

Platinum Bowtie Nanostructure Arrays for Massively Parallel Single Molecule Detection Based on Fluorescence Enhancement Phenomena

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ABSTRACT

We fabricated platinum bowtie nanostructure arrays producing fluorescence enhancement and evaluated their performance using two-photon photoluminescence and single molecule fluorescence measurements. A comprehensive selection of suitable materials was explored by electromagnetic simulation and Pt was chosen as the plasmonic material for visible light excitation near 500 nm, which is preferable for multicolor dye labeling applications like DNA sequencing. Observation of bright two-photon photoluminescence from each Pt nanostructure induced by irradiation at 800 nm with a femtosecond laser pulse clearly indicates that a highly enhanced local field is created near the Pt nanostructure. Between the Pt triangles of each nanostructure attachment of a single dye molecule was attempted by using selective immobilization chemistry. The fluorescence intensities of the single dye molecule localized on the nanostructures were measured. A highly enhanced fluorescence, which was increased by a factor of 30, was observed.

Keywords: single molecule detection, fluorescence enhancement, nanostructure, near field, plasmonics

1 INTRODUCTION

In order to enable massively parallel single molecule analysis, we have fabricated Pt bowtie nanostructure arrays which produce fluorescence enhancement. In addition, we have evaluated the performance using two-photon photoluminescence (TPPL) and single molecule fluorescence measurements. To enable the use of multicolor dye labeling, visible light excitation around 500 nm was preferable. Au is well known as plasmonic material and Au bowtie nanostructures show ultra high local field enhancement induced by light excitation around 800 nm, although the enhancement is small around 500 nm [1]. We explored suitable plasmonic materials for bowtie nanostructure comprehensively by electromagnetic simulation and consequently chose Pt. The calculation result indicates that Pt shows about 5 times higher enhancement than Au. A very thin titanium layer (thickness: ~ 5 nm) exists between the Pt triangles. Attachment of a single dye molecule (Atto532) was

attempted between the gaps of each nanostructure using selective immobilization chemistry.

Observation of bright TPPL (500–600 nm wavelength region) from each Pt nanostructure induced by irradiation at 800 nm with a femtosecond laser pulse indicates that a strongly enhanced local field was created near the nanostructure. The high fluorescence enhancement factor (ratio of fluorescence intensities of single Atto532 molecule with and without the nanostructure) up to 30 was observed. The distribution of the fluorescence enhancement factor was rather wide than estimated by simulation considering the variation of the gap size of the nanostructure. Position variation of the dye molecule attached on the nanostructure may influence the wide distribution of the fluorescence enhancement factor.

2 MATERIALS AND METHODS

2.1 Electromagnetic Simulation

The electromagnetic field near the nanostructure was simulated by the finite element method (FEM). The FEM calculation was executed using COMSOL Multiphysics 3.4® and the RF module (COMSOL AB). The nanostructure was on the interface of the quartz and water. The nanostructure was excited by 500-nm light, irradiated from the quartz side with an incident angle of 67.5°. In the electromagnetic field simulation, optical properties of materials were defined by the complex refractive index. The refractive indices for materials were collected from literature reports. In the case of a material for which the complex dielectric constant was only shown in the literature, the complex refractive index was calculated from the complex dielectric constant.

2.2 Fabrication of the Pt Bowtie Array

A Pt bowtie nanostructure array was formed on a quartz wafer. Thin films of Ti, Pt, and W with thicknesses of 5, 30, and 5 nm, respectively, were deposited on a quartz wafer 50 mm in diameter, through magnetron sputtering deposition. The resist mask pattern of the bowtie nanostructure array was formed on the wafer through electron beam lithography. Each bowtie nanostructure consists of a pair of two rounded triangles. The wafer was adhered onto a 500-nm-thick

SiO₂-deposited Si wafer of 200 mm diameter by a thermal release tape (REVALPHA, Nitto Denko). Thereafter, the wafer was processed by commercial fabrication systems for 200-mm Si wafers. The W layer was etched using an Ar plasma with the addition of 5 % SF₆, and the Pt layer of the wafer was etched using an Ar plasma through physical sputter etching, where the pressure was 1 Pa, and the peak-to-peak voltage of the AC wafer bias was 1 kV with the frequency of 800 kHz. The W thin film plays the role of a hard mask during Pt etching. If the W hard mask was not employed, the size of the bowtie nanostructure would be shifted and the gap distance between the two Pt nanostructures would be wider through the faceting of the resist mask shoulder, resulting in a lower plasmonic effect. The Ti layer was etched by Ar plasma with the addition of 10 % SF₆. The residual resist mask was ashed by O₂ plasma without applying the wafer bias. The wafer was heated to 170 °C, which released the quartz wafer from the Si wafer and the W hard mask was removed by wet etching using 30% H₂O₂ solution. The quartz wafer was diced to a 10 × 10-mm size and the device chips of the bowtie nanostructure array were thus fabricated.

