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Single-molecule surface-enhanced Raman spectroscopy of 4,4'-bipyridine on a prefabricated substrate with directionally arrayed gold nanoparticle dimers

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1 Single-molecule surface-enhanced Raman spectroscopy

- 2 of 4,4'-bipyridine on a prefabricated substrate with
- 3 directionally arrayed gold nanoparticle dimers
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9 In this study, single-molecule detection on a prefabricated substrate through surfaceenhanced Raman spectroscopy (SERS) with 4,4'-bipyridine molecules was achieved. The 10 11 use of a substrate with directionally arrayed gold nanoparticle dimers was proposed for the 12single-molecule detection and identification of a wide range of bio/chemical molecules. 13Around 50 Raman measurements and statistical analyses were performed to demonstrate a single-molecule SERS. At 10⁻¹¹ M, the distribution was fitted by three Gaussian curves, 14whereas the distribution of Raman intensities was fitted by one Gaussian curve at 10^{-5} M. 15The probability of molecule detection is consistent with the Poisson distribution. This result 1617indicates the possibility of detecting 0, 1, and 2 molecules. Thus, we confirmed that the 18 developed substrates achieved single-molecule SERS detection and identification.

20 **1. Introduction**

In recent years, a highly sensitive molecule detection of extremely low molecular 21concentration has been used for various fields such as medicine, biology, and environment.¹⁻ 22⁵⁾ Its medical and biology applications include the detection of biomolecules such as 23metabolites, proteins, and DNA bases. Its environmental application includes the chemical 2425detections of residual pesticides, explosive substances, and environmental detrimental substances. For these trace analyses, surface-enhanced Raman spectroscopy (SERS) has 26been expected because it has the potential to highly sensitively detect and identify molecules. 2728Raman spectroscopy is a powerful tool for molecular identification because a Raman 29spectrum includes molecular structural information. The Raman scattering light from a small number of molecules is significantly weak. In SERS, however, the Raman scattering light 30 31 can be enhanced by plasmonic resonance, which is generated on metal nanostructure surfaces.^{1,6)} Therefore, SERS analysis enables us to perform the high-sensitivity rapid 32detection and reliable identification of bio/chemical molecules without labeling, including 33 single-molecule detection and identification.⁷⁻¹⁰ 34

As a metal nanostructure for SERS on a substrate, numerous reports have been reported thus far. Most of the reported structures have been fabricated by self-organization processes. The fabrication procedures used are categorized into two methods, namely, (i) in-liquid formation⁷⁻¹⁰⁾ and (ii) on-substrate fabrication.^{4,11-13)}

39 In the formation method (i), an analyte solution and a colloidal nanoparticle solution are mixed so that particles form particle dimers or agglomerates in liquid with a molecular 40 bridge between particles. The particles gap is less than 1 nm. This nanogap results in a 41 42marked Raman enhancement because it exponentially increases as the nanogap decreases. The mixed solution is used for in-liquid SERS measurement, or is placed and dried on a 4344substrate followed by on-substrate SERS measurement. In this manner, single-molecule detection has been reported using the dimer configuration obtained when the polarization 45direction of an incident light is matched to a particle-particle connection direction.^{9,10} Many 46 theoretical studies have supported this polarization-dependent Raman enhancement.^{14,15} In 4748this method, the following problems emerge depending on the applications. One problem is that long-time incubation is required after mixing the analyte and colloidal solutions for 49 50dimer or agglomerate formation. Therefore, the SERS substrate obtained by on-substrate

fabrication (ii) is suitable for practical applications of single-molecule SERS. Another problem is that the connection direction cannot be controlled on a substrate for on-substrate SERS measurement.^{10,14)} Thus, it is necessary to adjust the polarization direction to the connection direction of particles after a scanning electron microscopy (SEM) or an atomic force microscopy (AFM) observation for each SERS structure.

For a prefabricated SERS substrate obtained by the formation method (ii), self-organized 56methods such as particle aggregation,^{4,11} carbon nanotube (CNT) aggregation,¹² and 57nanoporous fabrication¹³⁾ on a substrate have been reported. The structures involved in these 58method include numerous nanogaps called hotspots. The polarization-dependent property of 59Raman enhancement, however, has been unconcerned. Structures with a large effect on the 60 61 Raman enhancement factor are randomly formed, and the induction probability of the 62 marked enhancement is extremely low. Therefore, the self-organized structures result in sensitivity and reliability limitations especially for low-concentration trace detection and 63 single-molecule analysis. 64

