SURFACE-ENHANCED RAMAN SPECTROSCOPY ANALYSIS OF DNA BASES USING ARRAYED AND SINGLE DIMER OF GOLD NANOPARTICLE

Kohei Ikegami, Koji Sugano, and Yoshitada Isono
Department of Mechanical Engineering, Kobe University, Kobe, JAPAN

ABSTRACT

This paper reports an ultrasensitive surface-enhanced Raman spectroscopy (SERS) for detecting and identifying four kinds of DNA bases: adenine, cytosine, thymine, and guanine using a gold nanoparticle dimer. The gold nanoparticle dimer was directionally arrayed on a substrate in order to achieve huge enhancement according to the polarization-dependent enhancement. 10^{-11} M limit of detection for four DNA bases and 0.1 s rapid detection for adenine were achieved. Additionally, a separated single dimer was fabricated and evaluated. We clarified single-molecule-level SERS detection and identification was possible using the locally enhanced single hotspot of the single dimer with the 1-nm nanogap.

INTRODUCTION

The determination of the nucleobase sequence of DNA is of great importance. A surface-enhanced Raman spectroscopy (SERS) [1] has been expected for the identification of individual DNA bases because it is high sensitivity, high identification capability, and label-free method. Single molecule detection is possible because the extraordinary Raman enhancement can be obtained from nanogap between metal nanostructures, less than 1 nm, so called hot spot, adjusting a polarization direction of an incident light [1]. Electron-beam lithography-based process cannot fabricate such narrow nanogaps [2]. Therefore, various nanogaps using self-organization process have been reported so far, which are constructed with nanoparticles, nanoporous and so on. A nanoparticle dimer have been frequently used as the nanostructure which achieves the highest Raman enhancement at particle–particle contact when the connection direction of the particles is matched to the polarization direction of the incident light [3]. However, the particles form random geometry in random directions on a substrate in the conventional studies. Those structure has insufficient enhancement for single molecule detection. Therefore concentration limit of detection for four DNA bases has been reported to be 10^{-8} M [4]. We proposed the gold nanoparticle dimer array, as shown in Fig. 1(a), which has been reported in MEMS2015 [5] for highly sensitive detection of a pesticide material. This configuration allows for the straightforward adjustment of the polarization direction to the particle connection direction, resulting in a huge Raman enhancement that can be obtained with all dimers. The enhancement occurs locally at the 1-nm nanogap between particles according to the simulation result as shown in Fig. 1(b). In this study, we applied the regularly and directionally arrayed gold nanoparticle dimers to DNA bases detection. All dimers axes are matched to a polarization direction. Furthermore the single dimer was developed and evaluated.

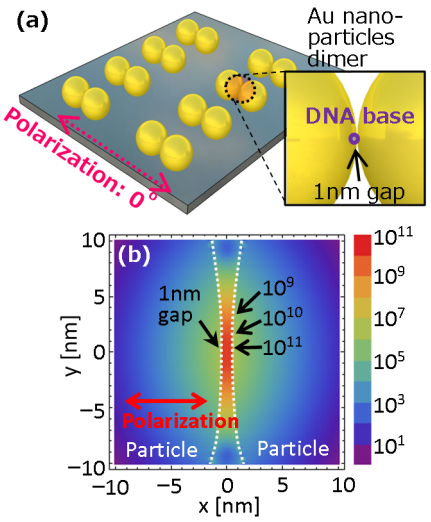

Figure 1: Gold nanoparticle dimer. (a) Schematic of a gold nanoparticle dimer array on a Si substrate fabricated by a nanotrench-guided self-assembly. (b) Simulated contour plot of Raman enhancement around a nanogap.

EXPERIMENTAL

Fabrication of Arrayed and Single Nanoparticle Dimer

The proposed structure was fabricated according to the self-assembly process shown in Fig. 2. Gold nanoparticles were arranged onto nanotrenches using nanotrench-guided self-assembly [6-8]. Colloidal gold nanoparticles of mean diameter 100 nm dispersed in water were used. A gold solution was introduced into a Si substrate with the fabricated nanotrenches and a glass substrate. Drying the aqueous dispersion between the substrates causes the water surface line to move backward, and the particles are concentrated near the meniscus edge. The interfacial forces drag and press the particles onto the Si substrate. When the meniscus passes over the nanotrenches, the particles are trapped on them. Then water bridges form between the

