analytical chemistry

Article

pubs.acs.org/ac

Sensitive Detection of a Tumor Marker, α-Fetoprotein, with a Sandwich Assay on a Plasmonic Chip

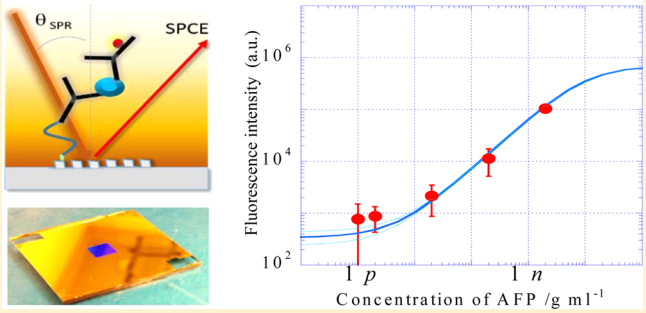
Keiko Tawa,*[,†,§] Fusanori Kondo,[†,§] Chisato Sasakawa,[†] Kousuke Nagae,[‡] Yukito Nakamura,[‡] Akitoshi Nozaki,[‡] and Takatoshi Kaya[‡]

[†]Health Research Institute, AIST, 1-8-31 Midorigaoka, Ikeda, Osaka 563-8577, Japan

[§]Department of Chemistry, Graduate School of Science and Technology, Kwansei Gakuin University, Sanda, Hyogo 669-1337, Japan

[‡]Konica Minolta Inc., No.1 Sakuramachi, Hino-shi, Tokyo 191-8511, Japan

ABSTRACT: Two types of plasmonic silver- and gold-coated grating biosensor chips (plasmonic chip) were applied in the detection of α-fetoprotein (AFP) with a sandwich imunoassay and surface plasmon field-enhanced fluorescence. On the plasmonic chip, unlabeled marker in the sandwich immunoassay was first quantitatively detected over a wide range between 10^{-12} and 10^{-8} g/mL. The affinity constants between AFP and anti-AFP antibody, which were obtained by fitting the experimental data to the Langmuir isotherm adsorption curve, were 1×10^{8} g^{-1} mL regardless of the kind of metal in the plasmonic chips. Although the fluorescence intensity on the silver plasmonic chip was 5 times larger than that on the gold plasmonic chip, the limit of detection (LOD) was on the order of 10^{-11} g/mL and not improved with a silver plasmonic chip. Herein, we used a new setup that generated less dispersions of both the fluorescence intensity for nonspecific adsorption and the background (optical blank) signal and improved the LOD of AFP to 4 pg/mL (55 fM) with the silver plasmonic chip. With the highly sensitive detection in the sandwich immunoassay, the development of a plasmonic chip for clinical diagnosis by a blood test is promising.



Recently, various types of immunosensors have been developed. The construction of highly sensitive detection systems that consist of simple and compact instrumentation is an important goal for the application of such immunosensing systems in clinical diagnosis. Although the limit of detection (LOD) required depends on the kind of antigen to be detected, a LOD of 10^{-12} to 10^{-15} M may be needed for early detection of diseases such as cancer. Surface plasmon resonance (SPR) methods have become generally available in laboratories since the development of "Biacore" in the 1990s.[1] However, conventional SPR (such as Biacore) methodology has a poor LOD. When a better LOD is required for an assay, the use of enzyme-linked immunosorbent assays (ELISA)[2] is often adopted; this technique is useful in spite of its complex and lengthy operation. Surface plasmon field-enhanced fluorescence (SPF) spectroscopy[3,4] is a candidate method for providing excellent LODs in an operationally simple manner because of the superior surface-selective enhanced fluorescence of this technique compared with conventional fluorescence methods measured on a cover glass or a plastic plate. The origin of the enhanced fluorescence is the surface plasmon field under resonance conditions. In the propagated SPR forms,[5] prism-coupled SPR (PC-SPR) and grating-coupled SPR (GC-SPR),[6,7] GC-SPR provides direct coupling between surface plasmon polaritons and the radiation modes. For application in immunosensors, GC-SPR has a number of advantages: it involves a simple optical setup that, in contrast to PC-SPR, does not require a prism, and it can be used to achieve highly sensitive levels of detection by using surface plasmon-coupled emission (SPCE).[8,9] We have previously developed devices consisting of a substrate with a metal-coated grating structure, which we termed a "plasmonic chip,"[11,12] and we have used such devices for immunosensing applications.[13,14]