2.3 TPPL Measurement

The excitation light source was a femtosecond-pulsed Ti:Al₂O₃ laser (Mai Tai, Spectra Physics), with an output wavelength of 800 nm (tunable wavelength range of 710-990 nm), repeat frequency of 80 MHz, and output pulse width of below 100 fs. The laser beam was passed through a variable ND filter, the first 1/2 wavelength plate, a polarization beam splitter, and further divided by a beam splitter. The transmitted beam was passed through the second 1/2 wavelength plate and de-focusing lens, reflected by a dichroic-mirror and irradiated onto a sample substrate using an objective lens. Luminescence emitted from the sample substrate face was collected with the object lens, passed through the dichroic mirror and two colored glass filters, and detected by a CCD camera.

2.4 Single Molecule Fluorescence Measurement

To immobilize the dye molecule to only the titanium layer but not to quartz layer, poly(vinylphosphonic acid) (Polyscience Inc., PVPA) was used as a linking molecule because phosphonic compounds can be attached to titanium but not to SiO₂ [20]. The PVPA was biotinized using a coupling reagent (EZ-Link TFPA-PEG3-Biotin, Thermo Scientific). The Pt bowtie nanostructure substrate was placed in biotinized PVPA solution (weight % of 3), and then was washed with distilled water. Then, a 5 μl droplet of an avidin (ImmunoPure NeutraAvidin, TechnoChemical KK) solution (100 nM) was placed on the substrate for fifteen minutes. The unreacted avidin was washed from the substrate and 5 μl of the Atto532-biotin (ATTO-TEC GmbH) solution (1nM) was applied to the substrate. Eventually the dye (Atto532) molecules were immobilized

on the titanium of the nanostructures. The substrate attached with dye was placed on the quartz prism. Irradiation using 532 nm from a YAG laser (10mW) was focused through a 1/4 wavelength plate onto a quartz prism in total reflection conditions, and the produced evanescent light irradiated the nanostructure substrate. Fluorescence from the nanostructure substrate was accumulated by an objective lens (60X) and focused onto the CCD camera. The fluorescence image of the sample substrate was acquired at a resolution of 1344 pixels x 1024 pixels (2×2 binning) and the image magnification was 60.

3 RESULTS AND DISCUSSIONS

3.1 Electromagnetic Simulation

We comprehensively examined materials that were suitable for fluorescence enhancement with excitation at 500 nm. At first, we chose candidate elements based on the following criteria: (1) metal elements, (2) not lanthanide, actinide or elements with atomic numbers greater than 103 since many of those elements have problems with availability or radioactivity, (3) metals with higher melting points than 100 °C, and (4) metals stable in water near room temperature since the nanostructure was used in aqueous solution. There were 34 elements that passed all four criteria. For 21 out of the 34, we obtained the complex refractive index at 500 nm as shown in Table 1. These 21 candidate elements were examined in the electromagnetic simulation shown below.

The field enhancement factor at the center of bowtie structure was calculated by changing the real part of the complex refractive index, *n* from 0.1 to 3.0 and the imaginary part, *k* from 0 to 10, respectively. The enhancement factor showed roughly unimodal distribution.

Taking not only the enhancement factor, but also the chemical stability and ease of dry etching into consideration, we chose noble metals. Noble metals were preferable from the point of view of selective immobilization chemistry. An adhesive layer was necessary between the quartz substrate and the metal layer, and the small amount of adhesive layer remained in the gap region after fabrication of the bowtie nanostructure by dry etching since the etching rate was much slower in the narrow gap region. If the adhesive layer and the metal layer have a different chemical property, selective molecule immobilization onto only the adhesive layer remained in the gap region can be realized. Metal oxide (a naturally formed oxide layer on the surface of metal) and noble metal have different properties in some cases. Therefore, we chose Pt for the metal layer and titanium for the adhesive layer because of their established reputation as materials in semiconductor fabrications. The simulation indicated that the Pt bowtie shows about a 5-fold higher enhancement factor than that of Au when they are irradiated by 500 nm light.

3.2 SEM Observation of Pt Nanostructure Array

Figure 1 shows a SEM image of the Pt bowtie nanostructure array. Each nanostructure was arranged in a grid pattern with a $2 \mu\text{m}$ pitch. The shape parameters of each nanostructure were determined by SEM observation. These values are summarized in Table 1. Three types of nanostructures with different gap sizes from 5.8 to 12.5 nm (average value) were fabricated on one quartz substrate. The sizes of the sides of the triangle were almost same (140 to 160 nm) among three types of nanostructures. Variation on the size of the sides is quite small (CV: 0.8 to 3.5%). On the other hand, the variation of the gap size between the two triangles of the bowtie nanostructures depends on the gap size. Smaller gaps showed larger variation.