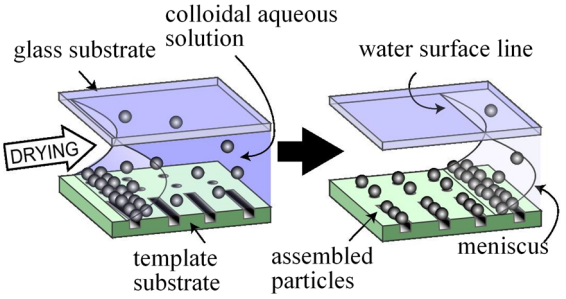

Figure 2: Experimental method for nanoparticle dimer arrangement using a nanotrench-guided self-assembly.

trapped particles. During removal of the remaining water between the particles, the particles attract each other and form particle–particle contacts, which act as hot spots. The colloidal particles used in this study were synthesized by a citrate reduction method. This synthesis method forms a uniform layer of adsorbed molecules on particle surfaces. The adsorbed molecules on the surfaces are citrate and its decomposition such as actonedicarboxylic acid and acetoacetic acid [9,10]. During the drying suspension between particles, the adsorbed molecule layer acts as a spacer between particles, forming nanogap between particles. The thickness of the citrate groups is around 0.5 nm [11]. Therefore around 1 nm nanogap forms between particles.

The nanotrenches are fabricated on a Si substrate by EB lithography and subsequent Si dry etching. The nanotrench array was designed in order to fabricate the particle dimer array as shown in Fig. 3(a). A center-to-center distance of dimers (trenches) is set to 400 nm and 200 nm in longitudinal (x axis) and transverse (y axis) directions of dimers. The nanotrenches are arranged in 5 μm × 5 μm region. The single dimers were arranged with 25-μm interval in the x and y directions owing to no interference as shown in Fig. 3(b).

Raman Spectroscopy Experiments

Raman spectra were obtained using a micro-Raman spectrometer equipped with a 632.8 nm wavelength laser with a 2 μm beam spot. For the dimer array we evaluated limits of molecular concentration and measurement time of detection. The laser spot was located at the center of the array region 5 μm × 5 μm. Before the SERS experiments for DNA bases, ultraviolet (UV)/O_3 treatment was performed to remove the adsorbed molecules. We confirmed that this treatment was able to remove the molecules. For the single dimer the adsorbed molecules on the particles was used for a mapping measurement and an evaluation of a polarization angle effect. For the mapping measurement, the interval was set to 1 μm. At the all SERS measurements, a polarization direction was adjusted to the parallel to the particle connection direction.

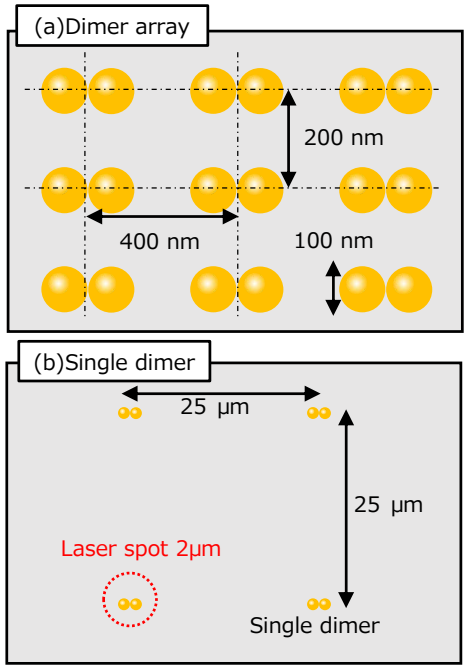

Figure 3: Schematics of arrangement. (a) a gold nanoparticle dimer array and (b) a single gold nanoparticle dimer on a Si substrate.

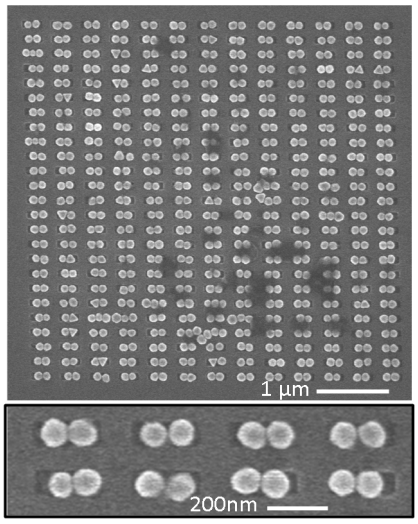

Figure 4: SEM images of gold nanoparticle dimer array. (a) 5 μm×5 μm area, (b) magnified image.