In the present study, an unlabeled marker was detected by using a sandwich immunoassay on a plasmonic chip. To date, wide detection ranges of 5−6 orders, i.e., 10^{-7} to 10^{-11} M, have been achieved for green fluorescent protein (GFP),[13] and detection ranges of 10^{-8} to 10^{-13} M were shown for epidermal growth factor receptor (EGFR)[14] in a fluorescently labeled marker system. In contrast, for unlabeled marker detection with the sandwich immunoassay, a narrow detection range of only three orders (10^{-12} to 10^{-14} M) was obtained for interleukin-6 (IL-6), as reported in our previous paper.[15]

In this study, we wanted to analyze the tumor marker α-fetoprotein (AFP) as an unlabeled marker and anticipated that the AFP could be detected over a wide concentration range and also at lower concentrations by using a sandwich immunoassay based on a plasmonic chip. This study is the first report of

Received: December 14, 2014
Accepted: February 26, 2015
Published: February 26, 2015

DOI: 10.1021/ac504642j
Anal. Chem. 2015, 87, 3871−3876

fluorescence immunosensing with a sandwich immunoassay for a wide concentration range on a plasmonic chip under illumination from the top panel of a chip. Silver-coated plasmonic chips have been considered theoretically and are expected to be superior to their gold-coated counterparts because of the dielectric constant of the former at a wavelength of 633 nm. The experimental results for detection of cy5-labeled streptavidin with a biotinylated plasmonic chip show that the fluorescence intensity generated by the silver plasmonic chip was more than three times larger than that generated by the gold chip (data not shown). In this study, both types of plasmonic chip, gold and silver, were fabricated and the fluorescence intensity and LOD for AFP detection were compared. We investigated whether the plasmonic chip providing a brighter fluorescence can reach the lower LOD, and the calibration curves were plotted for both plasmonic chips to determine affinity constants. Furthermore, the optical instrumentation was improved with respect to a horizontal sample stage and decreased laser power, and the apparatus was optimized to collect fluorescence more effectively, resulting in a lower LOD.

EXPERIMENTAL SECTION

Fabrication of Plasmonic Chips. Plasmonic chips were fabricated using UV nanoimprint lithography (UV-NIL) as described previously.[16] Gold and silver were used for coating, and the surfaces were prepared at a layer thickness of 150−180 nm with an rf-sputter setup (Cryo-back, custom-made). Both devices were finally covered with a silica overlayer with an adhesion layer of Cr or Ti for Au and Ag, respectively. The thickness of the silica layer was approximately 20 nm to sufficiently suppress quenching of fluorescence. The topography of the plasmonic chips was measured by using scanning probe microscopy (Figure 1). The pitch of the grating, the groove depth, and the duty ratio (ratio of the convex part to the pitch) were 430 nm, 30 nm, and 0.50, respectively.

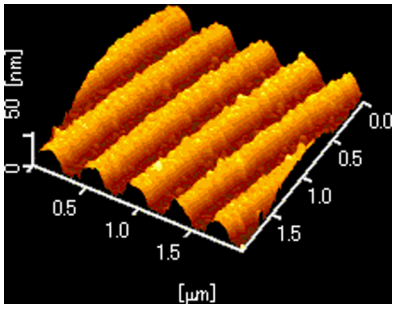


Figure 1. AFM image of a plasmonic chip.

Measurement of Fluorescence. Two types of instrument configuration were used: setup (I), based on the previously reported system[10−12] (Figure 2a), and the new setup (II) (Konica Minolta custom-made, Figure 2b). The former setup is described in detail elsewhere[10−12] and is briefly explained here: p-polarized light from a He−Ne laser (λ = 632.8 nm) was incident at 1 mW. To suppress the influence of fluorescence bleaching, the fluorescence intensity was measured for a constant exposure time under the same conditions. A plasmonic chip was set vertical to the sample stage on a goniometer. The incident angle was fixed relative to the resonance angle, and the fluorescence intensity was measured against the detection angle with a photomultiplier tube (PMT). In the new setup, a collimated laser beam of 637 nm wavelength from a laser diode (HL6322G, Opnext, Inc., CA, USA) passed through the band-pass filter (DIF-BPF-2 (half width; 630 ± 8 nm), Optical Coating Japan, Tokyo, Japan) and polarizer (USP-20C-01, Sigma Koki, Saitama, Japan). p-Polarized light was incident at 10 μW, and the illuminated area was a 1 mm diameter spot on the grating surface substrate. The diode laser was mounted on the rotational arm, making the incident angle variable. A plasmonic chip was set horizontally on the sample stage located at the rotational center. The light reflected from the grating surface substrate was monitored with a CCD camera (STC-MB33USB, Sentech Co., Ltd., Kanagawa, Japan) through a long cylindrical aperture mounted on the second arm. The fluorescence was measured with a PMT (H7421-40, Hamamatsu Photonics K.K., Shizuoka, Japan) mounted on the same arm as the CCD, and an emission filter (DIF-BPF-1 (half width; 668 ± 5 nm), Optical Coating Japan, Tokyo, Japan) was inserted in front of the PMT. The incident angle was fixed to the resonance angle, and the fluorescence intensity was measured against the detection angle with a PMT mounted on the second rotational stage. The cylindrical aperture played an important role in preventing stray reflected or scattered light from entering the detector.

