according to the LSP excitation. In addition, another peak appears at 625 nm when a tiny amount of dye molecules, for example, 30 molecules/Ag particle, adsorbs to give pronounced SERS signal as in Figure 1A and 1B. Actually, the enhancement factor observed for the Raman bands of R6G at 1654, 1574, 1510, or 1365 cm^{-1} was estimated to be 10^8–10^9 compared to the signal intensity at bulk solution state. Very interestingly, the scattering peak at 625 nm disappears when the SERS activity is lost because of diffusion or desorption of the dye molecules as depicted in Figure 1A, 1B, and 1C. Furthermore, intermediate scattering spectrum was observed for partially active state. These distinct elastic scattering spectra are inherently referred to the SERS activity, since similar features were observed for every hot or blinking particle according to cooling down of the activity. Likewise, the additional peak was observed at ca. 590 nm for another SERS-active particle (Figure 2), which extinguished after enough lengthy duration of measurement. In this case, slight changes of the peak position and intensity were observed at 475 nm as

well as at 700 nm, which arises from the coupling of the LSP at different particles. These features were confirmed for the blinking particles as indicated in Figure 3. Although two distinct LSP peaks were observed at ca. 475 and 630 nm according to rather complicate morphology of touching particles, significant spectral changes were observed at ca. 500–600 nm. Namely, a definite scattering peak that emerged at ca. 590 nm by the addition of R6G is extinguished when the SERS activity was lost as depicted in Figure 3A, 3B, and 3C. Experimental errors such as fluctuation of the signal intensity because of instable alignment of the optics are negligibly small, as confirmed for Ag particles at entirely inactive state (not shown). The additional peak in the elastic scattering spectra observed at the SERS active state probably arises from the electronic absorption of the dye molecules, locating at slightly shorter wavelength for thin film of R6G, that is, ca. 560 nm (main peak) and 530 nm (shoulder) (we will be back to this point). The LSP does not give such additional peaks unless quite formidable coalescence or morphology changes are induced by the dye adsorption. Since only a few molecules are attached and desorbed on each Ag particle that are immobilized on the glass substrate under ambient conditions, such enormous changes in morphology or aggregation state are not induced. Recently rather similar results were observed for Ag aggregates composed from several tens of Ag particles in water solution.[18]