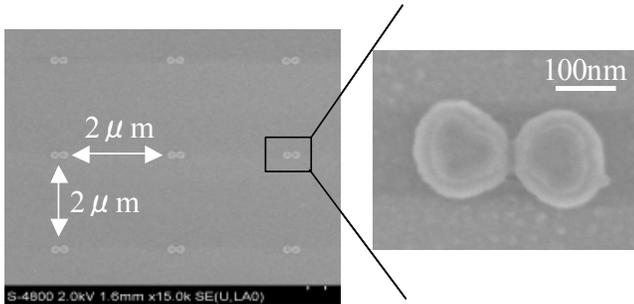


Figure 1: Images of scanning electron microscopy (SEM) of a Pt bowtie nanostructure array.

3.3 TPPL Measurement

It is possible to estimate the strength of the local electromagnetic field using surface plasmon excitation by external light irradiation and observing the nonlinear optical phenomena occurring near the nanostructure due to the high sensitivity against electric field strength (dependence on power law of field strength). For example, a strongly enhanced local field due to the excitation of surface plasmons in rough films, sharp tips, and nanoparticles induce two-photon absorption in Ag and Au [3]. Also, Moerner et al. recently demonstrated that Au bowtie nanostructures showed TPPL around 600 nm when they were irradiated by an 830 nm femtosecond laser pulse, and they confirmed that the TPPL intensity increased with

decreasing gap size, indicating that the strongly enhanced local field was due to the excitation of surface plasmon created [4].

We measured the photoluminescence of the Pt bowtie nanostructure. Figure 2 shows the photoluminescence image of the Pt bowtie nanostructure array excited by an 800 nm femtosecond laser pulse. Although there were few reports on the photoluminescence of Pt, we observed a transition near 600 nm similar to that of Au [4]. A bright spot was observed at each nanostructure and its brightness was different among the nanostructures. The intensity of the photoluminescence depended on the square of the excitation light intensity, suggesting that the two-photon absorption occurred in the Pt nanostructure.

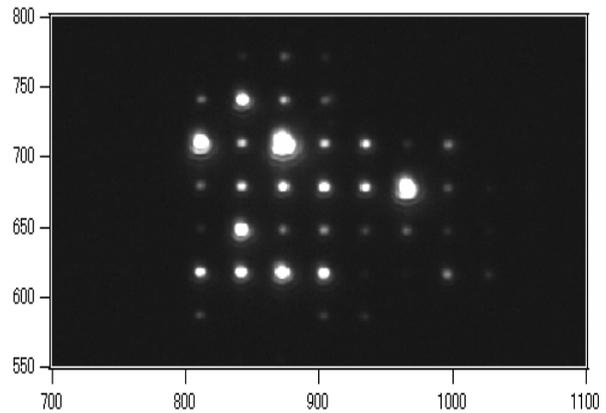


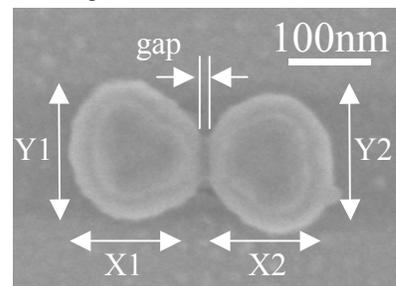
Figure 2: An image of TPPL of a Pt bowtie array

3.4 Single molecule fluorescence measurement

The fluorescence image of the Pt bowtie nanostructure array attached by Atto532 dye was obtained. Fluorescence from the dye was observed from almost 100 % of the nanostructure, indicating the selective attachment chemistry worked well. We examined the time profile of each fluorescence spot and chose only a single molecule fluorescence spot by examining the photobleaching due to one molecule. Eventually, single molecule immobilization with efficiency of approximately 20 % was observed. The fluorescence enhancement factor was evaluated by the ratio of fluorescence intensities of single dye molecule with and

Table 1: Shape parameters of the bowtie nanostructures determined by SEM observation. Shape parameter, X1, Y1, X2, Y2 and gap are depicted in the right SEM image.