Preparation of AFP Sandwich Assay. The day before preparing the platform modified with a capture antibody, as shown in Figure 3, 20 μL of *N*-(2-aminoethyl)-3-aminopropyltrimethoxysilane (Merck Schuchardt OHG) prepared as a 2% solution with 1 wt % acetic acid was first dropped onto the plasmonic chip surface, and then, the chip was incubated for 2 h at 40 °C to aminate the silica surface. After rinsing, the modified chip was incubated for a further 2 h at 80 °C. Prior to setting the custom-made cuvette (well-type) onto the plasmonic chip as shown in Figure 4, double-sided adhesive tape was positioned there without removal of the outside cover film. The custom-made cuvette was then placed into position; 80 μL of *N*-hydroxysuccinimide-PEG cross-linker (NOF; Sunbright DE-034CS) solution prepared at 4 mM with dimethyl sulfoxide (Wako Pure Chemical Industries) was dropped into the well, and the chip was incubated for 20 min on a shaker at room temperature. After rinsing with ethanol, 80 μL of anti-AFP capture antibody solution (Mikuri Immunology Laboratory, 1D5) prepared at 200 μg/mL with pH 8.0 tris-buffered saline including 0.05% Tween 20 (TBST, Fisher Scientific) solution was injected and the assay product was incubated for 30 min on the shaker. Considering the concentration of capture antibody, it was fully concentrated to be densely bound to the chip platform. After rinsing with TBST, the cuvette was removed from the chip and the background signal intensity was measured after injecting Milli-Q water into the space between the chip and a temporary cover glass, as shown in the right side of Figure 4. After removal of the cover glass, the chip was recombined with the cuvette, and AFP (Acris Antbodies) solution prepared with pH 7.4 phosphate-buffered saline solution (PBS, Nippon Gene) including blocker BSA (Pierce) was injected into the well. The assay system was then incubated for 1 h on a shaker. To determine the fluorescence intensity for nonspecific adsorption, AFP was not added, and only PBS blocker BSA solution was injected and rinsed in the same way. After rinsing with TBST, 2.5 μg/mL Alexa Fluor 647-labeled anti-AFP detection antibody (Mikuri Immunology Laboratory, 6D2) solution

DOI: 10.1021/ac504642j
Anal. Chem. 2015, 87, 3871−3876

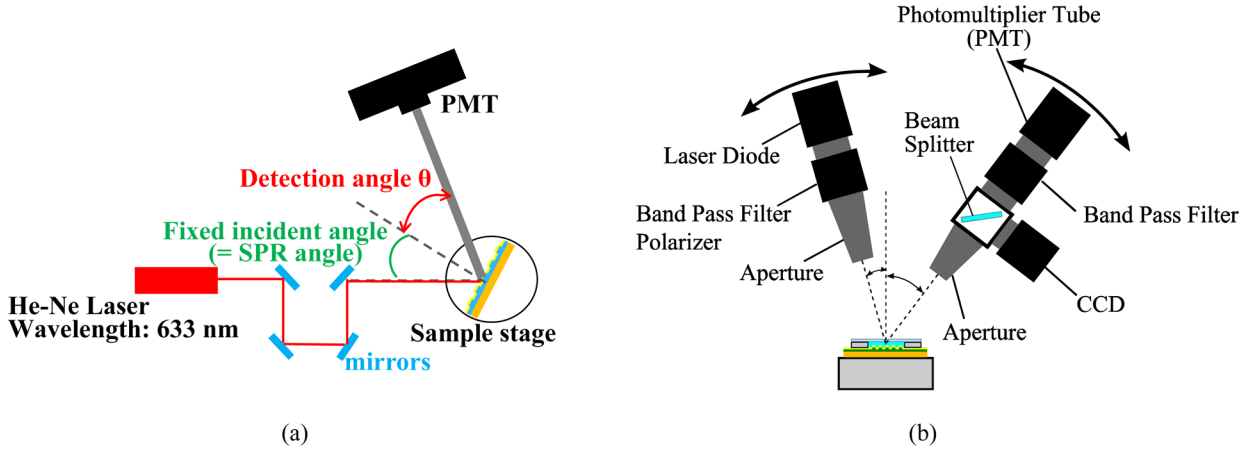


Figure 2. Two kinds of setup, (a) (I) and (b) (II), for angle-scanning SPF measurement: (a) top view and (b) side view.