Accordingly, we observed significant spectral variation in elastic scattering according to the on/off features of the enormous SERS activity. To get insight into the origin of the blinking and the vast enhancement in SERS, it is critically important to discuss why these spectral changes take place and how one can explain them. Concerning this, it was reported that the extinction spectra of Ag evaporated films change upon additional deposition of extremely thin dye film of <1 nm.[24] They qualitatively demonstrated the spectral changes on the basis of the effective medium theory. The electromagnetic coupling between the metal particles and dye molecules indeed
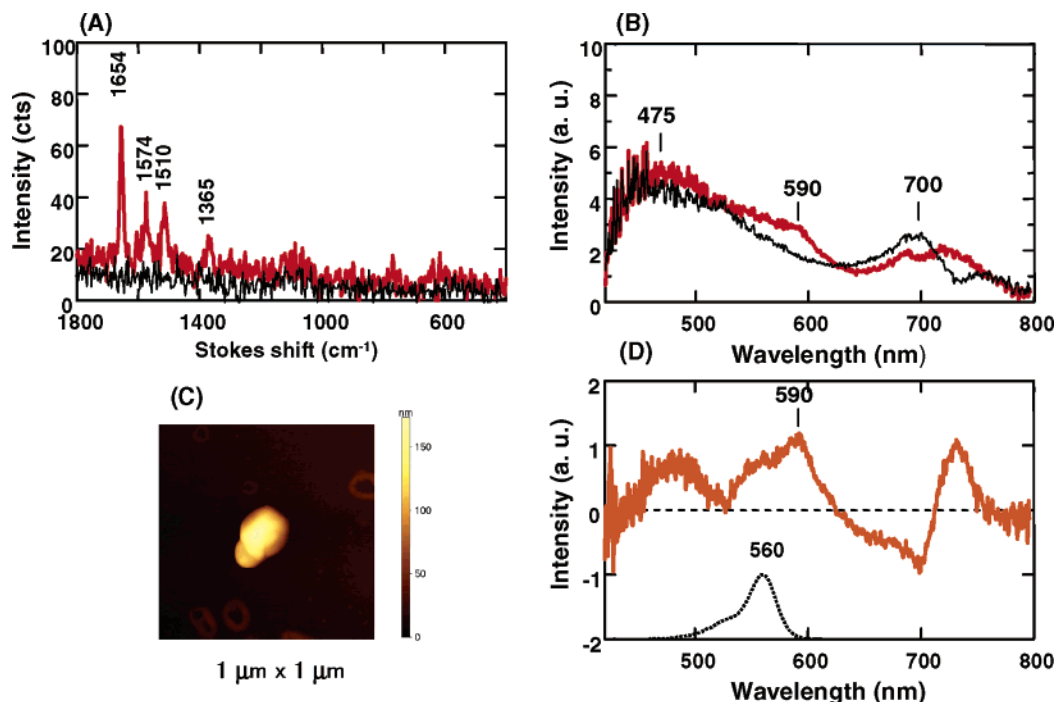
Figure 2. Topography and scattering spectra from the same hot Ag particles with a small amount of dye (30 molecules/Ag particle): (A) SERS spectra, (B) elastic scattering spectra, (C) differential scattering spectra in B between SERS active and inactive particles, and (D) AFM images of the hot particles. The bold (red) and thin (black) line show the spectra under SERS active and inactive states, respectively. The dotted line in D denotes the absorption of dye at bulk state.
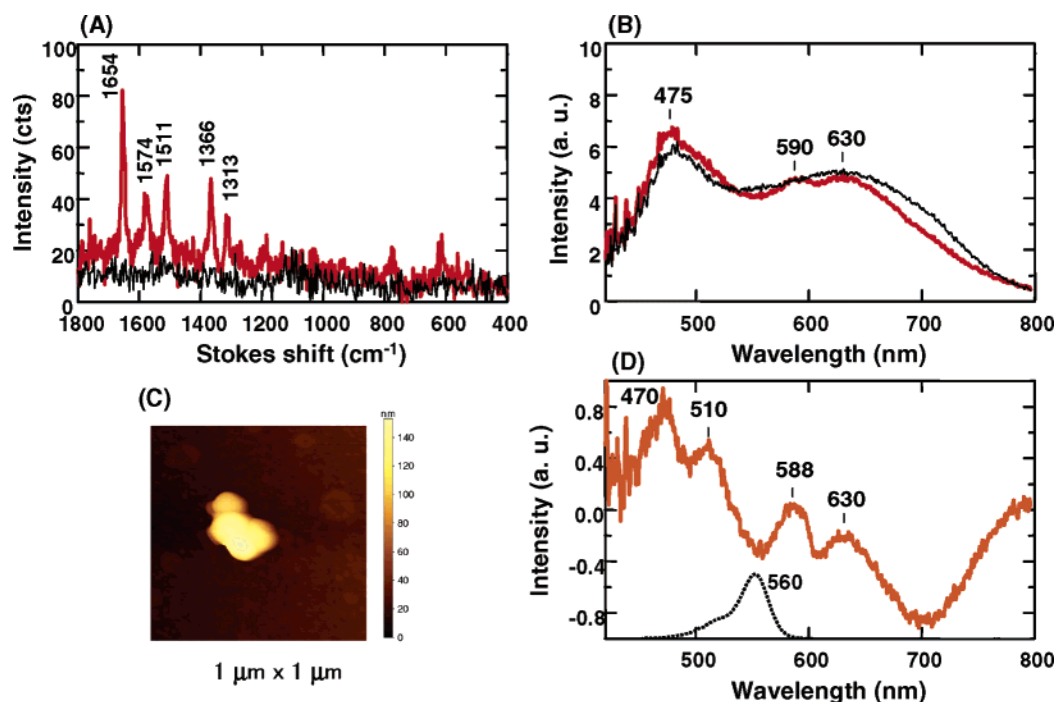
Figure 3. Topography and scattering spectra from the same blinking Ag particles with a tiny amount of dye (3 molecules/Ag particle): (A) SERS spectra, (B) elastic scattering spectra, (C) differential scattering spectra in B between SERS active and inactive particles, and (D) AFM images of the hot particles. The bold (red) and thin (black) line show the spectra under SERS active and inactive states, respectively. The dotted line in D denotes the absorption of dye at bulk state.

accounts for the apparent spectral changes via effective dielectric constants for the whole films, consisting of irregularly shaped Ag particles with various sizes and aggregation states.

(2) Numerical Simulations. The scattering cross section for nanoscale particles is given by the solution for Maxwell differential equations under particular boundary conditions. For example, a sphere covered with a cocentric spherical shell of different material has a polarizability, α, denoted by the next

equation,[19] which yields the induced dipole responsible for the elastic scattering.

$$\alpha = a^3 \frac{(\epsilon_2 - 1)(\epsilon_1 + 2\epsilon_2) + q^3(2\epsilon_2 + 1)(\epsilon_1 - \epsilon_2)}{(\epsilon_2 + 2)(\epsilon_1 + 2\epsilon_2) + q^3(2\epsilon_2 - 2)(\epsilon_1 - \epsilon_2)} \quad (1)$$