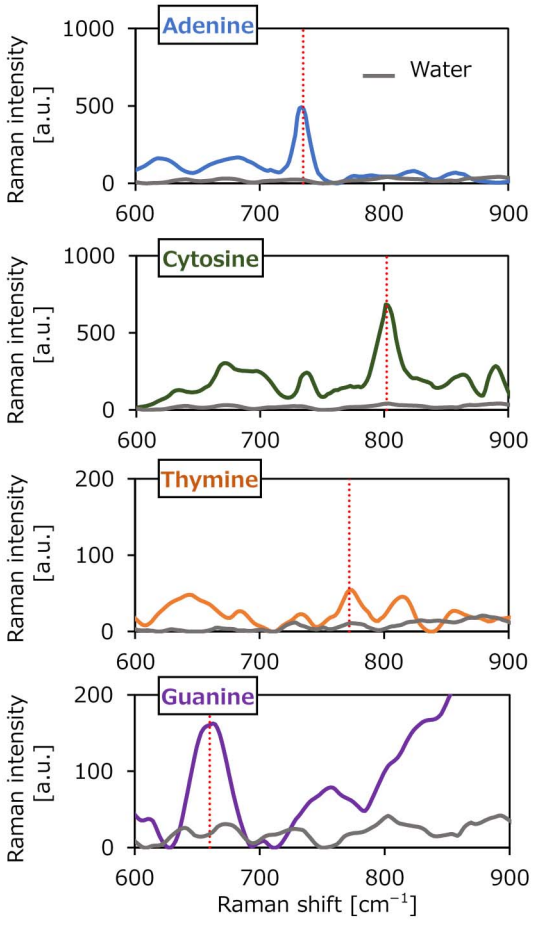

Figure 5: Raman spectra of four DNA bases. Red dotted and gray lines indicate Raman peaks of each DNA base and Raman spectra of pure water, respectively.

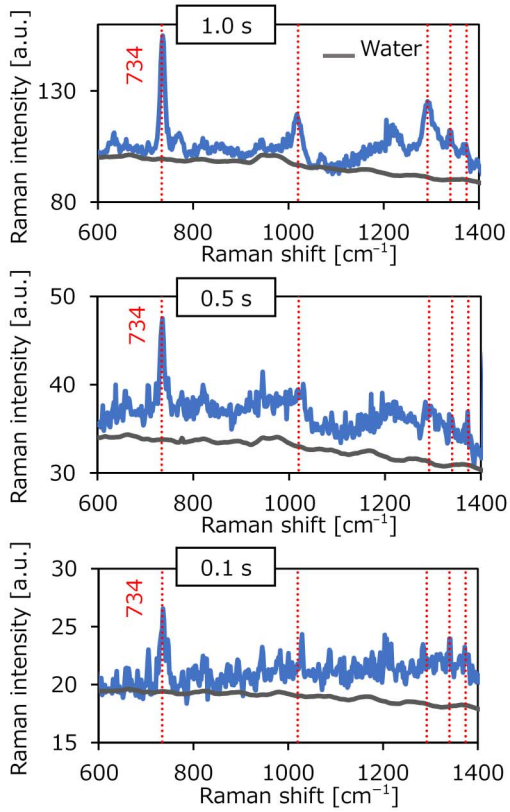

Figure 6: Raman spectra of Adenine at each measurement time. Red dotted and gray lines indicate Raman peaks of adenine and Raman spectra of pure water, respectively.

RESULTS AND DISCUSSION

Arrayed Dimers

Figure 4 shows the fabricated dimer array. All dimer s were arranged in one direction. Our previous study confirmed the Raman intensity depended on the polarization angle [5]. Figure 5 shows the Raman spectra from four kinds of DNA bases. Raman peaks from adenine, cytosine, thymine, and guanine were clearly detected at Raman shifts of 734, 801, 772, and 660 cm^{-1}, respectively, at 10^{-11} M concentration comparing with water spectra. These Raman peaks are assigned to ring breathing, which shows the strongest peaks [12]. The minimum concentration reported so far was 10^{-8} M [4]; the developed substrate shows 1000-times sensitivity. Even at the measurement time of 0.1 s, the adenine-derived peak was clearly observed at 734 cm^{-1} from 10^{-11} M solutions as shown in Fig. 6.

Single Dimer

The single dimer was evaluated. Figure 7 shows the fabricated single dimer on a Si substrate. We observed no particles other than the single dimer in a laser spot.