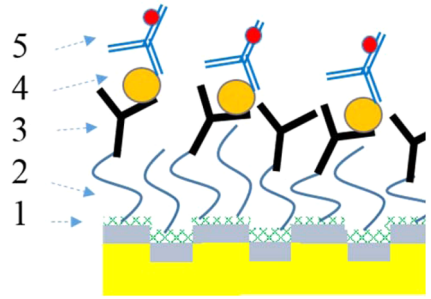


Figure 3. Procedure of surface modification: (1) *N*-(2-aminoethyl)-3-aminopropyltrimethoxysilane, (2) *N*-hydroxysuccinimide-PEG cross-linker, (3) Anti-AFP capture antibody, (4) AFP, and (5) Alexa647-labeled AFP detection antibody.

prepared with PBS solution including blocker BSA was injected and the cell was incubated for 10 min. After rinsing with TBST, the cuvette was smoothly removed and subsequently 10 μL of TBST solution was dropped onto the chip surface. A cover glass was immediately placed over the cuvette and fixed by removing the outside film of the double-sided adhesive tape prepared in advance. The chip was then set on the sample stage for fluorescence measurement. The labeled anti-AFP detection antibody was prepared in advance with an Alexa Fluor 647 Monoclonal Antibody Labeling Kit (Molecular Probes).

RESULTS AND DISCUSSION

AFP Detection with Sandwich Assay on the Gold and Silver Plasmonic Chips under Optical Setup (I). At first, resonance angles were investigated for AFP immunoassays on the gold and silver plasmonic chips. As shown in Figure 5a, on the gold plasmonic chip, the resonance angle was observed at 4 degrees. Therefore, the incident angle was fixed at this angle for measuring fluorescence intensity. The fluorescence peaks corresponding to the SPCE were observed at 11−12 degrees as shown in Figure 6a, and the SPCE peaks were detected in the AFP concentration range from 200 pg/mL (2.8 pM) to 10 pg/mL (140 fM). In more dilute AFP solutions, i.e., those prepared at 5 pg/mL (=70 fM) or for samples without AFP, the clear peaks could no longer be observed.

In contrast, the resonance angle was observed at 7 degrees with the silver plasmonic chip (Figure 5b). Under irradiation at the resonance angle, the fluorescence peak shown in Figure 6b was observed at 13 degrees. The peaks were always found at 12−13 degrees over a wide AFP concentration range (until 2 pg/mL). The fluorescence curve for nonspecific adsorption without AFP did not give rise to a signal.

The mean values of the fluorescence peaks measured against the detection angle shown in Figure 6 (F_{AFP}) were corrected for mean fluorescence intensity for nonspecific adsorption (F_{NS}) by subtraction, and the resulting values were plotted against the concentration of AFP (Figure 7). Error bars show the standard deviation from a mean fluorescence intensity for a chip-to-chip variation. The silver plasmonic chip produced more than 5 times higher fluorescence intensity compared with that from the gold plasmonic chip. In the AFP concentration range from 20 ng/mL to 2 pg/mL, all the measured values were more than three times the standard deviation (3SD) of the mean values for nonspecific adsorption. Therefore, quantitative analysis suggests the LOD was in the order of pg/mL for both chips.

Calibration Curves for AFP Detection Measured under Setup (I). From the calibration curve of a fluorescence intensity measured against the marker concentrations, the affinity constant is usually evaluated by using the Langmuir isotherm adsorption model.[17,18] The amount of marker adsorbed onto the surface of the sensor chip is proportional to the fluorescence intensity of labeled antibody binding to the marker, and the isotherm curve can be described as eq 1:

$$F(c) = F_{max}(cK_a)/(1 + cK_a) \quad (1)$$

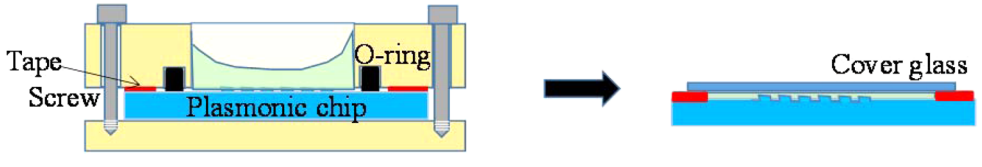


Figure 4. Schematic of (left) custom-made cuvette (well-type) for shaking solutions and (right) thin-space cell for measurement.