In this study, we propose the SERS structure shown in Fig. 1, which shows a high 65 sensitivity for single-molecule SERS analysis. The SERS structure consists of gold 66 nanoparticle dimers that are directionally arrayed on a substrate for a marked 67 electromagnetic enhancement. It is fabricated by nanotrench-guided self-assembly with a 68 high yield.¹⁶⁻¹⁸⁾ It enables us to utilize the effect of polarization on the enhancement for 69 single-molecule SERS. Although electron beam (EB) lithography or focused ion beam (FIB) 70 71has been used for fabricating orderly nanostructures, it cannot be used for fabricating a nanogap of around 1 nm, which shows a marked electromagnetic enhancement. 72

In this paper, the method of fabricating the proposed substrate and single-molecule detection using 4,4'-bipyridine molecules by the statistical analysis of Raman intensities are reported. As a SERS probe for single-molecule SERS studies, probe molecules with large Raman cross sections, such as rhodamine 6G (RH6G) and crystal violet (CV) have been used.¹⁹⁾ In this study 4,4'-bipyridine molecules were used; these molecules have been introduced as molecules with small Raman cross sections in the literature.^{20,21)}

79

80 **2. Experimental and analytical methods**

81 2.1 Proposed structure

82 We proposed the use of directionally and regularly arrayed gold nanoparticle dimers as the 83 SERS substrate shown in Fig. 1. In this study, gold nanoparticles with mean particle diameters of approximately 100 nm were arrayed on a Si substrate by a nanotrench-guided 84 self-assembly. The effect of SERS on particle diameter has been investigated.^{22,23)} A 85 common finding is that the Raman intensity increased with particle diameter up to 100 nm, 86 as indicated in Refs. 22 and 23. Although the quadrupole mode appears for larger particles, 87 88 the dipole mode is dominant for 100 nm particles. The nanoparticles are arranged along the 89 template nanotrenches so that the connection direction of the arranged particles is easily 90 matched to the polarization direction of the incident light without SEM and AFM 91observations.

92 2.2 Fabrication method

The nanotrench-guided self-assembly used in this study is shown in Fig. 2. A colloidal 93 nanoparticle solution was injected between a cover glass and a template substrate of Si with 9495an array of nanotrenches fabricated by EB lithography and dry etching. On drying the aqueous particle dispersion between the substrates, the water surface line moved backward 96 97 and the particles became concentrated near the edge of the meniscus. The drag force pressed the particles onto the template substrate. When the meniscus passed over the templates, the 98 99 particles were trapped on the template nanotrenches. Then, the water-bridge force acted on the trapped particles during drying, connecting the particles to each other. 100

101 The gold nanoparticles were synthesized by a citrate reduction method. The synthesized 102 particles showed negatively charged surfaces because acetonedicarboxylic acid and 103 acetoacetictic acid molecules uniformly formed and attached to particle surfaces without 104 vacancy during synthesis.^{24,25} Nanoparticle aggregation was minimized by electrostatic 105 repulsion between the nanoparticles.

During the removal of the remaining water between the particles, the particles attracted each other and formed particle–particle contacts, which acted as hotspots. A nanogap of around 1 nm formed between the nanoparticles because the molecules attached to the surfaces acted as spacers. The fabricated structures were used for SERS measurements after removing the attached molecules by UV/O₃ treatment.

Figure 3 shows the SEM images of the fabricated nanostructures. We observed that gold nanoparticles were arranged onto the nanotrench template and gold nanoparticle dimers were

arrayed directionally and regularly. Although the length of the nanotrench was 260 nm, two

114 particles with a mean dimeter of 100 nm were connected by water bridge force during the

- 115 drying process.
- 116 2.3 Simulated electromagnetic enhancement

We performed an electromagnetic simulation using the commercially available finite-117 difference time-domain (FDTD) simulation software. The simulation was carried out using 118 119 a three-dimensional model with 400 and 300 nm lengths for the x- and y-axes, respectively. 120A gold nanoparticle dimer was placed at the center of the x- and y-axes on a Si substrate. The diameter and gap between particles were 100 and 1 nm, respectively. We used CRC and 121122Palik data for the refractive indices of Au and Si, respectively, which are available in the 123software. Periodic boundary conditions along the x- and y-axes, and perfectly matched layers 124(PML) along the z-axis were used. A plane wave source was set with a wavelength range of 400-800 nm. A 0.2 nm mesh size was used for the nanogap area. We obtained 125electromagnetic field enhancement factors $|E|^2$ at a nanogap. 126

Figure 4(a) shows the simulated spectra of the electromagnetic field enhancement factor 127128as a function of polarization angle at the hotspot of two particles. The enhancement factor 129decreased with increasing the polarization angle. The proposed structure was observed to be 130suitable for SERS with the 632.8 nm laser used in this study. Figure 4(b) shows the contour plots of electromagnetic field enhancement. The incident light was localized at a nanogap 131132between particles in both cases of polarization angles of 0 and 90 degrees in the simulation. The electromagnetic field enhancement factors at the nanogap for the polarization angles of 1330 and 90 degrees were around 1.1×10^2 and 3.5×10^5 times at the incident light wavelength of 134632.8 nm, respectively, indicating a ratio of around 3.3×10^3 times. This result indicates that 135directionally arrayed dimer structures are expected to induce a marked Raman enhancement 136 137 compared with randomly arranged structures.