For the evaluation of the polarization direction effect and the mapping measurement the originally and uniformly adsorbed molecules on particle surfaces were used. The Raman spectra of the adsorbed molecules and the relative Raman intensity are shown in Figs. 8 and 9, respectively, depending on the polarization angle from 0° to 90°. The adsorbed molecules show strong Raman intensity at Raman

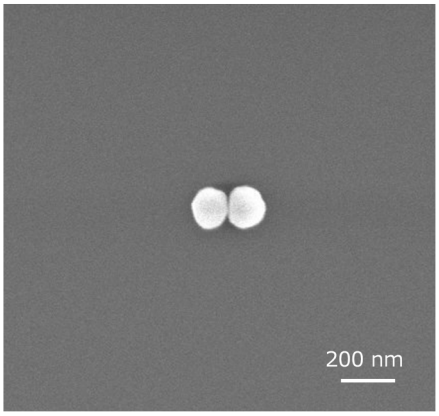

Figure 7: SEM image of a single gold nanoparticle dimer.

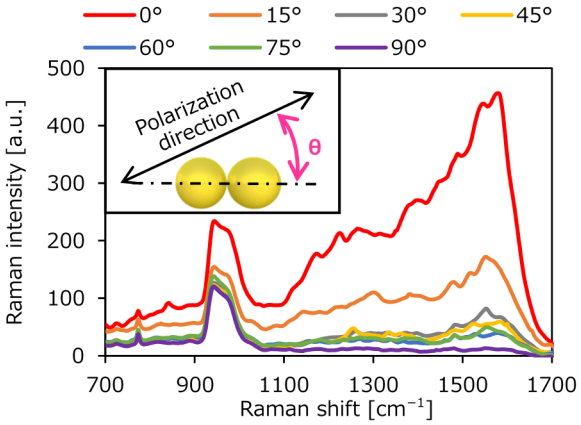

Figure 8: Average Raman spectra of acetone dicarboxylic acid at each polarization angle in a single dimer.

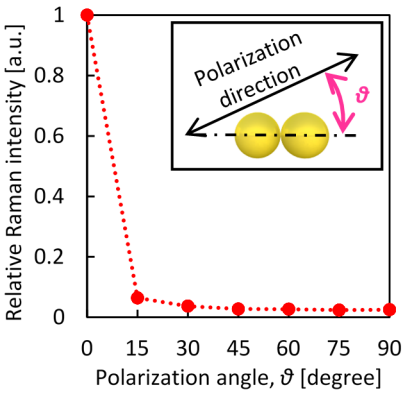

Figure 9: Relative Raman intensity from single dimer as a function of polarization angle.

shift from 1500 to 1700 cm^{-1}. Therefore the relative Raman intensity was calculated integrating from 1500 to 1700 cm^{-1}. The relative Raman intensity at 0° was much higher than those of other angles. We confirmed the polarization-dependent property. Figure 10 shows the result of the mapping measurement. We observed locally enhanced Raman intensity. These results indicate the Raman signal was derived from the single dimer. Finally the adenine molecule at 10^{-3} M and 10^{-11} M was detected using the single dimer as shown in Fig. 11. 10^{-3} and 10^{-11} M concentration correspond to one molecule per volume of a

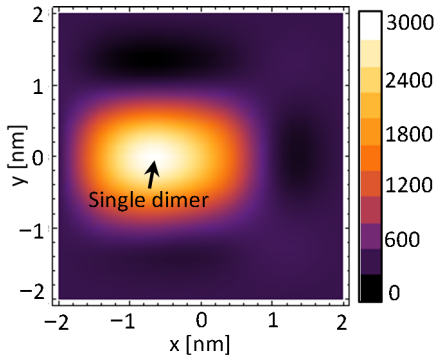

Figure 10: Mapping result of Raman intensities on single dimer substrate with mapping interval of 1 μm and laser spot of 2 μm (interposed).

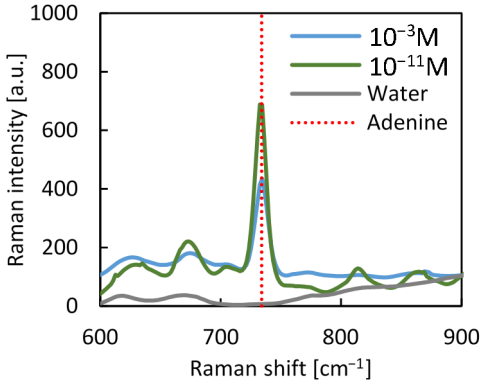

Figure 11: Raman spectra of adenine solutions at concentrations of 10^{-3} and 10^{-11} M, and pure water from single dimer.

cube with a side 12 nm and 5.5 μm, respectively. This result indicates the fabricated single dimer realized single molecule SERS detection because (1) those intensities with much deferent concentration were comparable, and (2) 10^{-11} M concentrations is enough low compared with significantly small volume of local Raman enhancement as shown in Fig. 1(b).