DOI: 10.1021/ac504642j
Anal. Chem. 2015, 87, 3871−3876

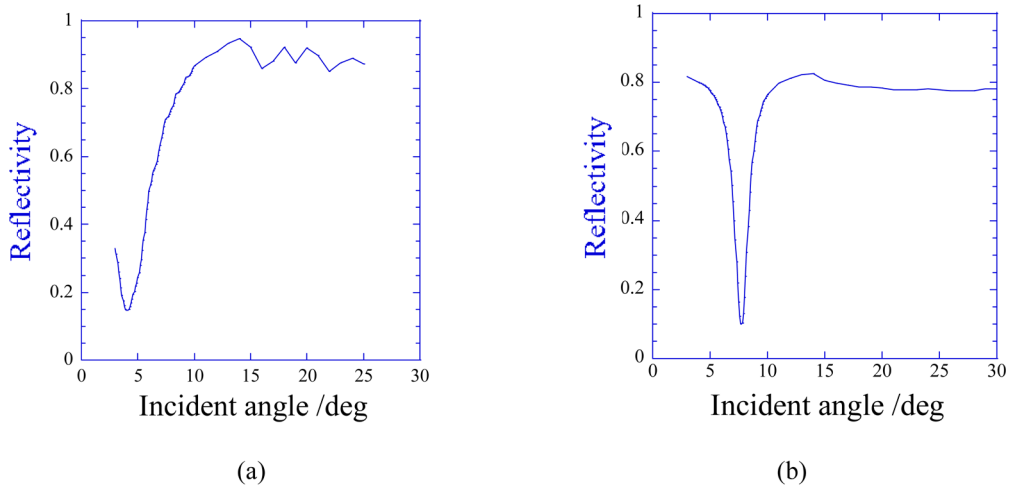


Figure 5. Reflectivity measured against incident angle for (a) gold and (b) silver plasmonic chips.

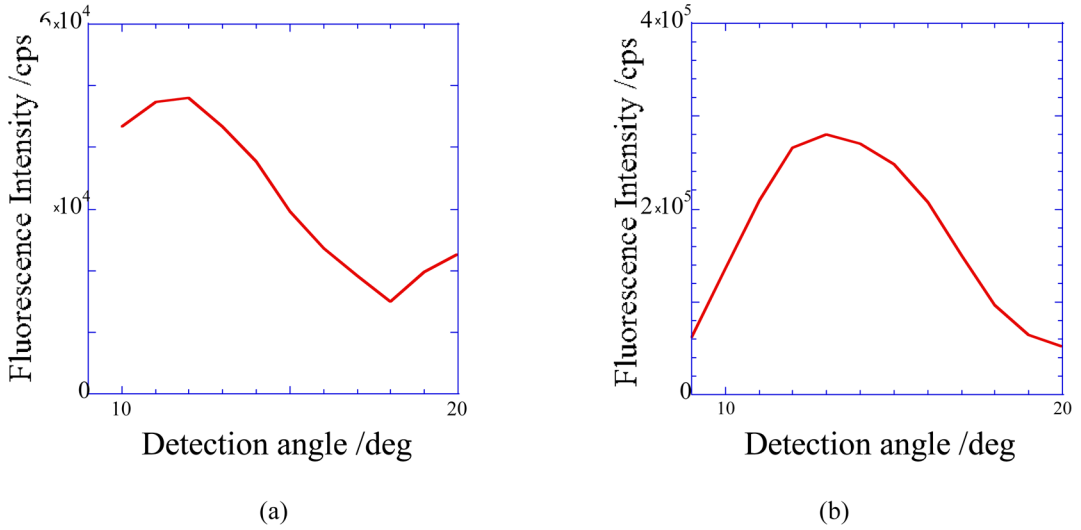


Figure 6. Fluorescence curves measured against the detection angle in the (a) gold and (b) silver plasmonic chips for AFP at 200 pg/mL.

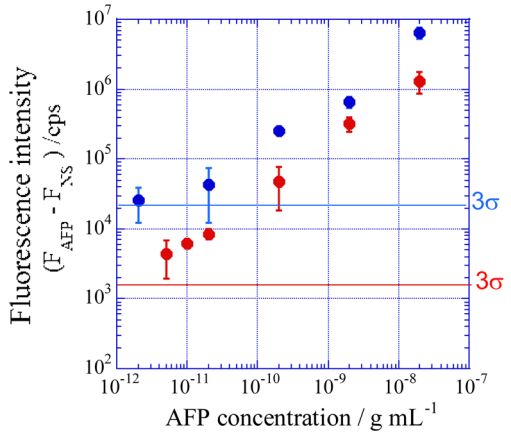


Figure 7. Calibration curves for AFP detection with the gold plasmonic chip (red circles) and silver plasmonic chip (blue circles). The fluorescence values (F_{AFP}) after subtracting fluorescence intensity for nonspecific adsorption (F_{NS}) were plotted. The solid lines correspond to three times the standard deviation (3SD) of mean F_{NS}.