Here, ϵ_1, ϵ_2, and ϵ_3 (=1) are dielectric constants for the inner
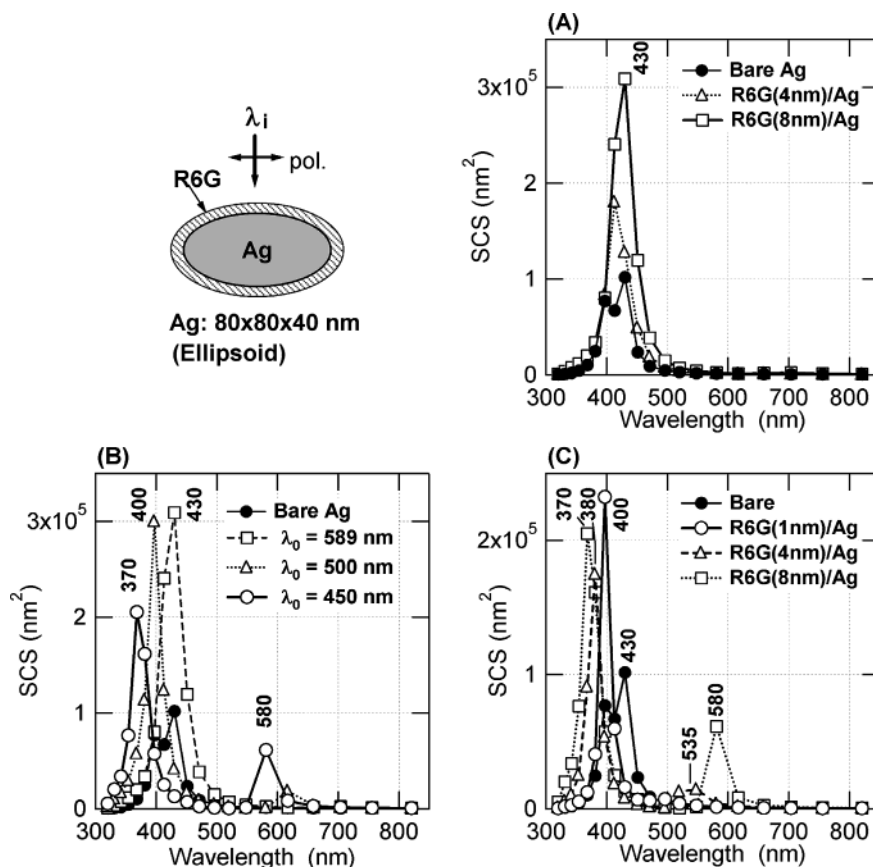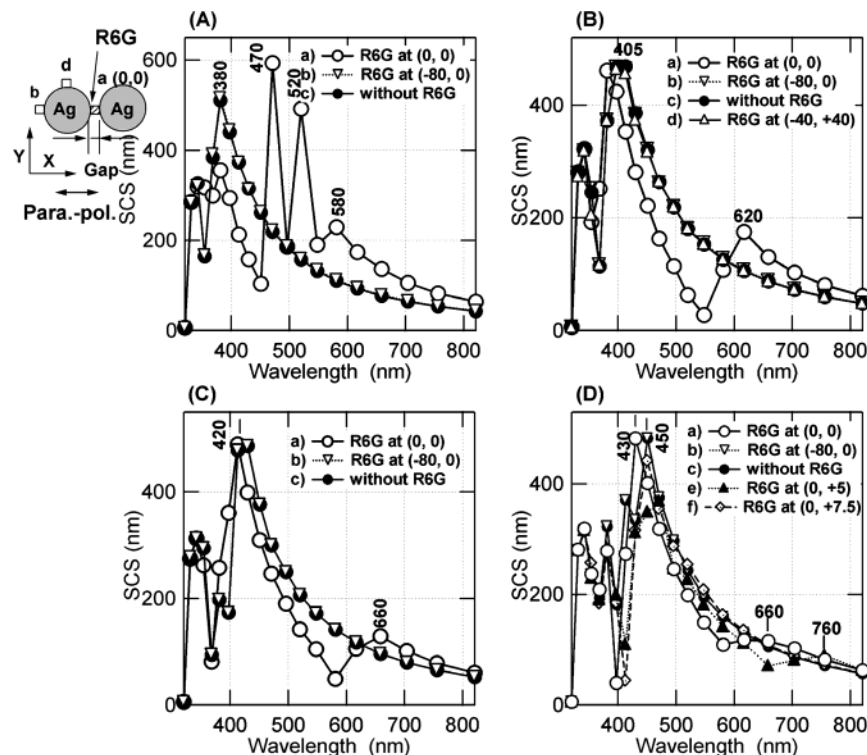
Figure 4. Elastic scattering spectra calculated for the Ag isolated ellipsoid (major/minor axes of 80/40 nm) with or without dye films using the FDTD-3D method: (A) for dye absorption peak (ω_{R6G}) at 589 nm with different thickness, (B) as a function of the peak wavelength of dye absorption from 589 to 450 nm, and (C) as a function of thickness of dye for the fixed absorption wavelength (450 nm).