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3. Results and discussion

A 632.8 nm laser-equipped micro-Raman spectrometer was used in this study. As a target
molecule of detection, 4,4'-bipyridine was used, which is a pesticide material.

We performed around 50 Raman measurements for each measurement time (1 or 0.05 s)

143 and molecule concentration (10^{-5} or 10^{-11} M). Figure 5 shows some examples of the obtained

spectra. Dotted lines indicate the Raman shifts derived from 4,4'-bipyridine.²⁶⁾ Then, statistical analysis was performed as shown in Fig. 6. The relative Raman intensities were calculated using the average Raman intensity for each condition. All spectra were discriminated on the basis of the presence (red column) or absence (blue column) of a peak at around 1609 cm⁻¹ that corresponded to the Raman shift of the target molecule.

At a 10^{-5} M concentration, all spectra exhibited clear peaks in both cases of measurement 149times (1 and 0.05 s). The distribution of Raman intensities was fitted by one Gaussian curve 150as shown in Figs. 6(a-1) and 6(a-2). At a 10^{-11} M concentration, the spectra with and without 151Raman peaks were observed as shown in Fig. 5. The experimental data at 1 and 0.05 s were 152fitted by three and two Gaussian curves, respectively as shown in Figs. 6(b-1) and 6(b-2). 153Since the first frequency peak in Figs. 6(b-1) and 6(b-2) comprises a data set without Raman 154peaks (blue columns), it is treated as a background intensity. It is considered that the second 155156and third peaks in Fig. 6(b-2) correspond to the data sets of 1 and 2 molecules detection, respectively, for the measurement time of 1 s. The average relative intensities of 0, 1, and 2 157molecules were 0.89, 1.06, and 1.28 at 1 s, respectively. The net relative intensity of 2 158molecules was calculated to be 1.28-0.89=0.39, which is 2.3 times as high as that of 1 159160 molecule (1.06-0.89=0.17).

161 Then, the Poisson distribution was calculated for each measurement time as shown in 162Table I. The experimental statistical distribution was consistent with the Poisson distribution. 163At the detected molecular number of 3 at the integration time of 1 s, the calculated frequency 164in the Poisson distribution was 0.7. The experimental frequency of 0 was relevant. The experimental frequencies at 0.05 s were also relevant to the detected numbers of 2 and 3. 165The statistical distribution at 10^{-11} M was explained by the Poisson distribution. The 166frequency of the second peak decreased with decreasing measurement time from 1 to 0.05 s 167168 as shown in Figs. 6(b-1) and 6(b-2). These results indicate that a single molecule was stochastically detected. 169

We calculated the Raman enhancement factor. The experimental Raman enhancement factor was calculated as $EF = (I_{SERS}/C_{SERS})/(I_{non-SERS}/C_{non-SERS})$. Here, *I* and *C* are Raman intensities per integration time and concentration, respectively. The subscripts *SERS* and *non-SERS* indicate the presence and absence of nanostructures, respectively. The measured Raman intensity without nanostructures was 2.0 counts/s at the concentration of 175 10^{-2} M and the integration time of 100 s for the Raman shift of 1609 cm⁻¹. The Raman 176 enhancement factors calculated from the experimental results were 9.0×10^{11} and 1.0×10^{12} at 177 the integration times of 1 and 0.05 s, respectively.

The simulated Raman enhancement factor was 6.3×10^{10} at 1609 cm⁻¹, calculated by 178 $|E_I|^2 \times |E_R|^2$ according to the spectrum of the electromagnetic enhancement shown in Fig. 1794. Here, $|E_I|^2$ and $|E_R|^2$ are the electromagnetic enhancement factors at the wavelengths 180 of the incident light (632.8 nm) and Raman scattering light (704 nm corresponding to 1609 181 cm⁻¹), respectively. Note that the experimental Raman enhancement includes the chemical 182enhancement due to charge transfer between a molecule and a gold surface in addition to the 183 electromagnetic enhancement. It has been reported to be 10–100.²⁷⁻³¹) Therefore, the total 184 Raman enhancement is 6.3×10^{11} - 6.3×10^{12} in the calculation. The Raman enhancement 185factors calculated from the experimental results are consistent with the simulation result. 186