CONCLUSION

We fabricated and evaluated the arrayed dimers and the single dimer of gold nanoparticles for SERS detection and identification of DNA bases. The gold nanoparticles were connected in one direction with 1-nm gap in order to obtain the huge Raman enhancement for the single molecule sensitivity. The arrayed dimer observed Raman peaks derived from four kinds of DNA bases at 10^{-11} M concentration. Adenine molecule was detected at 10^{-11} M concentration with 0.1 s. Ultrasensitive and rapid detection was confirmed.

We confirmed that the single dimer was active for SERS detection from the mapping measurement and the polarization angle dependency results using the originally adsorbed molecules on particle surfaces. The single dimer also detected adenine molecule at 10^{-11} M concentration at the locally enhanced hotspot with the 1-nm nanogap. These results indicates the fabricated nanostructure was possible to detect and identify DNA bases with single molecule sensitivity.

ACKNOWLEDGEMENTS

A part of this study was supported by The Canon foundation, KIST-BIC (Kyoto integrated Science and Technology Bio-Analysis Center), and Micro/Nano Fabrication Hub in Kyoto University.

REFERENCES

[1] S. Nie, S. Emory, "Probing Single Molecules and Single Nanoparticles by Surface-Enhanced Raman Scattering", *Science*, vol. 275, pp.1102–1106, 1997.

[2] M. G. Banaee, K. B. Crozier, "Mixed Dimer Double Resonance Substrates for Surface-Enhanced Raman Spectroscopy", *ACS NANO*, vol.5, pp.307–314, 2011.

[3] L. Tong, H. Xu, M. Käll, "Nanogaps for Sers Applications", *MRS Bulletin*, vol.39, pp.163–168, 2014.

[4] S. E. J. Bell, N. M. S. Sirimuthu, "Surface-enhanced Raman spectroscopy (SERS) for sub-micromolar detection of DNA/RNA mononucleotides", *J. Am. Chem. Soc.*, vol.128, pp.15580–15581, 2006.

[5] K. Sugano, D. Matsui, T. Tsuchiya, O. Tabata, "Ultrasensitive Surface-Enhanced Raman Spectroscopy with Directionally-Arrayed Gold Nanoparticle Dimers", *Proc. MEMS2015*, pp. 608–611 (2015)

[6] T. Ozaki, K. Sugano, T. Tsuchiya and O. Tabata: "Versatile Method of Submicroparticle Pattern Formation Using Self-Assembly and Two-Step Transfer", *J. Microelectromechanical Syst.*, vol.16, pp.746−752, 2007.

[7] K. Sugano, T. Ozaki, T. Tsuchiya and O. Tabata: "Fabrication of gold nanoparticle pattern using combination of self-assembly and 2-step transfer", *Sensor. Mater.*, vol.23, pp.263−275, 2011.

[8] K. Sugano, K. Suekuni, T. Takeshita, K. Aiba, and Y. Isono, "Surface-Enhanced Raman Spectroscopy Using Linearly Arranged Gold Nanoparticles Embedded in Nanochannels", *Japanese Journal of Applied Physics*, vol.54, 06FL03, 2015.

[9] Munro, C., Smith, W., Garner, M., Clarkson, J., and White, P., 'Characterization of the Surface of a Citrate-Reduced Colloid Optimized for Use as a Substrate for Surface-Enhanced Resonance Raman Scattering', *Langmuir*, vol.11, pp. 3712–3720, 1995.

[10] S. Kumar, K. S. Gandhi and R. Kumar: "Modeling of Formation of Gold Nanoparticles by Citrate Method", *Ind. Eng. Chem. Res*, vol.46, pp.3128−3136, 2007.

[11] M. Giersig, P. Mulvaney, "Preparation of Ordered Colloid Monolayers by Electrophoretic Deposition", *Langmuir*, vol.9, pp. 3408–3413, 1993.

[12] C. Otto, T. J. J. van den Tweel, F. F. M. de Mul, J. Greve, "Surface-enhanced Raman spectroscopy of DNA bases", *Journal of Raman Spectroscopy*, vol.17, 289–298, 1986.

CONTACT

*K. Sugano, tel: +81-78-803-6458;
sugano@mech.kobe-u.ac.jp