sphere, shell, and outer media (air), respectively, while a and ($q \times a$) denote the radii of the outer cocentric shell and inner sphere. The scattering cross section is given as a ratio of the scattered to the incident light intensity. However, Maxwell equations have not been analytically solved for complicated structures, such as touching sphere with a small amount of dye with given shapes and sizes. The effective medium theory that describes averaged dielectric properties on the basis of the volume fraction of the constituents is conveniently utilized to yield optical response from composite or layered samples. Clearly, it is not relevant to evaluate the local electric field on the nanostructures as well as the scattering cross section from much complicated samples. In such cases, numerical simulation is valuable to provide the electromagnetic field intensity at any positions in the near field or the far field for given nanostructures as a response to the incident wave. Here, we adopted the FDTD method to evaluate the scattering cross section as well as the local electric field at various wavelengths. A small amount of the dye molecules are attached onto different sites on the isolated or touching Ag ellipsoid or spheres to feature the effect of adsorbed positions.

At first, the scattering spectra were computed for an isolated Ag particle in the absence and presence of adsorbed molecules using three-dimensional FDTD method (FDTD-3D). The Ag particle adopted is an isolated ellipsoid (oblate) with a semi-major/-minor axis length of 80/40 nm, and bulk dielectric properties. It gives the LSP extinction peak at 430 nm with the polarization parallel to the longer major axis. Rhodamine B molecule was adopted to evaluate the effect of dye adsorption. Dielectric constants of the dye were expressed using a dumped harmonic oscillator model, $\epsilon = 1 + S/(\omega_0^2 - \omega^2 - i\omega\Gamma\cdot)$, here

an oscillation strength (S) of 5×10^{31} sec^{-2}, damping (Γ) of 2.5×10^{14} sec^{-1}, and resonance wavelength (ω_0) of 3.2×10^{15} sec^{-1} ($\lambda_0 = 589$ nm).[24] For the isolated Ag particle, a detectable but extremely weak peak (<0.01 with respect to the LSP peak) appears at ca. 700 nm even for thick dye films (8 nm) in addition to the LSP peak (see Figure 4a). The peak wavelength is slightly longer than the absorption peak of dye at bulk state. Nevertheless, the additional peak originates from dye, since only the LSP along the semimajor axis is excited with the incident polarization as observed at 430 nm, and spectral features of the new peak are intrinsically dependent only on the absorption of dye, for example, peak wavelength or thickness (vide infra). Variable spectral features are not always surprising, since the scattering spectra are not a simple superposition of optical response of the individual silver and dye but rather complicate features as denoted in eq 1. For instance, the LSP peak intensity decreases to two-thirds of the original one, whereas the dye absorption increases by a factor of ca. 20 with decreasing the peak separation from 160 to 20 nm, by adjusting the absorption wavelength of dye. It gives pronounced peak shift for both bands, for example, 430 and 704 nm for $\Delta\omega = 160$ nm, or 370 and 580 nm for $\Delta\omega = 20$ nm as shown in Figure 4B. For the isolated Ag particles, quite a thick film of dye ($>$ca. 5 nm) is necessary to detect the spectral changes (peak height ratio of 1:0.07 for the LSP and dye absorption) even for the faint peak separation of 20 nm (Figure 4C). Thus, one cannot observe distinct spectral changes for thinner dye films, for example, 1 nm, since it gives extremely weak peak for the dye with a ratio of 1:0.025 compared to the LSP peak intensity even for very close separation ($\Delta\omega = 20$ nm) of these peaks. Such an additional scattering peak is obtained only if dye molecules are
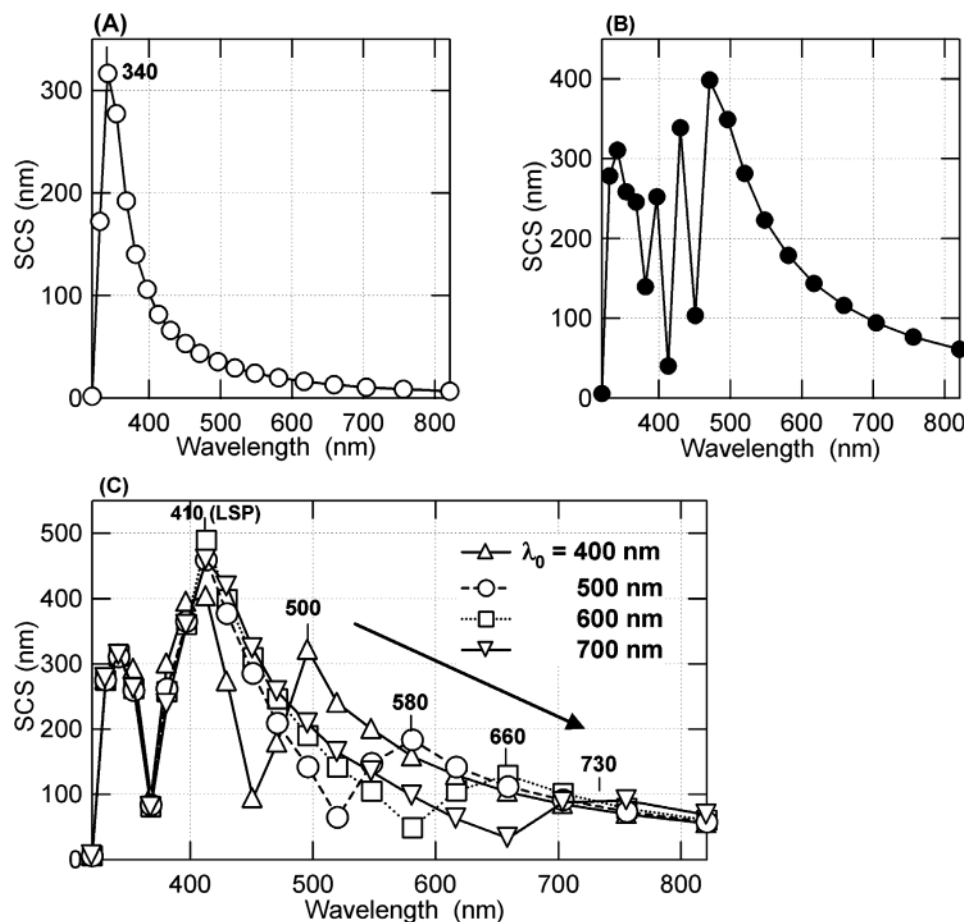
Figure 5. Elastic scattering spectra calculated for the Ag neighboring cylinders (diameter of 80 nm) for different gap sizes (g = 8 nm (A), 4 nm (B), 2 nm (C), and 1 nm (D)) using the FDTD-2D method. The dye molecules with a volume of $g \times g$ nm^2 are placed to fill the gap. Accordingly, the amount of dye decreases with decreasing the gap sizes. In addition, the effect of adsorbed position of dye was evaluated, e.g., left end position (denoted by the symbol of inversed triangle, b) in A−D, the top of the left particle in B, or at 5 nm and 7.5 nm apart from the gap center to vertical (*Y*) direction in D. The position of the dye molecules is denoted by the coordinate in A using a nanometer unit. Parallel polarization to the touching axis was used (see Figure 7 for perpendicular polarization).