The bare Raman cross sections of RH6G and CV have been reported to be on the order 187 of 10^{-26} – 10^{-27} cm²/sr.¹⁹) For a pyridine molecule, the bare Raman cross section is on the 188 order of 10^{-29} cm²/sr.²⁹⁻³¹) The chemical enhancement factors of both molecules are on the 189 same order.³²⁾ Therefore, the fabricated SERS structure in this study was proven to show a 190 marked Raman enhancement. The minimum Raman cross section of bio/chemical 191substances and the actual cross section necessary to single-molecule SERS are on the orders 192of 10^{-30} and 10^{-20} cm²/sr, respectively.¹⁹⁾ Therefore, the total Raman enhancement factor of 193 1.0×10^{12} obtained in this study is considerably sufficient for the single-molecule SERS of a 194 wide variety of bio/chemical substances. 195

196

197 **4. Conclusions**

In this study, we fabricated directionally arrayed gold nanoparticle dimers in order to 198 achieve a marked Raman enhancement, and evaluated the structures experimentally for 199 single-molecule SERS using 4,4'-bipyridine molecules. The dimer array was fabricated on a 200 Si substrate by the nanotrench-guided self-assembly of 100-nm-diameter gold nanoparticles. 201We confirmed the high-yield arrangement of the particle dimers with hotspots in one 202direction. The fabricated structure showed a high sensitivity with a 10^{-11} M and 0.05 s limit 203of detection. Then, around 50 Raman measurements and statistical analyses were performed. 204At 10^{-5} M, the distribution of Raman intensities was fitted by one Gaussian curve. At 10^{-11} 205

M, the distribution was fitted by three Gaussian curves. This distribution is consistent with the Poisson distribution. This indicates the probability of detecting 0, 1, and 2 molecules. From these results, we confirmed that the developed substrates achieved single-molecule SERS detection and identification. The calculated Raman enhancement factor was 1.0×10^{12} , which is consistent with the estimation result. The Raman enhancement factor is thought to be sufficient for the single-molecule detection of a wide variety of bio/chemical molecules.

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Figure Captions

- Fig. 1. Overview of proposed and developed SERS substrate with directionally arrayed dimers, which is prefabricated by nanotrench-guided self-assembly.
- 277

Fig. 2. Nanotrench-guided self-assembly. As the colloidal gold solution (mean diameter: 100 nm) is drying, the meniscus moves backward and then particles are trapped by interfacial force onto nanotrenches.

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Fig. 3. SEM image of directionally and regularly arrayed gold nanoparticle dimers in (a) 5 $\times 5 \ \mu m^2$ whole and (b) magnified areas.

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Fig. 4. (Color online) FDTD simulation results for electromagnetic enhancement factor depending on polarization angle θ to the connection direction of two particles. The particle diameter and the gap between the particles were set to 100 and 1 nm, respectively. (a) Spectra show the enhancement factor at a hotspot. The dashed line indicates the wavelength of the incident light used in this study. (b) Contour plot of electromagnetic enhancement factor $|E|^2$ at the polarization angles of 0 and 90° at the wavelength of 632.8 nm.

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Fig. 5. (Color online) Raman spectra at molecular concentrations of 10^{-5} and 10^{-11} M with and without peaks. Measurement times of (a) 0.05 and (b) 1 s were used. Dotted lines indicate 4,4'-bipyridine-derived Raman peaks.

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Fig. 6. (Color online) Statistical analysis of around 50 SERS measurements. The blue and red columns indicate frequencies without and with a peak at around 1609 cm⁻¹, respectively. The molecular concentration and measurement time are (a-1) 10^{-5} M and 1 s, (a-2) 10^{-5} M and 0.05 s, (b-1) 10^{-11} M and 1 s, and (b-2) 10^{-11} M and 0.05 s, respectively. The data are fitted by Gaussian curves.

301

Table I. Calculated Poisson distributions and experimental frequencies for the measurement times of (a) 1 and (b) 0.05 s at the molecular concentration of 10^{-11} M.



Fig.1. (Color online)



Fig.2. (Color online)



1 µm

Fig.3.



Fig.4. (Color online)



Fig.5. (Color online)



Fig.6. (Color online)

(a)			(b)		
Detected molecular No.	Poisson distribution	Experimental frequency	Detected molecular No.	Poisson distribution	Experimental frequency
0	28.8	29	0	56.6	56
1	15.3	14	1	7.8	9
2	4.0	6	2	0.5	0
3	0.7	0	3	0.03	0

Table I. Calculated Poisson distributions and experimental frequencies for the measurement times of (a) 1 and (b) 0.05 s at the molecular concentration of 10^{-11} M.