where c and K_a are the marker concentration and affinity constant between antigen and labeled antibody, respectively. $F(c)$ and F_{max} are fluorescence intensities measured at a concentration of c for marker (antigen) and at the surface saturated with marker proteins. Actually, in the sandwich immunoassay, both capture and detection antibodies are related to the affinity K_a, and therefore, eq 1 is strictly not correct. However, it can be a rough estimate as shown in the previous analysis for the sandwich immunoassay.[17,19] Although in AFP detection, when the AFP concentration was 0, some fluorescence signal was still detected due to nonspecific adsorption. Therefore, fluorescence due to nonspecific adsorption, F_{NS}, corresponding to the baseline, must be considered, and eq 1 is modified accordingly to give eq 2:

$$F(c)_{AFP} = F_{max}(cK_a)/(1 + cK_a) + F_{NS} \quad (2)$$

The mean values of F_{NS} obtained by fluorescence measurement in this study were 1200 and 13 500 cps for the gold and silver plasmonic chips, respectively. The affinity constants obtained in both types of plasmonic chip were predicted to be equivalent. Experimental values of $F(c)_{AFP}$ were plotted against concentrations of AFP, as shown in Figure 8. By fitting experimental values to the two isotherm curves in log−log plots individually, equivalent K_a values of 1×10^8 g^{-1} mL (i.e., 7×10^9 M^{-1}) were obtained for both gold and silver plasmonic

DOI: 10.1021/ac504642j
Anal. Chem. 2015, 87, 3871−3876

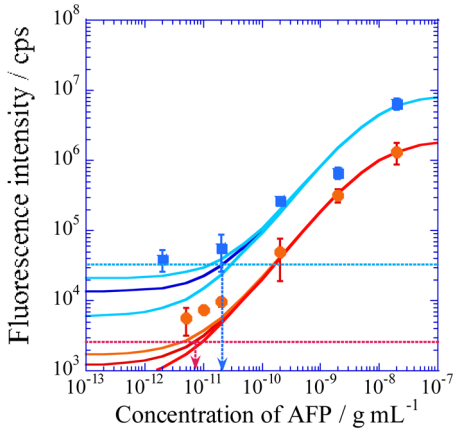


Figure 8. Log–log plots of fluorescence intensity ($F(c)_{AFP}$) measured with silver (blue squares) and gold (red circles) plasmonic chips vs AFP concentration. The middle lines show fitting to the Langmuir isotherm curves for silver and plasmonic chip, individually. The upper and lower curves binding a middle line show the error bars for nonspecific adsorption. Dotted lines correspond to individual 3SD of mean fluorescence values for nonspecific adsorption.

chips. This result indicates a dissociation constant (K_d) of 10 ng/mL (140 pM) irrespective of the type of metal.

The LOD is statistically determined as the value corresponding to the cross point between Langmuir isotherm adsorption curve and 3SD of a mean fluorescence intensity for nonspecific adsorption. The SDs of F_{NS} were 500 cps and 7500 cps for the gold and silver chips, respectively. Therefore, in this study, LODs for the gold and silver plasmonic chips were calculated to be 8 pg/mL (110 fM) and 20 pg/mL (280 fM), respectively, based on fitting of the data to Langmuir isotherm curves (Figure 8). Although a larger fluorescence intensity was obtained with the silver plasmonic chip, the LOD was not as good as that determined for the gold chip. The SD of nonspecific adsorption can determine the LOD, and the SD of nonspecific adsorption depends on the chemical assay and on the stability of signal intensity measured by the optical setup. The chemical assay on the surface for the silver and gold plasmonic chips was equivalent, and the difference presumably originates from the poor signal stability in silver, which was composed of the larger F_{NS} due to a larger fluorescence enhancement in the silver film and the larger deviation for background (optical blank). Why was the larger background provided in the silver plasmonic chip? In the silver plasmonic chip, the incident angle and the detection angle were very close, and it is considered to be difficult to collect fluorescence without influence from the reflection or scattering by using an optical setup (I).

On the other hand, for the silver plasmonic chip, deviation of the experimental values from the fitting curve was observed below the order of 10^{-11} g/mL, and it was in agreement with the LOD determined from 3SD of F_{NS}. In contrast, for the gold plasmonic chip, the experimental values already deviated from the fitted curve at concentrations of more than the LOD, i.e., 10^{-11} g/mL. Therefore, in the gold plasmonic chip, reliable quantitative AFP detection was possible to 10^{-11} g/mL. The LOD obtained in a gold chip was not considered to be absolutely better than that in a silver one, and the silver chip has an inherently greater ability to enhance fluorescence than the gold one.[20] We then studied the calibration curve by using the new setup with the aim of improving the LOD for the silver plasmonic chip.