sitting on Ag or other metal nanoparticles because of electromagnetic coupling of the LSP and absorption of dye. Actually, solely existing dye molecules or particles do not give any scattering peak. The essential points obtained here are summarized as follows: (1) the additional peak observed at 550−700 nm in the scattering spectra is essentially related to the absorption of dye and (2) much thicker dye ≫10 nm is required to reproduce the observed changes.

Next, the scattering spectra for two neighboring Ag particles were evaluated using a polarization parallel to the touching axis. In this case, two-dimensional (2D) simulation was used instead of three dimension to save the processing time, since 2D calculation gives essentially the same results for the polarization parallel to the cross section of the cylinder (circular base plane) as already confirmed.[13] Accordingly, Ag particles with cylindrical shape and diameter of 80 nm are adopted, while the amount of dye is restricted to fill the gap between the neighboring Ag particles, for example, 8×8 nm^2 for the gap size (g) of 8 nm as depicted in Figure 5A. The scattering spectrum from the adjacent Ag particles (g = 8 nm) without dye provides only a pronounced peak at ca. 380 nm corresponding to the LSP excitation, which is quite similar to that for an isolated Ag particle (see Figure 6A). However, remarkable and complicate features emerge when a small amount of dye is placed at the gap (8 nm) as shown in Figure 5A. The scattering spectra perturbed by the dye adsorption is allied closely to that observed for touching Ag particles (g = 0 nm) with the same size and shape but without dye (see Figure 6B). In fact, the touching Ag particles yield multiple peaks between ca. 320 and 500 nm because of efficient excitation of the LSP gap modes.[13,20] This is surprising because there is no efficient coupling of the LSP in each Ag particle at such a formidable gap size (8 nm) without dye. It indicates dye molecules located at the junction enor-

mously increase the coupling because of favorable electric conductivity (σ), for example, ca. 3.3×10^5 at 450 nm evaluated from the equation, $\sigma = \omega \, \epsilon_0 \, \epsilon''$, where ω, ϵ_0, and ϵ'' are frequency of the excitation light, free space dielectric constant, and imaginary part of dielectric constant in dye.[17] The absorption of dye, in other words imaginary part of dielectric constant, is crucial to yield such drastic spectral changes, since dielectric materials with fairly high refractive index without absorption like a glass material do not give any significant coupling, analogous to air in the gap (Figure 5A). Only a modest peak shift is anticipated even for a thick layer like 50 or 100 nm when the metal particles are encapsulated or soaked into the media with higher refractive index.[21,22] These discussions emphasize the importance of the junction, since the dye molecules at any other sites, for example, the left (or right) end of the left (or right) Ag particle, give exactly the same spectra observed for the bare Ag particles as depicted in Figure 5A−D. Clearly, these marginal positions do not increase the LSP coupling of neighboring Ag particles as prospected.