Shape parameter	Type A (n=51)		Type B (n=15)		Type C (n=3)	
	average (nm)	CV(%)	average (nm)	CV(%)	average (nm)	CV(%)
X1	143.5	3	144.6	1.7	151.4	1.4
Y1	152.8	2	154.3	1.5	157.7	1.9
X2	139.8	3.5	145.2	2.8	150	0.8
Y2	150.2	1.7	151.4	1.8	154.7	2.3
gap	5.8	31.9	10	15.9	12.5	14.2



without the nanostructure. We measured the fluorescence of individual dye molecules attached randomly to the quartz substrate and performed Gaussian curve fitting to determine the peak of the histogram compared to the fluorescence intensities of a single dye molecule without the nanostructure. Then fluorescence enhancement factor was calculated for each nanostructure and histogram shown in Figure 3 (Type A) was made. The distribution factor for higher enhancement regions was extended up to 30 and almost 20 % of the nanostructure showed enhancement by a factor greater than 10. The distribution was rather broad compared to the estimated distribution by electromagnetic field simulation considering the variation of the gap size (Table 1). Although the calculation results indicated that the field strength varied by a factor of 2, the fluorescence enhancement factor distribution was from 1 to 30, indicating that only the variation of the gap size did not cause the broad distribution of the fluorescence enhancement factor.

We also evaluated the enhancement factor in Type B and C and compared them as shown in Figure 4. With decreasing the gap size in order from Type C, B, to A, the distribution of the fluorescence enhancement factor clearly extends to larger region. This trend is consistent with that of TPPL and that of the local field strength calculated by electromagnetic simulation, indicating that the local enhanced field created in the nanostructure caused the fluorescence enhancement phenomena.

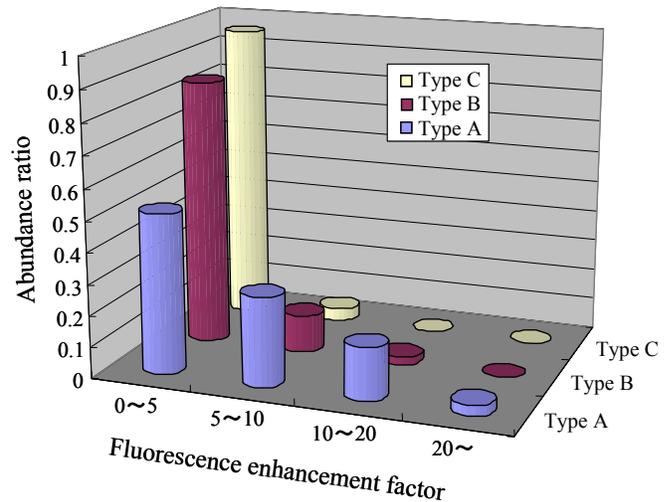


Figure 4: A comparison of distribution of fluorescence enhancement factor among Type A, Type B and Type C bowtie nanostructure.

REFERENCES

- [1] A. Sundaramurthy, K. B. Crozier, G. S. Kino, D. P. Fromm, P. J. Schuck and W. E. Moerner, *Phys. Rev. B*, 72, 165409, 2005.
- [2] P. H. Mutin, V. Lafond, A. F. Popa, M. Granier, L. Markey and A. Dereux, *Chemistry of Materials*, 16, 5670-5, 2004.
- [3] G. T. Boyd, Z. H. Yu and Y. R. Shen, *Phys. Rev. B*, 33, 7923, 1986.
- [4] P. J. Schuck, D. P. Promm, A. Sundaramurthy, G. S. Kino and W. E. Moerner, *Phys. Rev. Lett.*, 94, 017402, 2005.

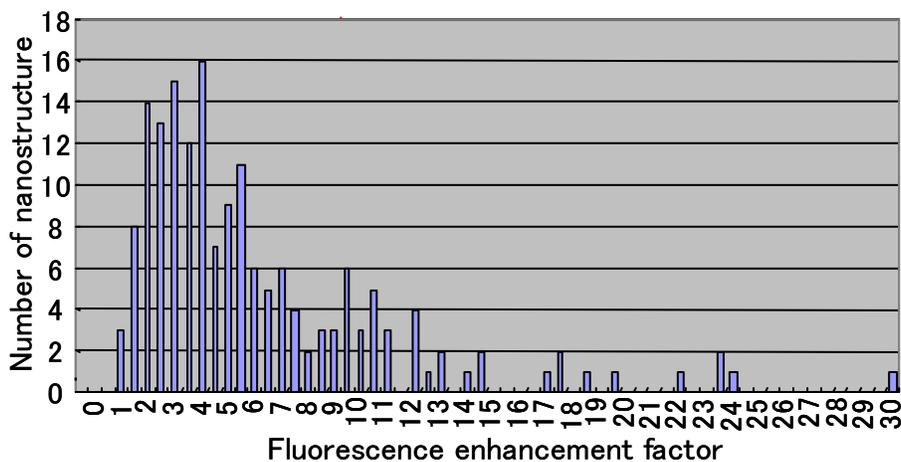


Figure 3: A histogram of fluorescence enhancement factor of Type A bowtie nanostructure