AFP Detection with Sandwich Assay on the Silver Plasmonic Chips under Optical Setup (II). All the data of fluorescence intensity, which have been accumulated and presented in our laboratory, were measured by optical setup (I), and the experimental results for AFP detection measured by setup (I) were required in order to compare with our previous ones. The new optical setup described above (Figure 2b) was constructed. The laser power was two orders lower than that used in setup (I), which suppressed fluorescence bleaching to below 1%. The bleaching rates for silver and gold plasmonic chips were <10% and <2%, respectively, during measurement using setup (I). The value of nonspecific adsorption was suppressed to 340 counts, and the SD of the F_{NS} was 100 counts. Especially, the cylindrical aperture played an important role in preventing stray reflected or scattered light from entering the detector, and therefore, the signal stability was dramatically improved.

The calibration curve was obtained by fitting the experimental data to the Langmuir isotherm (Figure 9), and

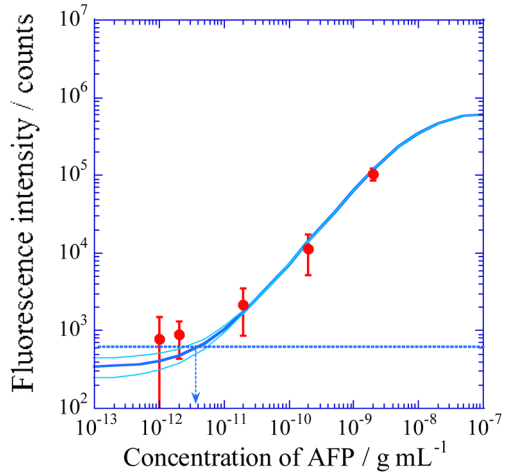


Figure 9. Fitting results to the Langmuir isotherm curves. The dotted line corresponds to 3SD of mean fluorescence values for nonspecific adsorption. The blue band shows the error bars for nonspecific adsorption.

equivalent affinity constants, $K_a = 1 \times 10^8$ g^{-1} mL, were determined irrespective of the experimental setup. The fluorescence intensity detected using this setup was one order lower than that detected using setup (I). The collection efficiency of fluorescence was considered to be superior to that in setup (I). The experimental values deviated from the fitted curve at a concentration of 10^{-12} g/mL, which is an order of magnitude lower than that obtained under the previous setup (I). The LOD determined from 3SD of F_{NS} was also 4 pg/mL (55 fM), as shown in Figure 9, which was in agreement with the value estimated by the deviation from fitted curve. The improvement of AFP concentration in the quantitative analysis by 1 order of magnitude, i.e., 10^{-12} g/mL, with setup (II) was considered to be mainly due to the suppression of signal intensity from the blank and consequent reduction in the SD. To improve the LOD, the stability of the fluorescence intensity, especially that for nonspecific adsorption, was found to be essential.

DOI: 10.1021/ac504642j
Anal. Chem. 2015, 87, 3871−3876

In our previous study, a LOD of 80 fM was reported for IL-6 detection with the sandwich immunoassay.[15] In this study, the LOD of 55 fM was achieved and AFP markers were detected in a wide concentration range of 5 orders of magnitude. This is the first study to show the marker detection in a wide concentration range by applying a plasmonic chip to a sandwich immunoassay system.

Furthermore, highly sensitive detection of AFP has already been reported previously.[21−23] Using gold nanolabels for chemiluminescence immunoassays[21,2] and multicolor quantum dot methods based on fluorescence polarization immunoassays,[23] the LODs were reported to be 10 or 5 pg/mL and 280 pg/mL, respectively. The results of this study are comparable or superior to those obtained by chemiluminescence, and further improvements in the GC-SPF method are anticipated.

CONCLUSIONS

Plasmonic chip constructs are known to provide fluorescence enhanced by an electric field under GC-SPR. In this study, plasmonic biosensor chips were first applied to marker detection in a wide concentration range with a sandwich immunoassay. The marker, AFP, was quantitatively detected with a sandwich immunoassay constructed on the plasmonic chip over a wide range of over five orders between 10^{-12} and 10^{-8} g/mL. Therefore, the development of a plasmonic chip for the clinical diagnosis by a blood test is promising.

Although the fluorescence intensity measured with the silver plasmonic chip was 5 times larger than that measured with the gold plasmonic chip, the LOD was on the order of 10^{-11} g/mL and not improved with the silver plasmonic chip because of the large signal deviation of the blank signals. The new optical setup suppressed the dispersions of nonspecific adsorption and the background signals, improving the LOD to 4 pg/mL. As a result, AFP can be quantitatively analyzed at concentrations of 10^{-12} g/mL. In conclusion, use of the detection system, leading to suppression of background signals and fluorescence signals for nonspecific adsorption, and subsequently reduced SD of signals were found to be important for improvement of LODs. In such an immunosensor, highly sensitive detection is usually required without contamination. Therefore, reusability of a plasmonic chip is considered to be difficult. Disposal is also more preferable than reuse due to the poor stability of thin metal and silica layers under the complete cleaning. The plasmonic chip described herein has great potential as a clinical diagnostic chip for use in sandwich immunoassays capable of contributing to early detection of diseases. In the further step for development to clinical diagnosis, the plasmonic chip will be required to be evaluated in a blood serum or plasma.