In contrast to the complicate features observed for thicker gap (8 nm, vide supra), smaller gap sizes that are filled by dye gave rather simple but still significant differences in the scattering spectra compared to the bare Ag particles. This is rationalized by increased coupling of the LSP for the neighboring particles at such gap sizes even without dye molecules.[13] For instance, dye molecules of 4×4 nm^2 on the Ag particles give the peak at ca. 620 nm additionally to the LSP scattering at ca. 405 nm. The peak wavelength markedly shifts to longer wavelength with decreasing the gap sizes, for example, $\lambda_0 =$ 620, 660, and 760 nm for the gap size of 4, 2, and 1 nm, respectively, whereas the LSP excitation shifts only slightly to longer wavelength, for example, from 405 nm (g = 4 nm) to 450 nm (g = 1 nm). More noticeable is that the additional peak
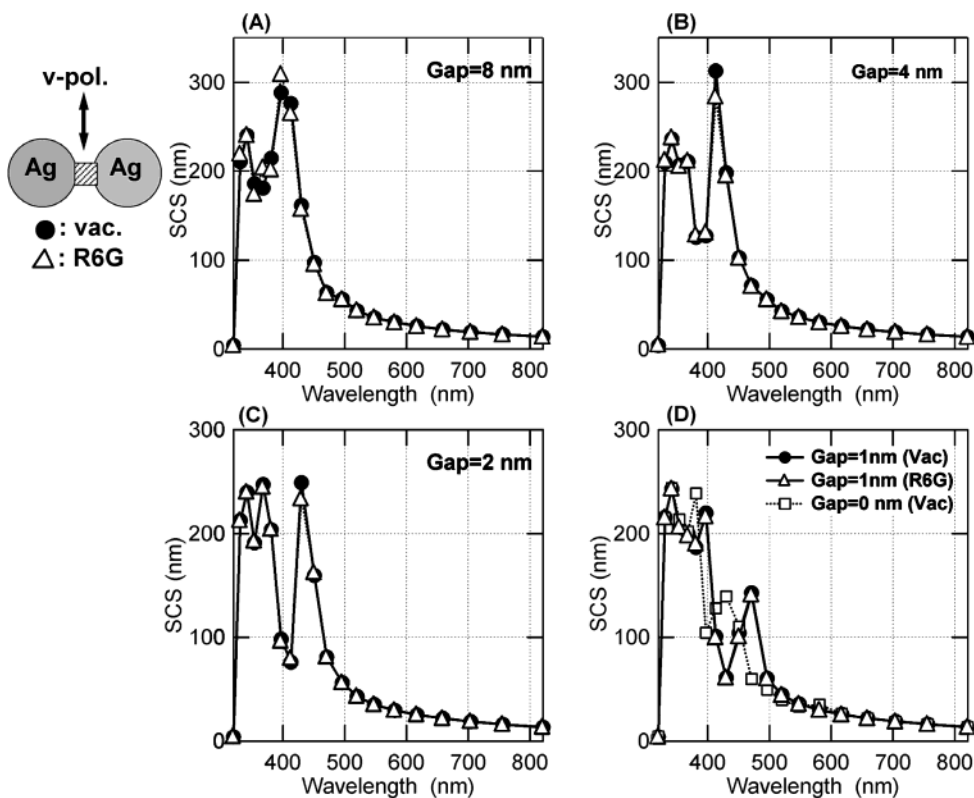
Figure 6. Elastic scattering spectra calculated for (A) isolated Ag particles with a diameter (φ) of 80 nm, (B) touching two Ag particles (φ80), (C) neighboring Ag particles (g = 2 nm) for various absorption peak wavelengths for dye. In Figure 6C, only the absorption peak position (λ₀) was tuned from 400 to 700 nm, while other parameters for the dye absorption spectra are the same as in Figure 5 (see also the text). Parallel polarization was used to the touching axis in Figure 6B and 6C.

position varies from 500 to 730 nm by tuning the absorption peak of dye from 400 to 700 nm as shown in Figure 6C in analogy with those for an isolated Ag particle. Conclusively, the additional peak position arises from the dye molecules at the junction. Even 1×1 nm^2 of dye molecules, approximately in accordance with the geometrical size of single R6G molecule, gives significant spectral changes as far as they are located at the junction. In addition, the spectral changes are sensitive to the dye position adjacent to the junctions. For instance, the upper or lower position apart from the center of the junction by ca. 5 nm gives the peak wavelength shift from 650 to 750 nm together with weakened peak intensity. Further distance by 2.5 nm gives the same spectra as that for the bare Ag particles. In other words, the additional peak at 650−750 nm is extinguished with the diffusion of molecules by 7.5 nm from the center of the gap, which can be used to monitor location of the adsorbates nearby the junction. These drastic changes together with the additional peak in the elastic scattering spectra were obtained only with parallel polarization to the touching axis. As shown in Figure 7 A−D, perpendicular polarization does not provide significant differences in the spectra. This is clearly due to faint coupling of the LSP for neighboring Ag particles with perpendicular polarization.[7,13,14] Accordingly, it is only when a dye with the absorption band close to the LSP peak is located at the junction that it gives an additional peak in elastic scattering spectra for parallel polarization to the touching axis. The validity of the two Ag particle models adopted in the simulation was confirmed by these polarization dependences as well as the fact that the blinking in SERS signal is a single molecule phenomenon.

Linearly aligned three or four Ag particles and two-dimensional array do not increase electric field at the gap as reported elsewhere.[13] Most probably, only one particular junction of touching Ag particles, consisting of three to four particles, observed in Figures 1−3 contributes to give significant changes in elastic scattering spectra and SERS spectra, which is aligned to the polarization direction of the incident light with the R6G molecule.

These results obtained for the scattering spectra correspond well with the SERS activity for the same Ag particles: (1) the vast enhancement in SERS was obtained only for Ag touching particles with the polarization parallel to the touching axis. (2) The blinking probably arises from thermal diffusion of adsorbed molecules between adsorption sites with distinct enhancement. (3) The vast electric field intensity on the Ag surfaces rapidly decreases with the distance from the gap center, for example, >10^{10} at the center or <10^{-4} at 5−7-nm separation. The additional peak at 550−700 nm is observed in the elastic scattering spectra only when the same sample gives the vast SERS activity. In contrast, exactly the same spectra were obtained for the inactive SERS particles as the bare Ag particles. Thus, we can conclude that the adsorption of dye molecules at the junction of touching Ag particles gives vast enhancement in SERS and additional scattering peak, whereas diffusion of adsorbed dye to ordinary (marginal) sites by >ca. 7.5 nm extinguishes the SERS activity and the additional peak.

As discussed here, the single molecule detection in SERS and the related phenomena such as the blinking or significant alternation in the scattering spectra are rationalized by the

Figure 7. Elastic scattering spectra for neighboring Ag particles with perpendicular polarization: (A) gap sizes of 8 nm, (B) 4 nm, (C) 2 nm, and (D) 1 nm. The symbols (●) and (△) are the results of the Ag particles without and with R6G at the gap, respectively. In contrast to the parallel polarization, negligible spectral changes were obtained by filling the gap with R6G because of faint LSP coupling of the Ag particles with this polarization.

enormous field enhancement due to the LSP resonance at the junction of touching metal particles. This is explicitly based on the so-called electromagnetic mechanism. Actually, our target is to explore the optimum nanostructures with single molecule sensitivity in SERS in the framework of SPP resonance. If it is possible, the electromagnetic enhancement is useful and convenient for various applications compared to the chemical enhancement, for example, using a charge-transfer resonance at specific sites on atomically roughened surfaces that is relevant only for particular adsorbates and metal surfaces. At this stage, it is useful to discuss about the possibility to get single molecule sensitivity (SMS) with the chemical enhancement. To our knowledge, there are two distinct approaches to explore the optimum metal nanostructures: (1) to find an appropriate particle with a fixed excitation wavelength[6−9] or (2) to find an optimum wavelength for particular metal particles.[5] The former group reported that only touching particles exhibit the vast enhancement on the basis of the AFM measurement and optical spectroscopy, while the latter accounted even isolated particles with particular sizes display the blinking or vast enhancement at different excitation wavelengths. For example, Doering and Nie ensured isolated gold ellipsoidal particles provide the blinking with particular sizes that are resonant with the excitation wavelength.[23] In this case, isolated Ag particles composed of spheres, ellipsoid, or cylinder should have only modest enhancement like 10^5 by means of the LSP resonance as described earlier.[7,13,14] Prominent enhancement can be obtained at sharp edge structures as in the triangular prism[13,14] in addition to chemical enhancement. Accordingly, they could increase the number of blinking particles by the addition of halide ions to the Ag particles immobilized onto the substrate, indicating increased effective sites for the chemical enhancement or extremely localized surface plasmon. Thus, for isolated metal particles, the chemical enhancement or nanoscale sharp edges are indispensable to yield SMS in SERS, although further conclusions await detailed nanoscale characterization on the surface morphology and electronic state. This issue is still obscure at present on account of insufficient spatial resolution in near-field spectroscopy. In contrast, the LSP coupling at the junction could provide the SMS in SERS for Ag touching particles irrespective of such additional enhancement.