AUTHOR INFORMATION

Corresponding Author

*Phone: +81-72-751-9243. Fax: +81-72-751-9628. E-mail: tawa-keiko@aist.go.jp.

Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

K.T. thanks TOYO GOSEI for providing the UV-curable resin PAK-02-A.

REFERENCES

(1) McDonnell, J. M. *Curr. Opin. Chem. Biol.* **2001**, *5*, 572−577.
(2) Bi, S.; Yan, Y. M.; Yang, X. Y.; Zhang, S. S. *Chem.—Eur. J.* **2009**, *15*, 4704−4709.
(3) Liebermann, T.; Knoll, W. *Colloids Surf., A* **2000**, *177*, 115−130.
(4) Yu, F.; Yao, D.; Knoll, W. *Anal. Chem.* **2003**, *75*, 2610−2617.
(5) Homola, J.; Yee, S. S.; Gauglitz, G. *Sens. Actuators, B* **1999**, *54*, 3−15.
(6) Raether, H. *Surface Plasmons on Smooth and Rough Surfaces and on Gratings*; Springer-Verlag: Heidelberg, Germany, 1988.
(7) Knoll, W. *Annu. Rev. Phys. Chem.* **1998**, *49*, 569−638.
(8) Malicka, J.; Gryczynski, I.; Gryczynski, Z.; Lakowicz, J. R. *Anal. Chem.* **2003**, *75*, 6629−6633.
(9) Matveeva, E.; Gryczynski, Z.; Gryczynski, I.; Lakowicz, J. R. *J. Immunol. Methods* **2004**, *286*, 133−140.
(10) Tawa, K.; Yokota, Y.; Kintaka, K.; Nishii, J.; Nakaoki, T. *Sens. Actuators, B* **2011**, *157*, 703−709.
(11) Tawa, K.; Hori, H.; Kintaka, K.; Kiyosue, K.; Tatsu, Y.; Nishii, J. *Opt. Express* **2008**, *16*, 9781−9790.
(12) Cui, X. Q.; Tawa, K.; Hori, H.; Nishii, J. *Adv. Funct. Mater.* **2010**, *20*, 546−553.
(13) Tawa, K.; Hattori, T.; Umetsu, M.; Kumagai, I. *Anal. Chem.* **2011**, *83*, 5944−5948.
(14) Tawa, K.; Umetsu, M.; Nakazawa, H.; Hattori, T.; Kumagai, I. *ACS Appl. Mater. Interfaces* **2013**, *5*, 8628−8632.
(15) Tsuneyasu, M.; Sasakawa, C.; Naruishi, N.; Tanaka, Y.; Yoshida, Y.; Tawa, K. *Jpn. J. Appl. Phys.* **2014**, *53*, 06JL05.
(16) Akashi, N.; Tawa, K.; Tatsu, Y.; Kintaka, K.; Nishii, J. *Jpn. J. Appl. Phys.* **2009**, *48*, 06FH17-1.
(17) Rucker, V. C.; Havenstrite, K. L.; Herr, A. E. *Anal. Biochem.* **2005**, *339*, 262−270.
(18) Tawa, K.; Yao, D. F.; Knoll, W. *Biosens. Bioelectron.* **2005**, *21*, 322−329.
(19) Vareiro, M. M. L. M.; Liu, J.; Knoll, W.; Zak, K.; Williams, D.; Jenkins, A. T. A. *Anal. Chem.* **2005**, *77*, 2426−2431.
(20) Touahir, L.; Jenkins, A. T. A.; Boukherroub, R.; Gouget-Laemmel, A. C.; Chazalviel, J.-N.; Peretti, J.; Ozanam, F.; Szunerits, S. *J. Phys. Chem. C* **2010**, *114*, 22582−22589.
(21) Yang, X. Y.; Guo, Y. S.; Bi, S.; Zhang, S. S. *Biosens. Bioelectron.* **2009**, *24*, 2707−2711.
(22) Du, D.; Zou, Z.; Shin, Y.; Wang, J.; Wu, H.; Engelhard, M. H.; Liu, J.; Aksay, I. A.; Lin, Y. *Anal. Chem.* **2010**, *82*, 2989−2995.
(23) Tian, J. N.; Zhou, L. J.; Zhao, Y. C.; Wang, Y.; Peng, Y.; Zhao, S. L. *Talanta* **2012**, *92*, 72−77.

DOI: 10.1021/ac504642j
Anal. Chem. 2015, 87, 3871−3876