Summary

Significant changes in elastic scattering spectra were observed during inactivation process of SERS: (1) an additional peak was observed at ca. 600 nm with a slightly longer wavelength than an absorption peak of dye only for hot or blinking particles, (2) this peak was extinguished by inactivation of SERS, and (3) with using FDTD calculation for isolated and neighboring Ag particles, the observed spectral changes were reproduced only if the dye is located at the junction. Such spectral changes result from the increased electromagnetic coupling of the localized surface plasmon (LSP) on neighboring Ag particles through dye molecules since (1) the peak position depends solely on the absorption peak wavelength of the dye and (2) the additional peak was obtained only with parallel polarization to the touching axis. The spectral changes essentially correlate with on/off features of the SERS activity, which is induced by thermal diffusion of individual molecules in and out of the junctions. Thus, we can conclude that adsorbates at the junction provide efficient LSP coupling and thus the enormous enhancement in SERS. Critical importance of the junction of Ag nanoparticles was confirmed concerning the origin of the blinking and mechanism of the enormous enhancement in SERS.

Single Molecule Sensitivity

J. Phys. Chem. B, Vol. 108, No. 35, 2004 **13127**

References and Notes

(1) Kim, Y.; Komeda, T.; Kawai, M. *Phys. Rev. Lett.* **2002**, *89*, 126104.

(2) Ushioda, S. *Solid State Commun.* **2001**, *117*, 159−166.

(3) Otto, A.; Mrozek, I.; Grabhorn, H.; Akemann, W. *J. Phys.: Condens. Matter* **1992**, *4*, 1143−1212.

(4) Kerker, M. *Surface Enhanced Raman Scattering*; SPIE, Vol. MS10, 1990.

(5) Krug, J. T.; Wang, G. D.; Emory, S. R.; Nie, S. *J. Am. Chem. Soc.* **1999**, *121*, 9208−9214.

(6) Kneipp, K.; Kneipp, H.; Itzkan, I.; Dasari, R. R.; Feld, M. S. *Chem. Rev.* **1999**, *99*, 2957−2975.

(7) Xu, H.; Aizpurua, J.; Käll, M.; Apell, P. *Phys. Rev. E* **2000**, *62*, 4318−4324.

(8) Michaels, M.; Nirmal, M.; Brus, L. E. *J. Phys. Chem. B* **2000**, *104*, 11965−11971.

(9) Maruyama, Y.; Ishikawa, M.; Futamata, M. *Chem. Lett.* **2001**, 834−835.

(10) Weiss, A.; Haran, G. *J. Phys. Chem. B* **2001**, *105*, 12348−12354.

(11) Bosnick, K. A.; Jiang, J.; Brus, L. E. *J. Phys. Chem. B* **2002**, *106*, 8096−8099.

(12) Maruyama, Y.; Ishikawa, M.; Futamata, M. *J. Phys. Chem. B* **2003**, *108*, 673−678.

(13) Futamata, M.; Maruyama, Y.; Ishikawa, M. *J. Phys. Chem. B* **2003**, *107*, 7607−7617.

(14) Kottmann, J. P.; Martin, O. J. F.; Smith, D. R.; Schultz, S. *Chem. Phys. Lett.* **2001**, *341*, 1−6.

(15) Krug, J. T., II; Sánchez, E. J.; Xie, X. S. *J. Chem. Phys.* **2003**, *116*, 10895−10901.

(16) Palik, E. D. *Handobook of Optical Constants of Solids*; Academic press: London, 1998; p 351.

(17) Kunz, K. S.; Luebbers, R. J. *The Finite Difference Time Domain Method for Electromagnetics*; CRC Press: Boca Raton, FL, 1992.

(18) Itoh, T.; Hashimoto, K.; Ikehata, A.; Ozaki, Y. *Appl. Phys. Lett.* **2003**, *83*, 5557−5560.

(19) Van de Hulst, H. C. *Light Scattering by Small Particles*; Dover: New York, 1981; p 74.

(20) Johansson, P. *Phys. Rev. B* **1998**, *58*, 10823−10834.

(21) Futamata, M.; Maruyama, Y.; Ishikawa, M unpublished.

(22) Itoh, K.; Asahi, T.; Masuhara, H. *Jpn. J. Appl. Phys.* **2002**, *41*, L72−L74.

(23) Doering, W. E.; Nie, S. *Anal. Chem.* **2003**, *75*, 6171−6176.

(24) Craighead, H. G.; Glass, A. M. *Opt. Lett.* **1981**, *6*, 248−250.